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Evaluation of Metformin and Nigella Sativa in Treatment of Type 2 Diabetes Mellitus (T2DM) and Hypothyroidism: A Hormonal and Biochemical Study in Male Rats

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Abstract:

Background:

Diabetes and hypothyroidism are common hormonal disorders that negatively affect metabolic function. The relationship between the two diseases is reciprocal, meaning that people with one are more likely to develop the other. This study aimed to investigate the synergistic effect of metformin and black seed (Nigella sativa) in the treatment of male rats with type 2 diabetes and hypothyroidism. The experiment was designed with 120 male Wistar rats (weighing 200-250 grams) divided to 6 groups (20 rats per group). The first groups were control group (G1), receiving no treatment other than normal saline. The remaining five groups underwent induction of type 2 diabetes mellitus (T2DM) with a single intraperitoneal dose of alloxan, and symptoms of diabetes were observed 72 hours after the alloxan injection. Following diabetes induction, the second group received no treatment. The third group was treated with metformin, the fourth with black seed extract, and the fifth with a combination of metformin and black seed extract, all administered once daily for 30 days. The sixth group was unique in its treatment duration. After induction of type 2 diabetes mellitus and observation of clinical signs confirming diabetes 72 hours later, hypothyroidism was induced through oral administration of carbimazole for 28–30 days. After conducting hormonal tests and confirming hypothyroidism in addition to clinical signs, they were treated with metformin and black seed extract together for thirty days. The hormonal and biochemical indicators were measured, including thyroid hormones (T3, T4, and TSH), fasting blood glucose levels and body weight. The measurements showed that the combined administration of metformin and black seed extract, as well as treatment with each alone, significantly regulated thyroid hormone levels and significantly decreased fasting blood glucose levels and improve body weight compared to the (G1) and untreated type 2 diabetes group(G2). The results demonstrate that the combined administration of metformin and black seed extract has a significant effect on enhancing metabolic and hormonal indicators in diabetic and hypothyroid rats.

Aims

This study aims to assess the pharmacological properties of Metformin and black seed extract, both individually and in combination, in a rat model of chemically induced diabetes associated with hypothyroidism. This will be achieved by examining changes in body weight, blood glucose levels, and thyroid hormones. This study seeks to fill a significant scientific gap in the field

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of therapeutic interaction between chemical drugs and herbal preparations within experimental veterinary medicine and to provide findings that may have future practical applications. The research focuses on the fact that the combined administration of metformin and black seed extract has a synergistic effect in improving hormonal and metabolic parameters in diabetic and induced hypothyroid mice and is more effective than either treatment alone.
Keywords: Type 2 diabetes mellitus, hypothyroidism, metformin, Nigella sativa, Wister male rats, thymoquinone, glycemic control.

Introduction

Veterinary medicine is an essential science that contributes directly to animal health, protective public health, and confirming food safety, mainly through the study of metabolic and endocrine diseases affecting companion and production animals (Redistils et al.,2007; constable et al., 2017). Among these disorders, type 2 diabetes mullite's(T2DM) was induced by a single intraperitoneal injection of alloxan (150 mg/kg) (Munna, 2017; Han et al., 2019) and hypothyroidism was induced in diabetic rats with the use of an antithyroid drug, carbimazole by administration orally at a dose of 30 mg/kg, (El-Bakry et al., 2016; Hassan et al., 2019), are among the most common hormonal disorders, due to their wide-ranging effects on metabolic balance, growth, and various organ functions. The pathological overlap between diabetes and hypothyroidism presents a complex case, as thyroid hormones directly affect glucose metabolism (Brent,2011; Duntas&Orgiazzi,2013), insulin sensitivity, and energy regulation, while chronic hyperglycemia disrupts the thyroid-pituitary axis. If not addressed thoroughly, this disruption could exacerbate clinical manifestations and hinder treatment efficacy. Metformin is a widely used and effective medication for treating type 2 DM, as it improves insulin sensitivity and reduces hepatic glucose production. However, its long-term use may be associated with varying effects on thyroid function, the dose of metformin 300mg/kg was administered orally (foretz et al.,2014; Vigersky et al.,2006). Metformin is broadly used and is known as a primary-drug for the treatment of (T2DM) due to its remarkable capability to low hepatic glucose production and improve insulin sensitivity in peripheral tissues. Metformin's mechanism of action is primarily through the Activation of Adenosine Monophosphate Kinase (AMPK), which plays a critical role in regulating cellular energy stability. Further studies have demonstrated an additional and important role for metformin beyond glucose regulation, such as its unique anti-inflammatory and antioxidant properties, which can negatively impact endocrine functions, such as thyroid hormone regulation (Foretz, M., et al., 2023). On the other hand, black seed (Nigella sativa) is receiving increasing scientific attention due to its active compounds, particularly thymoquinone (Ahmad et al.,2013; Hannan.,2007), which has demonstrated antioxidant, hypoglycemic, and endocrine-supporting properties. The dose of black seed extract(300mg/kg) was administered orally (Danladi et al.,2013; Sadiq et al.,2021). Recent studies and reports indicate that black seed improves

