A Physiological Study of the Effect of Bee Pollen on Albino Rats Induced Hypothyroidism

Ahmed Haleem kadhim[1], Aseel Najah Sabour[2]

[1,2]*University of AL-Qadisiya / College of Education /Department of Biology. Email: edu.bio.posta52@qu.edu.iq. aseel.najah@qu.edu.iq*

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ABSTRACT
The current study aimed to evaluate the therapeutic role of bee pollen (BP) in reducing the harmful effects resulting from hypothyroidism compared to the drug thyroxine on some physiological indicators and improving their levels, which included weight gain (gm), hormone level (T3, T4, TSH), level TPO, cholesterol levels, HDL, LDL, VLDL, and liver enzymes ALT, AST, Hypothyroidism was induced in (24) male rats using the drug carbimazole (CBZ) Which lasted for (15) days at 30 mg/kg of body weight. A random sample was chosen to confirm the occurrence of hypothyroidism, as the results of examining blood samples that were drawn from the animals showed a high concentration of the TSH hormone and Low concentrations of thyroid hormones in the serum compared to the negative control., and this indicates the occurrence of hypothyroidism. Then the animals in which hypothyroidism was induced were separated into 3 experimental collections in addition to the control group and a fifth group. Each group consists of (8) animals. The dosing period lasted thirty days orally. The negative control group, G1, was provided with a diet and water freely. G2 was continued to be dosed with the drug (CBZ) at a concentration of 30 mg/kg of body weight. G3 was continued to be dosed with the drug (CBZ) at a concentration of 30 mg/kg and solution (BP). At a concentration of 200 mg/kg body weight, G4 was dosed with levothyroxine at a concentration of 20 mg/kg body weight, while G5 was dosed with BP solution only at a concentration of 200 mg/kg body weight. At the end of the experiment, the animals were sacrificed and blood samples were obtained. The results were less than 0.05 in group G2 in body weight, the concentration of thyroid hormones T3 and T4, and the concentration of TPO and HDL, and a significant increase ($P<0.05$) in the concentration of TSH, Cholesterol, LDL, and VLDL, respectively, with an increase in the concentration of liver enzymes. AST and ALT compared to the control group. As for the G3 and G4 groups, the result appeared an original increase a total body weight and the concentration of the hormones T3, T4, and the TPO enzyme, a noticeable improvement in the lipid profile, and a low level of the ALT enzyme and the and thyroid-stimulating hormone compared to the G2 group. As for the group G5 has witnessed an increase in T4 concentration and a decrease in ALT concentration and body weight, and the concentrations of T3, TSH, and TPO are closer to control. We conclude from the current study that BP has a role in reducing the effects of hypothyroidism, increasing weight gain, and improving the lipid profile and the level of ALT liver enzymes. AST and Thyroid peroxidase (TPO) level.

1. Introduction

Foods from plant sources have gained great importance. Its safety and therapeutic ability are due to its high content of bioactive polyphenols, which depends mainly on its structure, and its main role in protecting against different kinds of diseases such as high blood sugar, cholesterol, blood lipids, and cancer[1], pollen collected by bees is considered Apis mellifera in powder form from plant flowers and beekeepers collect it using pollen traps. Because they are considered excellent sources of biologically active compounds that act as Anti-inflammatory, anti-microbial, anti-oxidant, and increases the activity of the immune system [2, 3]. According to the review by Thakur and Nanda (2020) of more than a hundred studies to determine the average BP content, it was as follows: carbohydrates 54.22%, proteins 21.30%, fats 5.31%, fibers 8.75%, ash 2.91%, as well as various percentages. Of glucose, fructose, sucrose, and mineral elements such as potassium, calcium, phosphorus, magnesium, zinc, and iron, the total content of phenol reached 30.59 mg\g. Pollen also contains phenolic compounds that have an effect in reducing the effects of metabolic syndrome in infected mice. It has also been used to prevent obesity in humans and non-alcoholic fatty liver disease [4]. Thyroid hormones affect the functions of almost all organs; Because they mainly control a different group of pathways that participate in the cellular metabolism of proteins, fats, and carbohydrates in many target tissues, They also regulate biogenesis and mitochondrial respiration, and any disturbance in these hormones will lead to an imbalance in these processes[5] thyroid disorders include hypothyroidism, which is a term that expresses a decrease in the amount of thyroid hormone in target tissues regardless of the cause [6]. Levothyroxine (LT4) is prescribed as the well-

