

ANTIHYPERGLYCEMIC EFFECTS OF THYMOQUINONE IN DIABETIC RATS

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ABSTRACT

To investigate the antihyperglycemic potent of thymoquinone (TQ), this study has been conducted in streptozotocin-induced diabetic male rats. Diabetes was induced by single injection with streptozotocin (60 mg/kg b.w., *i.p.*). Rat ≥ 200 mg/dl of blood glucose was used as diabetic. Sixty five adult male rats (aged 56 days and weighted 138 ± 8.8 g) were divided into five groups, non-diabetic control (were drenched with drinking water) and four diabetic groups (DM, TQ50, TQ100, and DMI) were drenched with drinking water, TQ (50 mg/kg, bw), TQ (100 mg/kg, bw), and injected with insulin (4 IU/animal), respectively, for 42 days. During the experiment, body weight gains were recorded and blood samples were obtained weekly for assessment of plasma glucose and insulin concentrations. TQ treated male rats showed normal activity and body health throughout the experiment. Significant decrease of body weight gain has been recorded in untreated diabetic (DM) and insulin treated diabetic (DMI) groups as compared with that of intact control (C) and TQ treated diabetic (TQ50 and TQ100) groups, started from the fourth day of experiment, while DM group registered the lowest body weight gain among the experimental groups. Results of blood glucose concentrations referred to significant elevation in diabetic groups as compared with intact control. While in comparison between the diabetic groups, blood glucose concentration decreased significantly TQ50, TQ100, and DMI groups compared with DMI group. It has been found that insulin treated (DMI) and TQ treated (TQ50 and TQ100) male rats recorded no significant difference in serum insulin concentration when compared with each other but they were significantly lower than that of intact control male rats (C), but the average means of these four groups were significantly higher than that of non-treated diabetic male rats (DM). These changes were time dependent during the studied experimental period. It can be concluded that drenching of 100 mg/kg of TQ has potent hypoglycemic effect in experimentally-induced diabetic male rats.

INTRODUCTION

Diabetes is the world's largest endocrine disorder associated with increased morbidity and mortality rate according to genotypic and phenotypic phenomena (1). Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia that caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. Diabetes is a metabolic syndrome of multiple etiologies characterized by chronic hyperglycemia with abnormalities in carbohydrate, fat and protein metabolism due to defect in insulin secretions (2). Diabetes mellitus is also associated with long term complications including retinopathy, nephropathy, neuropathy and angiopathy and several others (3).

In recent years, much effort has been dedicated to a search for natural or pharmacological preventive agents, which would attenuate diabetes mellitus (4). Epidemiological and experimental studies demonstrate the importance of compounds derived from plants in reducing the risk of diabetes mellitus (5) and inhibit the development and the multiplicity of diabetes mellitus in experimental animals (6). *Nigella sativa L.* is an annual herbaceous plant belonging to the Ranunculaceae family, commonly have been used traditionally in Middle Eastern folk medicine as a natural remedy for various diseases for over 2000 years (7).

TQ is the abundant *Nigella sativa* essential oil compound and it is known as the active principle which is responsible for many of the seed's antioxidant and anti-inflammatory effects (8). Current opinion is that trials should be conducted with treatment by TQ that may effective at onset of diabetes mellitus. Particularly, early detection is required to maximally preserve the remaining beta-cell mass, because the ability to secrete even small amounts of insulin can make the disease be controlled easier and help to minimize the complications of chronic inadequate glycemic control (9). The result from this study would provide more insight information on the therapeutic use of the black seed cumin extracts in diabetic rats. Thus, the present study investigates the potential anti-hyperglycemic in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

1. Experimental rats: Mature male Sprague-Dawley rats have been used in the experiment, and underwent one week acclimatization before beginning of experiment. Rats were nourished chewable concentrate and drinking water *ad libitum* throughout the experiment. Room temperature was maintained at $23 \pm 2^{\circ}\text{C}$, the light-dark cycle was on a 12:12 h throughout the experimental period.