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insulin production and enhances glucose uptake in peripheral tissues. It shares some of these mechanistic properties with metformin, thus enhancing its effects. Furthermore, it plays a role in protecting pancreatic B-cells from damage caused by oxidative stress. These characteristics made it a promising complementary treatment for metabolic disorders such as diabetes and thyroid dysfunction (Hannan et al., 2023; Leong et al., 2023). The expectation was that administering metformin alongside Nigella sativa would lead to a greater enhancement of metabolic and thyroid function compared to when either agent is used independently. Accordingly, black seed, also identified as Nigella sativa or black cumin, contains many biologically active substances, the most important of which is thymoquinone, which plays an effective role in many therapeutic activities (Figure 1). NS oil It is extracted using the cold method to obtain the highest oil thymoquinone content (Ahmad et al., 2015; Danladi et al., 2013; Sadiq et al., 2021). This research seeks to contribute valuable evidence to veterinary clinical practice and potentially offer new insights relevant to both animal and human health.

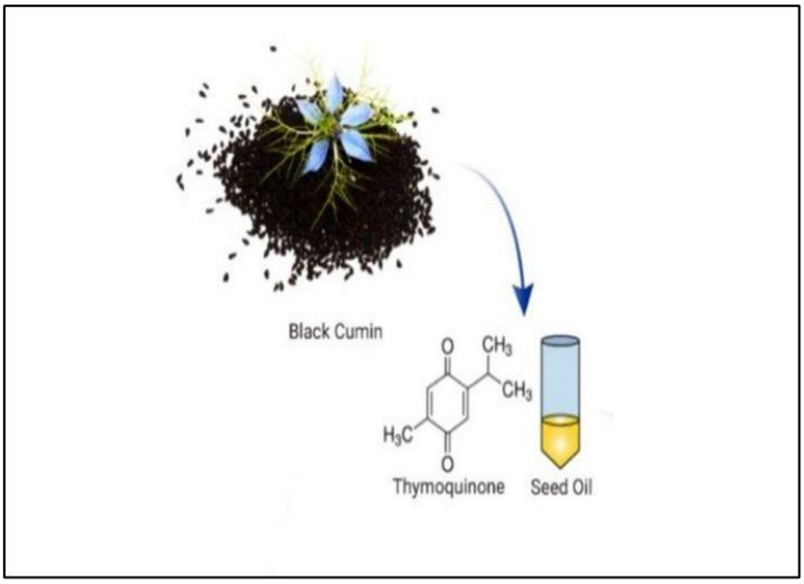

Figure 1: Black seed, Seed oil and the Chemical structure of active compound of Thymoquinone (Adapted from Hannan et al.,2021)

The image above shows black seed, also called black cumin, and its most important active compounds, namely thymoquinone, its chemical structure, and its oil extract, which plays an effective role as an anti-oxidant and anti-inflammatory agent, and has a dynamic role in the endocrine system.

Materials and Methods

This study aimed to estimate and determine the properties of metformin and black seed extract, individually for metformin and black seed, and both together, on hormonal, metabolic, and thyroid measures in a male Wistar rat model of type 2 diabetes mullites (T2DM) associated with hypothyroidism The experimental design followed the previously mentioned number of animals and groups. Diabetes was induced first by using alloxan, and fasting blood glucose levels were measured