known treatment Due to a deficiency of thyroid hormones. [7], but its long-term use is associated with a higher risk of stroke and heart failure [8, 9]. As for hyperactivity, it is treated with carbimazole, which can cause nephrotoxicity and Hepatitis[10]. It causes programmed cell death in parts of the liver and also an increase in the number of Kupffer cells [11]. To reduce the side effects of chemotherapy used in many diseases in the body, such as endocrine diseases, plant-based products are used because of their therapeutic activity and their absence of almost any side effects or toxicity [12], whereas plants were used. Medicine has been around for as long as humanity has existed, and according to W.H.O. reports, about 80% of people use herbal products for prevention or treatment [13]. The current study aims to determine the effectiveness of bee pollen in reducing the harmful effects of hypothyroidism and compare it with the use of chemotherapy on weight gain, thyroid hormones, Thyroid peroxidase (TPO) profile, lipid profile, and liver enzymes AST and ALT in white male rats in which hypothyroidism was induced with the drug carbimazole. Thyroxine drug

2. Materials and Methods

Study design: The current study used 40 male rats of the *Ratts norvegicus* strain, with ages ranging between (10-11) weeks and weights ranging between (150-200) grams. The first experiment included inducing hypothyroidism in (24) male rats using the drug carbimazole. At a concentration of 30 mg/kg of body weight, the dosage continued for (15 days), after which a random sample was selected to confirm the occurrence of hypothyroidism by measuring the levels of hormones (T3, T4, and thyroid-stimulating hormone) in serum. The level of thyroid-stimulating hormone increased, accompanied by a decrease in the level of both T3 and T4 in laboratory results, which confirms the occurrence of hypothyroidism in these animals. The second experiment included the following groups: G1, numbered (8), representing the negative control group, G2, numbered (8), representing the positive control group, G3, numbered (8), and the group treated with bee pollen, G4, number (8) represents the group treated with Thyroxine-L, and G5, number (8), represents the group fed with pollen only. The weights of the animals were measured at the end of the first and second experiments, and after a day had passed since the last dose, a sample of blood using a five-ml medical syringe from the heart directly after anesthetizing them with chloroform, and the blood samples were placed in tubes devoid of clotting factors for (15- 20 minutes, then transferred to a centrifuge for (15) minutes at a speed of (3000) rpm to obtain the serum. It was placed in an Eppendorf Tube with a capacity of (1.5) ml and kept at a temperature of (-20) until it was used to measure biochemical tests.

Studied criteria:

Various Weight

Weights were recorded at the beginning and end of the second experiment according to the following equation: -

Weight gain (g) = final weight (g) - starting weight (g)

Hormonal parameters:

Measure T3, T4, TSH

Measured according to the method [14-17]

Measurement of blood fat profile:

Measuring cholesterol level

[20-18] Measured according to

Measure HDL level

The measurement was done according to the method [21].

LDL level measurement

The measurement was done according to the method [22].

Measurement of VLDL level.[23] Measurement was done according to the method of

Measuring the level of AST, ALT, and TPO

A colorimetric method was used to determine the activity of liver enzymes, which included AST and ALT by using the colorimetric method [25 ,24], and the percentage of TPO enzyme was measured by the enzyme immunofluorescence method (ELFA) at 450 nm using the VIDAS device.

Statistical analysis

The results of the study were analyzed statistically using the statistical program known as the Statistical Package for the Human Sciences (SPSS), version 32. The statistical test used the difference single analysis of variance (ANOVA) to compare the means of the studied physiological indicators, and to compare between any two rates, the statistical test used the least significant difference (LSD). The average and standard deviation were calculated for all indicators. In the groups included in the study, the results were based on a 5% significance level [26].