2. Preparation of TQ suspension: TQ suspension at a dose of 50 or 100 mg/kg bw (10) were prepared by dissolving 5 or 10 mg of TQ powder in 1 ml of drinking water to be used as 5 or 10mg/ 100 g bw, so that each 100 g bw will need drenching 1 ml of TQ suspension to be contain 5 mg (group TQ50) or 10 mg (group TQ100).

3. Induction of diabetes in rats: According to Mansford and Opie (11), diabetes has been induced in 52 male rats (weighted 138 ± 8.8 g and aged 56 days) by injection of single dose of STZ; Sigma Aldrich, England (60 mg/kg b.w., i.p.). STZ was dissolved in 1 M of sodium citrate buffer (pH 4.5). STZ induces diabetes mellitus within 3-5 days by destroying the beta cells of Langerhans islets in the pancreas. The rats with plasma glucose ≥ 200 mg/dl were considered as DM rats and used for experiment (12).

4. Experimental design: control non diabetic and STZ-induced male rats were classified into five equal groups (13 rats, each); intact control (C), diabetic control (DM), diabetic TQ treated (TQ50 and TQ100), and diabetic insulin treated (DMI) groups. Intact and diabetic control rats were injected with normal saline (100 μ l, *sc*) and drenched with drinking water daily for 42 days. Diabetic TQ treated rats were injected with normal saline (100 μ l, *s.c*) and drenched with TQ suspension (50 and 100 mg/ kg, b.w.) daily for 42 days. Diabetic insulin treated rats were injected with insulin (4 IU, *s.c*) and drenched with drinking water daily for 42. Daily body weights have been recorded during the experimental period. Blood samples have been obtained from tail vein for blood glucose determination and insulin concentrations.

5. Blood glucose assessment: Blood glucose was measured using GLUCOSE MR[®] kit (Cromatest, Spain).

6. ELISA technique for insulin assay in serum: Depending on the manufacturer instructions (ABO, Switzerland), serum insulin concentration has been estimated.

7. Statistical analysis: Results were expressed as mean \pm standard error of the mean (SDM). Comparisons were performed using one way analysis of variance (ANOVA-1) and Newman-Keuls to test all groups unpaired values. Differences were evaluated at $p < 0.05$. Statistical analysis was carried out using the GraphPad Prism (SAS Institute, Inc., USA).

RESULTS

1. Clinical observations: All male rats treated with TQ showed normal activity and body health throughout the experimental period in the present study. This findings revealed to TQ at the given doses (50 and 100 mg/kg b.w.) have no harmful effect on body function in normal rats, but instead of, had a positive ameliorated diabetic signs of STZ induced diabetic male rats. The results revealed diabetogenic effect of STZ when used in a single intraperitoneal injection of 60 mg/ kg as it

accompanied with clinical appearance of the symptoms of diabetes which was clearly seen within 2-4 days. In this, it has been shown that non-treated diabetic animals suffer from hyperglycemia, polyuria and polydipsia accompanied by weight loss. These observations initiated on the third day of the experiment.

2. Body weight: Results of daily body weights illustrated in figure (1) revealed to significant differences ($p < 0.05$) among experimental groups of the experiment. The differences were shown early in the third week of the experiment. These changes continued to the end of the experiment. Statistical analysis showed significant increase ($p < 0.05$) in the DMI group, which included diabetic insulin treated male rats, compared with other groups which showed no significant difference between them ($p > 0.05$), except DM group, which included diabetic non-treated male rats, showed significant decrease ($p < 0.05$) in comparison with other experimental groups. Figure (2) revealed significant decrease ($p < 0.05$) in final body weight gain of DM group compared with other groups which showed no significant difference ($p > 0.05$) when compared with each other.