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72 hours until they reached their peak. This was achieved by measuring blood glucose levels daily in the morning after the animals had fasted overnight for 72 hours. Clinical signs confirming the onset of diabetes were also observed. Treatments were then administered to each group according to its specific needs. For the 6 group, after inducing diabetes for 72h by alloxan, hypothyroidism was induced using carbimazole at a dose of 30mg/kg for 28-30 days. Following this, the group received the treatment as outlined in the introduction. The Wistar rats (n=120), (10-12 weeks old), (weighing 200-250 g), The choice of sample size was informed by earlier analogous experimental research. and studies to ensure robust statistical results and clearly identify differences between groups. The findings were further strengthened and supported by the application of the study to a larger number of animals. were attained from the Tikrit University College of Veterinary Medicine Animal House. The rats were kept in polypropylene containers (three rats per container) and the environmental conditions were kept the same throughout the experiment (Temperature= 25±2°; Humidity= 50-60%; 12- h light/dark cycle (light on at 07.00 hrs.)). During the acclimatization and the experiment, the rats had free access (ad libitum) to standard rodent feed (60% carbohydrate, 22% protein, 5% fat, 5% fiber, and 8% ash) and water. The experimental protocols were made to adhere to the strategies issued by the team for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the ARRIVE 2.0 reportage strategies (Percie du Sert et al., 2020) and were approved by the animal Ethics Committee (IAEC/2022/Pharm/001) of the University. The experimental protocols were done in such a way to mitigate the suffering on the animals, and the number of animals used in the experiment was kept at the minimum to be statistically sufficient. Blood samples were obtained using the method described in (Sharma, A., Fish, B. L., Moulder, J. E., Medhora, M., Baker, J. E., Mader, M., & Cohen, E.P, 2014) p. 117, and they punctured the retro-orbital sinus, and the blood was then poured into an anticoagulant-coated capillary tube and allowed to clot for 30 minutes at room temperature. Serum was obtained and centrifuged at 3000 rpm for 15 minutes, and the temperature was maintained at 4°C. The serum was then kept at -80°C pending the analysis.

Treatment Regimen :

Group 1: Healthy control Rats group (saline)
Group 2: Diabetic control Rats group (alloxan only)
Group 3: Diabetic Rats treated with metformin only (300 mg/kg)
Group 4: Diabetic Rats treated with black seed extract only (300 mg/kg)
Group 5: Diabetic Rats treated with the combination (300 mg/kg metformin + 300 mg/kg black seed extract
Group 6: Diabetic Rats with hypothyroidism treated with the combination (300 mg/kg metformin + 300 mg/kg black seed extract).

Experiment timeline in the following way:

First, on day 0, we gave rats a special shot of alloxan to make them diabetic. Each rat got 150 milligrams of alloxan per kilogram of their body weight.

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Next, on day 3, we took a blood sample from each rat to check their blood sugar levels. This helped us confirm that the diabetes had taken hold.

After that, we started the experimental treatments for the next 27 days. Each day, the rats in different groups got either just metformin, just a special herb extract called black seed, or a combination of the two.

One group of rats - let's call them group 6 - got an additional condition, called hypothyroidism. We did this on day 4 when we'd confirmed they had diabetes by giving them carbimazole, a 30-milligram dose per kilogram of body weight, every day for about a month.

Only after we'd confirmed the hypothyroidism through some lab tests did we start giving the rats in this group our special combination treatment of metformin and black seed extract. They got this treatment for 30 days.

When all that was done, we drew more blood from the rats for further testing and analysis .

Ethics approval:

This study was conducted after obtaining approval from the Animal Research Ethics Committee at the College of Veterinary Medicine, Tikrit University, and in accordance with ARRIVE guidelines for animal experimentation, with consideration given to minimizing animal suffering. The study period was more than two months, from September until the beginning of October 2025.

General Statistical Analysis

IBMSPSS Statistics version 26 was used to analyze the statistical data. The Shapiro-Wilk test was used to verify the normality of the data distribution, and the Levene test was used to determine the homogeneity of variance. ANOVA was used to compare groups, followed by the LSD test statistically significant changes were found at the $p < 0.05$ level.

Results

The findings indicated distinct variations between the different experimental groups. in relations of body weight, blood glucose levels, and thyroid function after different treatment periods. A significant improvement in the studied parameters was detected in the groups who treated compared to the untreated groups.