3. Results and Discussion

Weight gain

Group G2 treated with carbimazole only showed a significant decrease (P>0.05) in the rate of weight gain compared to the control group G1 and all treatments. The results also showed a significant decrease (P>0.05) in G3 and G4 treated with carbimazole with bee pollen and levothyroxine only, respectively, in comparison. With the negative control G1, There were no fundamental differences (P>0.05) between G3 and G4. The results also showed a statistically significant increase (P>0.05) in G5 treated with BP only compared to the hypothyroid groups G2, G3, and G4, as shown in Table No. (1).

Changes in hormone levels

Treatment G2 recorded a significant decrease of more than 0.05 in the concentration of T3, and T4, accompanied by a significant increase of more than 0.05 in the concentration of TSH in blood serum compared to control G1, and both groups G3, and G4 a statistically significant increase was recorded more than 0.05 in hormonal concentration. T3 and T4 were accompanied by a significant decrease (P>0.05) in the concentration of TSH compared to G2. As for the G5 group, its results were similar to the negative control group G1, as clarified in Table No. (1).

Table No. (1): Shows the effect of bee pollen on weight gain and T3, T4, and TSH.

Standard / Groups	Weight gain mg/ml	T3 mg/ml	T4 mg/ml	TSH µU/mL
G1	40.8±1.8a	1.74±0.07a	39.5±6.3ab	0.008±0.003b
G2	2.6±0.18d	1.20±0.04c	13.14±0.93d	0.104±0.05a
G3	30.4±1.45c	1.35 ±0.10b	21.66±3.4c	0.020±0.01b
G4	32.2±1.16c	1.44±0.10b	33.52±3.38b	0.013±0.004b
G5	36.8±2.24b	1.84±0.11a	33.96±7.4a	0.032±0.013b
LSD	2.68	0.125	6.42	0.034

- Numbers indicate mean ± standard error.
- Probability level 5%
- Different vertical letters between the totals indicate significant differences.

- Similar vertical letters between any two rates mean there are no fundamental differences

Changes in fat profile

The results are shown in Table No. (2) showed that the levels of both cholesterol, high-density lipoprotein (HDL), and Very low-density lipoprotein (VLDL) increased significantly (P>0.05) in treatment G2, accompanied by a significant decrease (P>0.05) in the level of HDL compared to control G1, while treatment G3 showed a significant decrease (P>0.05) in the levels of both LDL cholesterol and VLDL, compared to the G2 group. The G3 group also recorded a significant decrease in the HDL level, accompanied by a significant increase in the LDL level, and the levels of both cholesterol and VLDL converged compared to the G1, while the G4 group recorded a significant decrease (P>0.05) in the levels of LDL and VLDL cholesterol was accompanied by a significant increase (P>0.05) in the HDL level compared to the G2 group. The G4 group also showed a significant increase more of than 0.05 in the HDL level and a convergence in the levels of both LDL cholesterol and VLDL compared to G3. The G4 group also recorded a significant decrease in the level of HDL, accompanied by a significant increase in the level of LDL, and the levels of both cholesterol and VLDL converged compared to the control group G1, while the G5 group witnessed a convergence of the levels of both cholesterol and HDL, LDL, and VLDL in comparison. With the control group G1, group G5 also recorded a significant decrease of more than 0.05 in the levels of cholesterol and HDL, LDL, and VLDL compared to group G2. The results of group G5 also showed a significant increase of more than 0.05 in the level of HDL accompanied by a decrease. Significant (P>0.05) in the LDL level and the levels of both cholesterol and VLDL were close compared to group G3. In addition, group G5 witnessed a significant (P>0.05) decrease in the LDL level accompanied by a significant (P>0.05) increase in the HDL level. There was a similarity in the levels of both cholesterol and VLDL compared to the G4 group.

Table 2. Shows the effect of bee pollen on the lipid profile in rats with induced hypothyroidism.