3. Blood glucose concentration: After injection of streptozotocin, blood glucose concentrations were monitored for five days. Blood glucose has been detected to confirm the presence of diabetes mellitus, whose levels that exceed 200 mg/dl was considered to be the zero day of the experiment. Figure (3) reveals significant decrease ($p < 0.05$) of weekly assessed blood glucose concentration in insulin treated (DMI group) and TQ treated (TQ50 and TQ100 groups) male rats started from 3rd week of experiment but still significantly ($p < 0.05$) above control concentration. These decrement continued in TQ50 and TQ100 groups to reach near the normal levels within six weeks of the experiment.

4. Blood insulin concentration: Results shown in figure (4) clarify the insulin concentration (ng/ml) of the experimental groups at the end of the experiment. It has been found that insulin treated male rats (DMI group) and TQ treated male rats (TQ50 and TQ100 groups) recorded no significant difference ($p > 0.05$) when compared with each other but they were significantly lower ($p < 0.05$) than that of intact control male rats (C group) (1.99, 1.72, 1.77 and 2.27 ng/ml, respectively), but the average means of these four groups were significantly ($p < 0.05$) higher than that of non-treated diabetic male rats (DM group) (0.48 ng/ml).

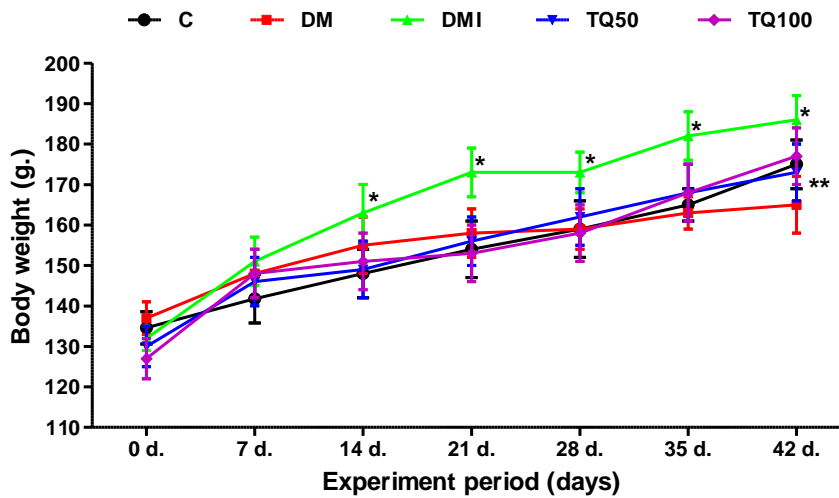


Figure (1): Effect of TQ administration for 42 days on body weight (g) in streptozotocin- induced diabetic mature male rats.

*Significantly higher than control (p<0.05).

** Significantly lower than control (p<0.05).

C(Intact control rats) and **DM**(non treated diabetic rats): drenched with drinking water (500 µl) and injected with normal saline (100 µl s.c) daily for 42 days.**DMI** (insulin treated diabetic rats): drenched with drinking water (500 µl) and injected with insulin (4 IU) daily for 42 days.**TQ50** and **TQ100** (TQ treated diabetic rats): drenched with TQ suspension (50 and 100 mg/kg bw, respectively) and injected with normal saline (100 µl s.c) daily for 42 days.

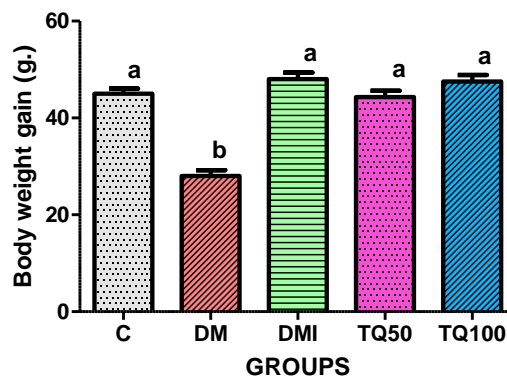


Figure (2): Effect of TQ administration for 42 days on body weight gain (g) in streptozotocin- induced diabetic mature male rats.