Table 1: Comparison between difference groups in Body weight and Blood glucose

Group	Mean ±SE	
	Body weight (g)	F.B.S. (mg/dl)
G1: Control	227.79 ±4.12 a	95.13 ±2.42 e
G2: DM Type 2 (without metformin)	177.51 ±3.79 d	214.58 ±8.51 a
G3: DM Type 2 (with metformin)	185.18 ±3.56 cd	127.60 ±0.82 c
G4: DM Type 2 (with Nigella sativa)	189.34 ±3.56 bc	154.35 ±1.36 b
G5: DM Type 2 (with metformin + Nigella sativa)	190.56 ±3.56 bc	118.25 ±0.55 cd
G6-1: DM Type 2+hypothyroidism before treatment	196.98 ±3.61 b	216.68 ±8.30 a
G6-2: DM Type2 + hypothyroidism after treatment (with metformin + Nigella sativa)	219.63 ±4.13 a	114.55 ±0.72 d
L.S.D.	10.550 *	12.974 *
Means having with the unlike letters in same column differed significantly. *(P≤0.05)		

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Table 1 demonstrations the results, where fasting blood glucose levels showed a significant decrease in the metformin and black seed treatment groups, particularly the combined treatment group, associated to the untreated diabetic group, with statistically significant alterations ($P \leq 0.05$). This group also demonstrated better blood glucose control compared to the monotherapy group. Regarding body weight, the treated groups showed gradual weight recovery compared to the untreated diabetic groups, with the combined treatment group showing improvement closer to the controller group, with statistically significant alterations ($P \leq 0.05$)

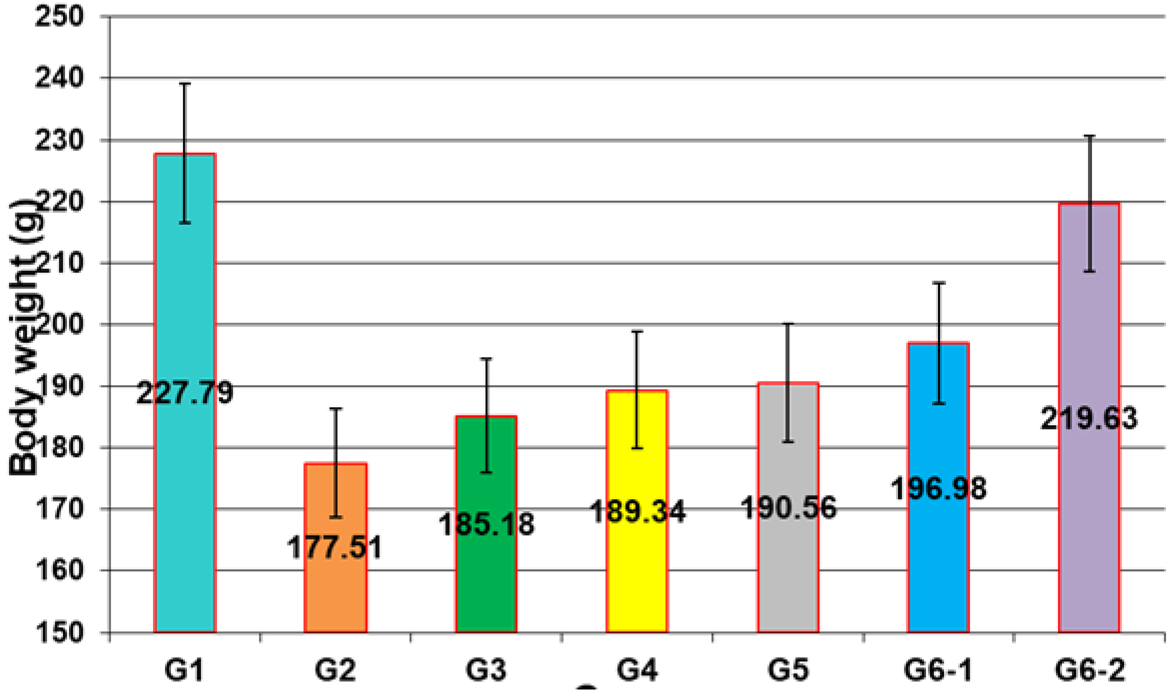

Figure 2: The data revealed significant differences in fasting blood glucose levels between the experimental different groups.

The group treated with metformin and black seed showed a more pronounced decrease, nearer to that of the control group, compared to the group with untreated diabetes, which had the highest blood glucose levels.