Standard / Groups	Cholesterol mg/ml	HDL mg/ml	LDL mg/ml	VLDL mg/ml
G1	43.12 ±3.4ab	31.32±2.59 a	6.2±2.77c	5.6±1.51b
G2	52.6 ±1.92a	20 ±0.44 c	22.6±1.34a	10 ±0.89a
G3	44 ±2.53b	22±2.68 bc	15 ±1.09b	7±1b
G4	48 ±4.08ab	25.2 ±5.16 b	17 ±5.5b	6.4±1.34 b
G5	43.7±12.4b	32.32±3.35a	4.4±0.89c	7±0.7b
LSD	8.2	4.25	3.81	1.49

- Numbers indicate mean ± standard error.
- Probability level 5%
- Different vertical letters between the totals indicate significant differences.
- Similar vertical letters between any two rates mean there are no fundamental differences.

Changes in liver Enzymes AST and ALT

Group G2 witnessed a significant increase (P>0.05) in the levels of both ALT and AST in the serum compared to the control group G1. Treatment G3 and G4 also recorded a significant decrease (P>0.05) in the levels of AST and ALT in the blood serum compared to group G2. Group G3 did not record significant differences in the concentration of ALT and AST compared to group G4, while group G5 recorded a decrease in the concentration of ALT, but it did not record a significant difference (P>0.05) in the concentration of AST compared to the control group G1. It also showed a significant decrease. In the concentration of ALT, AST compared to G2, no significant differences

(P>0.05) were observed in group G5 in the concentration of ALT, AST compared to groups G3, G4, as shown in Table No. (3).

Changes in the level of the enzyme Thyroid peroxidase (TPO)

Group G2 recorded a significant decrease of more than 0.05 in the level of the enzyme Thyroid peroxidase (TPO) compared to the control group G1, while groups G3 and G4 witnessed a significant increase in the level of this enzyme compared to group G2, but this increase did not reach the level of the enzyme (TPO) In the negative control group G1, group G5 did not show significant differences of more than 0.05 in the level of this enzyme compared to the G1. As shown in Table No. (3).

Table 3. Shows the effect of bee pollen on the liver enzymes AST, ALT, and thyroperoxidase (TPO) in male rats with induced hypothyroidism.

Standard / Groups	AST U/L	ALT U/L	TPO IU/mL
G1	77.2 ±6.63c	57±3.53b	0.90±0.15a
G2	144.4±12.7a	92.26±9.2a	0.30±0.07c
G3	98.8±7.46b	41.78±2.16c	0.58±0.19b
G4	106.04±13.6b	37.78±10.7c	0.70±0.10b
G5	86.2±5.76 bc	41.9±5.01c	0.90±0.12a
LSD	12.94	9.19	0.179

- Numbers indicate mean ± standard error.
- Probability level 5%
- Different vertical letters between the totals indicate significant differences.
- Similar vertical letters between any two rates indicate that there are no significant differences.

The Effect of Bee Pollen on The Rate of Weight Gain

The results indicated a decrease in the rate of weight gain in group G2 Which is carbimazole doses compared to the G1. This can be attributed to a decrease in feeding compared to the rest of the groups. This is the same as what a study found [27], and this is a result of the lack of Energy consumption and an increase in the level of fat resulting from hypothyroidism. This leads to a rise in the hormone level of the hormone Leptin in the blood, which gives a feeling of fullness and suppresses the feeling of hunger. It is secreted from adipose tissue and intestinal cells when the level of fat rises to regulate the balance of energy consumption and a decrease in mass. Fat within adipocytes [28, 29]. Furthermore, the decrease in the levels of thyroid hormones is directly linked to a decrease in appetite, which causes a decrease in appetite for food and leads to a decrease in weight [30]. Weight gain in body weight increased in G3 treated with CBZ and bee pollen together compared to G2 treated with carbimazole only, and This is similar to what he found [31], which indicates the role of pollen in improving levels of metabolic hormones such as increasing insulin. -like growth factor-1, Insulin, and T3, T4 in the blood plasma of rabbits [32, 33]. Many studies indicated the role of BP in increasing muscle mass and metabolism. In rats that suffer from a poor diet, it also contributes to an increase in the levels of proteins, globulin, and albumin, and thus helps well in fattening chickens [34, 35], and the phenolic compounds in grains also work. The vaccine protects liver cells from chemicals and free radicals and reduces the harmful effects of fatty degeneration [36]. The results did not show a significant difference in weight gain between the G3 and the G4 that was treated with thyroxine, which worked to treat hypothyroidism and reduce its