Different letters represent significantly lower than control (p<0.05).

C (Intact control rats) and **DM**(non treated diabetic rats): drenched with drinking water (500 µl) and injected with normal saline (100 µl s.c) daily for 42 days.**DMI** (insulin treated diabetic rats): drenched with drinking water (500 µl) and injected with insulin (4 IU) daily for 42 days.**TQ50** and **TQ100** (TQ treated diabetic rats): drenched with TQ suspension (50 and 100 mg/kg bw, respectively) and injected with normal saline (100 µl s.c) daily for 42 days.

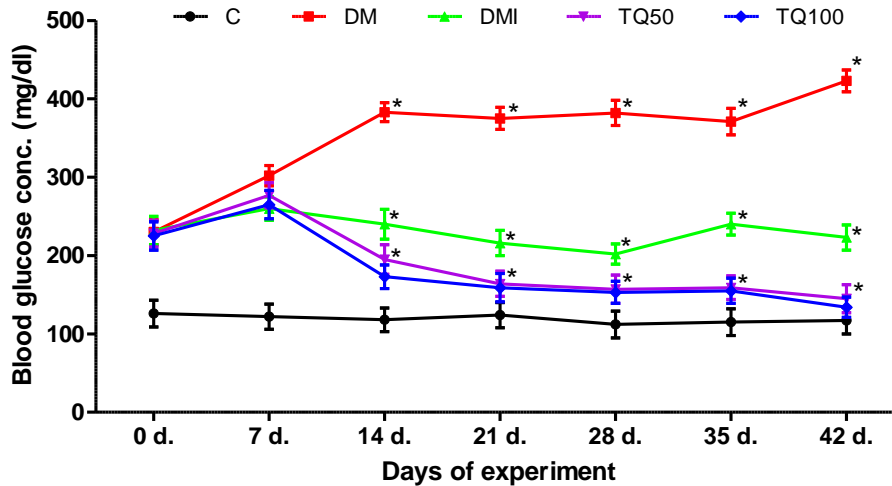


Figure (3): Effect of TQ administration for 42 days on blood glucose concentration (mg/dl) in streptozotocin-induced diabetic mature male rats.

* Significantly higher than control (p<0.05).

C (Intact control rats) and **DM**(non treated diabetic rats): drenched with drinking water (500 µl) and injected with normal saline (100 µl s.c) daily for 42 days.**DMI** (insulin treated diabetic rats): drenched with drinking water (500 µl) and injected with insulin (4 IU) daily for 42 days.**TQ50** and **TQ100** (TQ treated diabetic rats): drenched with TQ suspension (50 and 100 mg/kg bw, respectively) and injected with normal saline (100 µl s.c) daily for 42 days.

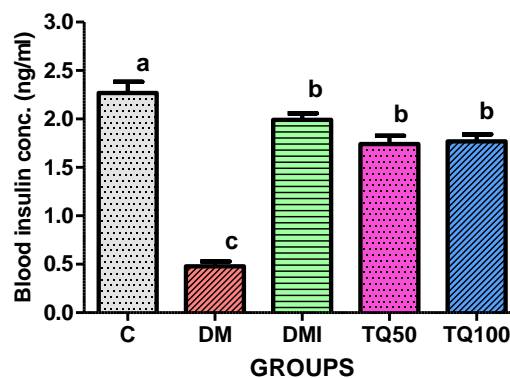


Figure (4): Effect of TQ administration for days on blood insulin concentration (ng/ml) in streptozotocin-induced diabetic mature male rats.

Different letters represent significantly lower than control (p<0.05).

C (Intact control rats) and **DM**(non treated diabetic rats): drenched with drinking water (500 µl) and injected with normal saline (100 µl s.c) daily for 42 days.**DMI** (insulin treated diabetic rats): drenched with drinking water (500 µl) and injected with insulin (4 IU) daily for 42 days.**TQ50** and **TQ100** (TQ treated diabetic rats): drenched with TQ suspension (50 and 100 mg/kg bw, respectively) and injected with normal saline (100 µl s.c) daily for 42 days.