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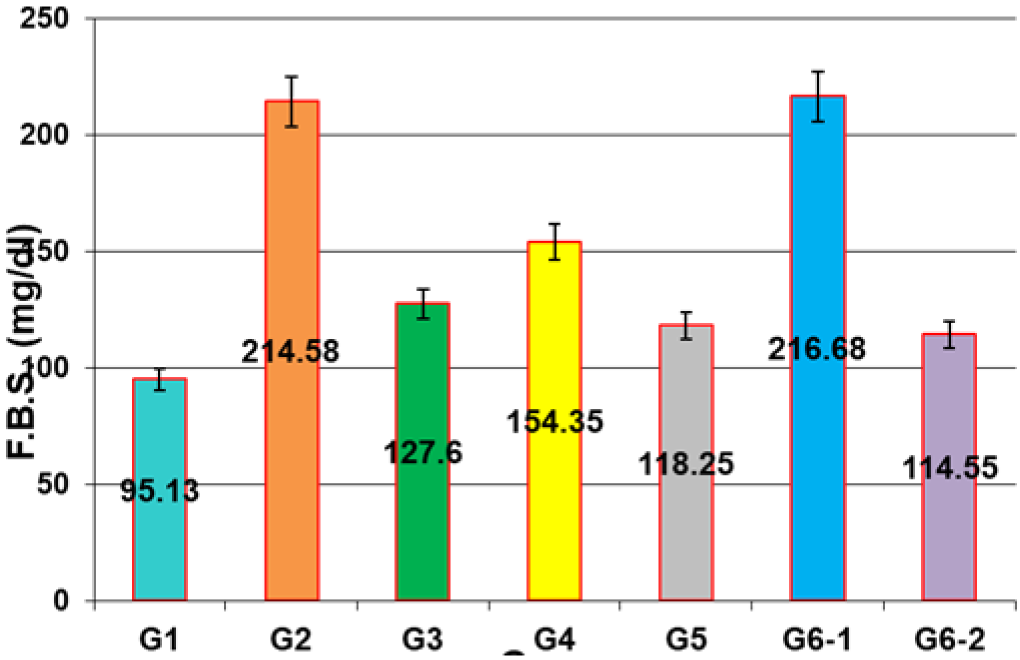

Figure 3: Comparison of F. B. S in differences groups.

The figure illustrates the weight changes in the experimental groups. The untreated diabetic group exposed a significant decline in body weight, while the treated groups demonstrated gradual recovery, particularly the group who received a combined treatment from metformin and black seed (Nigella sativa).

Table 2: Comparison between difference groups in Hormone's level

Group	Mean ±SE		
	T3 (ng/dl)	T4 (µg/dl)	TSH (ng/dl)
G1: Control	0.782 ±0.02 a	4.75 ±0.08 a	0.0306 ±0.001 c
G2: DM Type 2 (without metformin)	0.399 ±0.01 b	3.74 ±0.04 c	0.0309 ±0.002 c
G3: DM Type 2 (with metformin)	0.530 ±0.01 bc	4.19 ±0.01 b	0.0424 ±0.001 b
G4: DM Type 2 (with Nigella sativa)	0.623 ±0.02 cd	4.77 ±0.02 a	0.0321 ±0.001 c
G5: DM Type 2 (with metformin + Nigella sativa)	0.704 ±0.01 de	4.85 ±0.02 a	0.0276 ±0.001 c
G6-1: DM Type 2+hypothyroidism before treatment	0.192 ±0.01 e	1.92 ±0.08 e	0.0604 ±0.004 a
G6-2: DM Type2 + hypothyroidism after treatment (with metformin + Nigella sativa)	0.472 ±0.02 f	3.35 ±0.03 d	0.0463 ±0.001 b
L.S.D.	0.1357 *	0.1368 *	0.0047 *
Means having with the different letters in same column differed significantly. * ($P \leq 0.05$).			

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Table 2 shows the results, where thyroid hormone levels (T3, T4, and TSH) showed significant improvement in the treated groups, approaching normal levels, particularly in the metformin group with black seed extract, compared to the untreated groups. Statistical analyses showed statistically significant differences between groups in all calculated parameters ($P \leq 0.05$). Tables 1 and 2 summarize the mean and standard deviation of the calculated parameter values and show the effect of different treatments on metabolic and hormonal indices.