effects. This is confirmed by a study [37] which indicates the effect of levothyroxine in treating thyroid-induced hypothyroidism in rats. Hypothyroidism and increased bone and periosteal growth in long bones compared to the group treated with carbimazole only[37], which explains the Increase in weight when compared to G2. As for the G5 Feeding on pollen grains only, there was a significant increase in weight gain compared to all hypothyroidism groups (G2, G3, G4). The weight gain is attributed to the fact that bee pollen is a rich source of powerful antioxidants such as phenolic compounds and flavonoids. Amino acids, furthermore it a large group of nutrients such as proteins, fats, carbohydrates, fibers, and micronutrients with biological activity such as vitamins and mineral elements [38, 39], and BP is characterized by containing more than 250 active substances [40].

The Effect of Bee Pollen on The Concentration of T3, T4, and TSH Hormones

The decrease in the concentration of T3 and T4 thyroid hormones in treatment G2 compared to the control group G1 and the rest of the treatments is attributed to the role of the drug carbimazole (CBZ), which is converted into methimazole (MMI) and is used to treat excessive thyroid secretions, as it works to reduce the concentration of thyroid hormones through. Reducing hydrogen peroxide, Which must be available to work off the Thyroid peroxidase (TPO) enzyme, and inhibiting it from adding iodine to Tyrosine and linking it to Thyroglobulin, which is the process necessary for biosynthesis of thyroid hormones [41, 42], as for TSH concentration It increased significantly compared to control G1, and this is consistent with the study [43] to compensate for the decrease in hormone concentration caused by the drug (CBZ). The concentration of T3 and T4 increased significantly in the G3 group compared to the G2 treatment, and this is consistent with the study [44], which indicated the role of flavonoids, which are one of the components of bee pollen, as they work to increase the absorption of iodine in the thyroid gland and Consequently, there is an increase in the production of hormones within the physiological rate, in addition to an increase in the level of Insulin-like growth factor-1 and Insulin in the plasma of rabbits fed bee pollen [32, 33].

In the fourth treatment, the concentration of T3 and T4 hormones increased significantly compared to treatment G2. This is due to the role of the drug L-thyroxine, which is considered a substitute for the T4 hormone and has the same physiological role as the thyroxine hormone. It is used to treat hypothyroidism caused by lymphatic goiter or patients who have had their thyroid removed [45], and this also explains the lower concentration of TSH in this treatment given the increased concentrations of thyroid hormones and the inverse relationship between them. The G5 group treated with pollen only did not witness significant differences in the concentration of hormones (T3, TSH) compared to the negative control group G1. This is consistent with the study [46], which indicated the effectiveness of bee pollen in improving changes in the thyroid gland and its hormone concentrations within the average range. Natural, in addition to increasing the total antioxidants also stimulates the body's immunity[47], In contrast, BP shows an enzyme inhibitor role D1 deiodinase enzyme in the liver, which converts tetraiodothyronine to triiodothyronine, thus increasing the concentration of (T4) [44].

The Effect of Bee Pollen on Lipid Profile

The G2 group treated with carbimazole only witnessed a significant increase in the levels of both cholesterol LDL and VLDL compared to the control group G1. This is consistent with the study [48], and could be the reason for the decrease in the thyroid hormones T3 and T4 and their effect. On metabolic pathways and controlling the metabolism of fats, proteins, and carbohydrates [49], a high level of TSH is directly related to the level of the lipid profile [50]. The results also showed a significant decrease ($P>0.05$) in the concentration of LDL, VLDL, and Cholesterol and a significant increase ($P>0.05$) in the HDL concentration in the G3 group treated with pollen compared to the G2 group, which continued to be dosed with carbimazole throughout the experiment, and this could be a result of effectiveness of bee pollen in increasing fat metabolism by increasing the concentration of related hormones such as thyroxine, insulin, and testosterone, and this leads to lowering the level of fat [33]. This is consistent with the study that indicates the role of pollen in lowering the level of fat