DISCUSSION

1. Clinical observations

This study was undertaken to assess hyperglycemic potent by loading dose TQ as the most important active ingredient of *Nigella sativa* in streptozotocin induced diabetic mature male rats as a model for mammals. When looking for potential health hazards, examining various generations of a test subject all at once can provide more definitive proof of a product's safety or potential threat. Overseers such as the Federal Food and Drug Administration (FDA) require this level of proof before even considering human clinical trials. Agca and Critser (13) showed that rats considered as important models in a wide range of disciplines, including physiology, behavior, and endocrinology. All TQ treated male rats, examined under diabetic condition, showed normal activity and good general body health throughout the experimental period extended for 6 weeks. While untreated male rats, examined under diabetic condition showed variable results. The overall outcome results under diabetes revealed that administration of TQ (50 and 100 mg/ kg, bw) cause more beneficial positive effect in ameliorating the effect of single injection of streptozotocin (60 mg/ kg, bw).

2. Body weight:

Administration of diabetic male rats TQ caused insignificant changes in body weight gains in comparison with intact control male rats and significant elevation compared with diabetic non treated male rats. On the other hand, the high significant body weight gain in insulin treated diabetic male rats was unexpected and the reason for this increase is unclear. It might be explained by the increment of glucose entrance to inside the cells and its contribution in glycolysis which may finally increase the shift of acetyl CoA for lipid and protein biosynthesis. However, the insignificant changes in body weight gain of TQ treated diabetic male rats compared with control may attributed to the hyperlipidemic treated effect that resulted from TQ (14).

The decrement of body weight gain in diabetic non treated male rats may be explained either by the dehydration of plasma fluid compartment, or increment of serum glucose concentration. Regarding to the dehydration, it has been supported by the polydypsia which had been shown in male rats compared with that of other groups. On the other hand, the suggested hyperglycaemia may attributed to the blockage of TNF- α receptor 1 (TNFR1), which is the major mediator responsible for the TNF- α pathophysiological action in insulin resistance (15, 16). This blockage may results in insulin resistance and increase blood glucose concentration. This will increase glomerular filtration rate of kidney nephrons. These events may result in polydypsia and loss of

energy through appearance of glucose in urine (glucoseurea). It has been reported that *N. sativa* oil treatment to mice for 6 weeks significantly reduced the body weight compared to normal mice (17).

3. Blood glucose and insulin concentration

The non treated diabetic group exhibited hyperglycemia, with significantly increased serum glucose levels and significantly decreased serum insulin levels compared with control at different time points. However, treatment of diabetic male rats with the TQ (TQ50 and TQ100 groups) resulted in a significant decrease in serum glucose levels compared with the non treated diabetic group after 10 days treatment. After 30 days, serum glucose levels had decreased to levels that did not differ significantly from basal levels seen in the control group. However, treatment with TQ for 30 days had no significant effect on insulin levels compared with those in the insulin treated diabetic group (Group MDI), levels in both Groups TQ100 and MDI remained above those in the control group.

In the present study, the effect of TQ on diabetes and b-cells damage in streptozotocin induced diabetic male rats was evaluated both structurally and mollecularly. Diabetics and experimental animal models of diabetes exhibit high oxidative stress due to chronic hyperglycemia, which depletes the activity of the antioxidant defense mechanism, promoting the generation of free radicals (18). It has been reported that the excessive availability of free radicals, accompanied by a reduction in the antioxidant capacity, leads to cellular dysfunction (19). The beta cell cytotoxicity of streptozotocin is thought to be mediated by inhibition of free radical scavenging, which enhances the production of superoxide radicals, resulting in lipid peroxidation, DNA damage, and sulfhydryl oxidation (20).