Discussion

The results exhibited clear differences between the experimental groups, reflecting the important role of therapeutic interference in improving metabolic and hormonal parameters. The two diseases were chosen together due to their bidirectional relationship. Diabetes and thyroid disorders are among the most common endocrine diseases. Hypothyroidism negatively impacts blood sugar control because the thyroid gland plays a crucial role in regulating metabolism and implance insulin sensitivity and glucose metabolism. Similarly, diabetes affects thyroid function through metabolic and insulin disturbances, leading to altered levels of T3 and T4 hormones, and also impacting TSH levels through a negative feedback mechanism (Razvi et al., 2025; Biondi et al., 2021; Kaur et al., 2025). Body weight results showed that the diabetic rats, mainly those with hypothyroidism, experienced significant weight loss compared to the control group. This is attributed to impaired glucose metabolism and increased protein and lipid catabolism resulting from insulin deficiency (Scheen,1996; Han et al.,2019) and thyroid hormone imbalance. Interestingly, when rats received a combination of metformin and black seed extract, their body weight began to normalize. This points toward an overall enhancement of their metabolic processes. This observation aligns with previous studies indicating metformin's ability to improve insulin utilization and black seed's capacity to mitigate oxidative stress and better manage energy expenditure (Foretz et al., 2014; Ahmad et al., Fasting blood glucose levels were notably elevated in the untreated diabetic rats, a common consequence of alloxan-induced diabetes which compromises insulin-producing pancreatic cells. In contrast, treated rats exhibited reduced blood sugar levels, with the combined metformin and black seed extract proving most effective. This synergistic effect is likely due to increased glucose uptake by muscles, diminished glucose production by the liver, and the hypoglycemic action of thymoquinone, a key component of black seed (Scheen, 1996; Hannan et al., 2007). Thyroid hormone profiles further supported the experimental model; rats with induced hypothyroidism displayed reduced T3 and T4 levels alongside elevated TSH, confirming the successful induction of an underactive thyroid using carbimazole. Treatment, particularly the combined therapy, significantly ameliorated these hormonal imbalances, bringing them closer to physiological norms. This improvement is hypothesized to stem from the treatment's ability to reduce oxidative stress and enhance overall endocrine function, corroborating research suggesting black seed's beneficial role in supporting the endocrine system and hormonal equilibrium (Brent a, 2011; Sadiq et al., 2021). The simultaneous enhancement of body weight, blood glucose levels, and thyroid function observed in the combination therapy group reinforces the idea that the use of metformin alongside black seed extract represents an efficient approach to managing diabetes mellitus accompanied by

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hypothyroidism. This treatment method may help lower the necessary dose of the chemical medication, thus reducing the likelihood of adverse effects when compared to single-drug therapy, which is consistent with current trends in the application of complementary treatments in veterinary medicine (Han et al., 2019). The synergistic effect between metformin and black seed can be interpreted through several possible mechanisms. Metformin acts by activate the AMP-activated protein kinase (AMPK), which is important for reducing production Hepatic glucose and increase insulin sensitivity. In contrast, the black seed extract, particularly thymoquinone, has antioxidant and anti-inflammatory properties that reduces oxidative stress and improve pancreatic B-cell function in the pancreas. This overlap may contribute to strengthening blood sugar and organization of thyroid functions. (Foretz et al., 2014; Ahmad et al., 2013). Despite the positive results shown by this study, there are some factors to be taken into consideration, including the period of the experiment, which may affect the generalization of results. In addition, the use of an animal model may not fully reflect the physiological response in humans, which requires longer future studies and on dissimilar model

Conclusion

The results proved that treatment with black seed extract and metformin showed a good response in blood sugar levels, in addition to the return of body weight to its normal level. An improvement in thyroid function was also observed in rats with diabetes and thyroid disorders. This is due to the effective role of the herb and the therapeutic substances used in the experiment, and this is consistent with the approach of experimental veterinary science.

Recommendation

Through this study, the integration of metformin with Nigella sativa extract could be a potentially effective treatment strategy. With thyroid problems associated with diabetes. Based on the results of this experiment, we suggest conducting several studies to determine the results of this mixture of black seed and metformin treatment and its effects.

Acknowledgment

I would like to express my gratitude to Tikrit University, College of Veterinary Medicine, for providing laboratory equipment and scientific support, which played an effective role in the work of this research, in addition to the staff of the Animal House for their support and assistance.

Conflict of Interest

There are no conflicts of interest of any kind, whether scientific, personal, or financial, that could affect the interpretation of this research and its result.

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Authors' Contributions

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Researcher Zahraa Ayoub contributed to the study and experiments in this research, in addition to analyzing the results. Supervisor Buthaina Abdul Hamid Abdullah contributed to the interpretation of the final results of this research.

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