and Glucose improves the tissue structure of the liver and is an anti-inflammatory [34, 51]. Pollen grains contain the compound Apigenin, which belongs to the flavone group known as 4′, 5, 7, -Trihydroxyflavone, which has an effective effect. In reducing the harmful effects of high blood glucose, and dyslipidemia, and improving liver and kidney functions in rats, as well as in autoimmune disorders [52, 53]. The lipid profile in group G4 decreased significantly due to levothyroxine, and These results are similar to his findings [54], which indicated the the action of levothyroxine in increasing the level of thyroid hormones in patients with hypothyroidism [45, 55], so high blood fat levels are associated with a low level of FT4, as the drug levothyroxine works to increase the level of FT4 and thus reduce the effects of hypothyroidism on imbalanced fat levels [56].

Effect of Pollen on AST, ALT, and TPO levels

The results of the study shown in Table (3) showed that there is a statistical increase (P>0.05) in liver enzymes for group G2 treated with carbimazole, which can cause hepatotoxicity [10] and cause an increase in the concentration of aspartate and aminotransferase enzymes in the serum [57], causing hypothyroidism. Liver disease, poor fat metabolism, hyperammonemia [58], and a high level of the ALT enzyme indicate damage to liver cells [59]. The level of the AST enzyme also increases in patients with hypothyroidism due to a defect in the liver. in hepatocytes [60]. Groups G3 and G4 did not show significant differences between them. This is due to the action of bee pollen pills, which is similar to The drug levothyroxine reduces the effects of hypothyroidism. The ALT and AST levels were significantly lower in group G3 treated with pollen and carbimazole compared to G2 as a result of the role of bee pollen pills. BP, works to restore its proportion to the physiological value, and this is consistent with the study [33], which indicated the role of bee pollen in reducing the harmful effects of carbimazole, and because of the antioxidant content of bee pollen, it plays a role. It enhances immunity, protects the liver, and heals wounds without side effects [61]. Phenolic compounds work to donate electrons to free radicals and limit their harmful activity [62]. On the other hand, compounds in bee pollen work to activate enzymes with antioxidant activity such as glutathione reductase (GSH) and glutathione peroxidase (GPX), Glutathione-S-transferase (GST), and Superoxide dismutase (SOD) [63, 64].

In group G4, the concentration of liver enzymes AST and ALT decreased significantly compared to group G2, and this is consistent with the study [65], as the drug levothyroxine improves liver function and reduces the effects of oxidative stress and hepatopathy caused by hypothyroidism [57, 66], and the level of ALT also decreased in the G5 treated with pollen only compared to the control group G1 and the G2, which is attributed to phenolic compounds that work to protect the liver and modify the density of microorganisms. In the intestines of rats, it also prevents non-alcoholic fatty liver disease Caused by eating a high-fat diet, as well as reducing oxidative stress and its effects such as inflammation [4]. In treatment G2, the level of the Thyroid peroxidase enzyme TPO decreased compared to control G1 resulting from treatment with carbimazole, which causes damage to the epithelial cells lining the follicles, their bleeding and damage, and a decrease in the glycoprotein inside them, with a lack of homogeneity of the colloidal material or its absence in some follicles [67]. The level of TPO in G3 treated with pollen and carbimazole is due to the role played by bee pollen in improving the Histological components of the thyroid and follicles and thus increasing the production of TPO, which reflects positively on the Raising the level of thyroid hormones, and this increase is similar to that of group G4 in which hypothyroidism is induced and is treated with thyroxine.

4. Conclusions

We conclude from the current study the effectiveness of bee pollen in reducing the effects of hypothyroidism, increasing weight gain, improving lipid profile, the level of Liver enzymes Which included both AST and ALT, and increasing the level of the TPO enzyme necessary for the synthesis of thyroid hormones. Hypothyroid patients may benefit from pollen. Bees improve the level of

thyroid hormones. With a need for more studies to know other possible therapeutic aspects of bee pollen.

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