From the present results, it is of interest to note that hypoglycemic effect began even before starting of insulin secretion, due to TQ administration. This may indicate that the observed hypoglycemic effect is not related directly to insulin action and may be mediated by another mechanism. Recently, Meddah *et al.* (21) showed that chronic oral administration of *Nigella. sativa* seeds to rats inhibited intestinal glucose absorption, which may contribute to the hypoglycemic effect of *Nigella. sativa*. Or it may caused an increased sensetivity of insulin receptors on cell membrane to the insulin. TQ may effective in treatment of diabetes mellitus type 2.

Houcher *et al.* (22) showed that the use of the commercial oil of *Nigella sativa* at a dose of 2.5 ml/ kg per day for 25 days significantly reduced blood glucose, especially during the first 10 days of treatment. Fararh *et al.* (23) demonstrated that *Nigella sativa* oil exhibited a significant hypoglycemic effect in streptozotocin plus nicotinamide induced diabetic hamsters after four weeks treatment, indicating that the hypoglycemic effect of the oil was time dependent. In addition, Fararh *et al.* (23) showed a significant increase in serum insulin levels. Comparing the results of Houcher

et al. (22) and Fararh *et al.* (23) with those of the present study indicates that the use of the active component of *Nigella sativa* oil for a longer period of time in the present study have produced similar results.

Fararh *et al.* (24) also suggested that the observed decrease in glucose after the first week of treatment with *Nigella sativa* oil may be due to decreased hepatic gluconeogenesis. However, after activation of b-cells in response to increased insulin levels, a significant decrease in glucose levels to normal was observed, which could be due to the combined action of decreased hepatic gluconeogenesis and activation of b-cells. This could be explained by a possible increase in the level and activity of other antioxidant enzymes.

In this study, treatment with TQ for 42 days restored both serum glucose and insulin levels to normal. Consistent with our results, Fararh *et al.* (24) showed that daily gastric administration of 50 mg/kg TQ for 30 days reduced both fasting glucose and glycated hemoglobin levels. In diabetics, the increased gluconeogenesis is related to increased expression of gluconeogenic enzymes (25). Fararh *et al.* (24) stated that TQ decreased the elevated gluconeogenesis by suppressing the synthesis of gluconeogenic enzymes.

Recently, Pari and Sankaranarayanan (26) have shown that daily gastric administration of 80 mg/kg TQ for 45 days produces a consistent, dose dependent, and significant decrease in plasma glucose concentrations and an increase in insulin levels in streptozotocin induced diabetic rats. Pari and Sankaranarayanan also showed that TQ decreased the activities of the gluconeogenic enzymes; glucose-6-phosphatase and fructose-1,6- disphosphatase, in the diabetic rats, in agreement with the results reported by Fararh *et al.* (24).

The present observations provided an explanation for the significant decrease in serum glucose levels seen after one week treatment with TQ in the present study, even though insulin levels at the time were not yet secreted under the influence of TQ treatment. The results emphasize that TQ is effective in reducing hyperglycemia in streptozotocin induced diabetic male rats and that the antidiabetic action is not related directly to insulin, but could be mediated, in part, by inhibition of gluconeogenesis. TQ protected and preserved beta cell integrity by decreasing oxidative stress. Thus, it may be concluded that the antidiabetic action of TQ could be due, in part, to amelioration of the cellular and subcellular structures of beta cells.

تأثير الثايموكوينون الخافض للسكر في ذكور الجرذان المصابة بداء السكري

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الخلاصة

من أجل تقييم فعالية مستخلص الحبة السوداء الثايموكوينون في خفض مستوى سكر الدم لذكور الجرذان المستحث فيها داء السكري تجريبياً باستخدام عقار الستربتوزوتوسين، أجريت الدراسة الحالية باستخدام 65 جرذاً ذكراً ناضجاً بعمر 56 يوماً ووزن 138 ± 8.8 غرام. أستخدمت داء السكري في 52 جرذاً باستخدام حقنة مفردة من عقار الستربتوزوتوسين (60 ملغم/كغم من وزن الجسم في البريتون). تم التأكد من حدوث داء السكري عن طريق قياس مستوى سكر الدم، إذ أن تجاوز التركيز لأكثر من 200 ملغم/100 مل يعد مصاباً بداء السكري. تم توزيع الجرذان السليمة والمصابة عشوائياً على خمس مجموعات متساوية العدد (13 جرذ لكل مجموعة)، ضمت الأولى حيوانات سليمة وعدت على أنها مجموعة سيطرة (C) وجرعت ماء الشرب وحقنت بالمحلول الفسلجي يومياً، وضمت المجموعات الأخرى حيوانات مصابة إذ تركت الثانية بدون علاج (DM) وجرعت ماء الشرب وحقنت بالمحلول الفسلجي يومياً، والثالثة (TQ50) جرعت معلق الثايموكوينون (بجرعة 50 ملغم/كغم من وزن الجسم) وحقنت بالمحلول الفسلجي يومياً، وجرعت الرابعة (TQ100) معلق الثايموكوينون (بجرعة 100 ملغم/كغم من وزن الجسم) وحقنت بالمحلول الفسلجي يومياً، وحقنت الخامسة (DMI) بهرمون الانسولين (بجرعة 4 وحدات دولية لكل حيوان) وحقنت بالمحلول الفسلجي يومياً. استمرت الدراسة لمدة 42 يوماً. تم تسجيل أوزان ذكور الجرذان يومياً وتم قياس تركيز سكر الدم كل ثلاثة أيام. بعد مرور 24 ساعة على آخر يوم من التجربة، تمت التضحية بالحيوانات وأخذت منها نماذج دم لغرض قياس آخر تركيز للكلوكوز وتركيز الانسولين في مصل الدم.

أظهرت جميع ذكور الجرذان المعاملة بالثايموكوينون فعالية طبيعية وصحة جيدة خلال مدة الدراسة، كما أظهرت النتائج انخفاضاً معنوياً في أوزان جرذان المجموعة المستحث فيها داء السكري تجريبياً وغير المعالجة والمجموعة المعالجة بالانسولين بالمقارنة مع مجموعة السيطرة والمجموعتين المعالجتين بالثايموكوينون والتي أظهرت أعلى معدل للكسب الوزني، ابتداءً من اليوم الرابع للتجربة. وفي نهاية التجربة أظهر التحليل الإحصائي حصول حيوانات السيطرة على أعلى معدل للكسب الوزني تلتها حيوانات المجموعتين المعاملتين بالمستخلص وتلك المعاملة بالانسولين الذين لم يظهر فرقاً معنوياً فيما بينهما. في حين كانت المجموعة الغير معالجة الأقل كسباً للوزن من بين مجموعات التجربة خلال مدة التجربة. أشارت نتائج تركيز كلوكوز مصل الدم إلى الارتفاع المعنوي في المجموعات المصابة بداء السكري بالمقارنة مع السيطرة. وعند المقارنة بين تلك المجموعات مع بعضها البعض أظهرت حيوانات المجموعات المعالجة (TQ50 و TQ100 و DMI) انخفاضاً معنوياً بالمقارنة مع المجموعة غير المعالجة (DM) خلال مدة الدراسة. كما بينت الدراسة أن ذكور الجرذان المصابة بداء السكري والمعالجة بالانسولين وتلك المعالجة بالثايموكوينون (TQ50 و TQ100) سجلت مستويات غير معنوية في تركيز الانسولين عند المقارنة فيما بينها إلا أنها كانت أقل معنوياً بالمقارنة مع السيطرة وأعلى معنوياً بالمقارنة مع ذكور الجرذان المصابة وغير المعالجة (DM).

يستنتج من الدراسة الحالية أن تجريع ذكور الجرذان بمعلق الثايموكوينون (100 ملغم/كغم من وزن الجسم) له تأثير فعال في خفض سكر الدم لذكور الجرذان المصابة بداء السكري.

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