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*Original Article*

# Comparing the Effects of Allicin and Thymoquinine on type 1 Diabetes

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## Abstract

**Introduction**: Nigella sativa (Black seed) and Allium sativum (garlic), are common dietary spices also traditionally used as a treatment for various diseases including diabetes mellitus. The antidiabetic activity of each individual spice is well documented.**Purpose**: This study aimed to compared the effect of the active ingredients of nigella sativa (Thymoquinone) and allium sativa (Allicin) on STZ induced type 1 diabetes in rats' model.**Materials and Methods**: Six equal sized groups of rats were used in this experiment. Five groups were injected with STZ to induce type 1 diabetes. Four groups received a daily dose of intraperitoneal injection of one of the following: 5mg/kg of Thymoquinone, 10mg/kg of Thymoquinone, 8mg/kg of Allicin, and 16 mg/kg of Allicin for four weeks. One STZ treated group was used as a positive control and the last non treated group was used as a negative control. At the end of the experimental period, the body weights, fasting glycose levels and insulin levels were tested and compared among these groups. **Results**: The results of the four treated groups were compared to the negative and positive control groups. The body weight for all four treated groups increased especially the group treated with 8mg/kg Allicin compared to the positive control group. FBG levels for all treated groups was also decreased. The group treated with 8mg/kg Allicin showed the best result where the FBS level were within the normal level by the end of the month. The group treated with 16mg/kg Allicin showed the best result for insulin level where it was restored by 79.26% compared to the control group. The other treated groups showed a very close results at the end of the month. The histology of pancreatic islets showed similar ameliorating effects in all of the four treated groups when compared to the positive control group. **Conclusion**:In conclusion, these experimental results indicate the use of Allicin showed the best impact on type 1 diabetes treatment.

**Keywords:** Thymoquinine, Allicin, Diabetes, STZ, Body weight, Pancreatic islets, Insulin, Fasting blood glucose.

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## Introduction

Diabetes mellites is the most common endocrine disorder diseases associated with abnormally high levels of glucose in the blood1.Type 1 diabetes or insulin-dependent diabetes mellitus is the most severe form of diabetes according to the World Health Organization (WHO)2. It is an autoimmune disease characterized by selective loss of insulin producing in the islet of Langerhans in the pancreas. The STZ is the most commonly used diabetogenic agent in experimental animals. This chemical is toxic to insulin-producing pancreatic cells by selective destruction of the β-cells and induces type 1 diabetes3.

Nigella sativa is a spice plant known by many different names like black seeds or black cumin; it has been used as a herbal medicine for more than 2000 years by different cultures for variety of applications including treatments and prevention of several diseases and illnesses. Both Nigela sativa and thymoquinone (TQ) (the main bioactive component of Nigella sativa) were effective in treating diabetes symptoms and lower blood glucose4–6 .
Allium sativum, commonly known as garlic (a bulb-forming herb) is a common spicy flavoring agent used since ancient times7,8. Allicin (diallythiosulfinate) is the major active principle of Allium sativum. Studies have shown that both Allium sativum and allicin have a variety of pharmacological activities including antidiabetic effects 5,8,9.

## The aim:

The aim of this study is to compare the effects of the active ingredients of two medical plants (Allicin from Allium sativum and Thymoquinone from Nigella sativa) in treating type 1 diabetes. Biological and histological tests were performed to find out if these two compound will mend the effects of type 1 diabetes induced by STZ in rats. Compared to the non-treated diabetic group, the effects of these two compounds on body weight, fasting blood glucose levels, insulin levels, and histology of pancreatic islets of rats with type 1 diabetes are investigated.

## Materials and Methods:

The total number of 36 male rats with a weight ranges from 150 grams to 250 grams were used. The rats were kept at controlled conditions for 3 weeks before the start of the experiment. By intraperitoneal injection of a single dose of streptozotocin (STZ) (65 mg/kg), Type 1 diabetes was induced in overnight fasted 30 rats. The rats were equally divided into five groups after four days. The first group was the STZ group without treatment as a positive control group. The second and third groups were treated with a daily dose of intraperitoneal injection of either 8mg/kg or 16 mg/kg Allicin for four weeks. The fourth and fifth groups were treated with a daily dose of intraperitoneal injection of either 5mg/kg or 10 mg/kg Thymoquinone for four weeks. The last group was a negative control group where this group did not receive any treatment. All groups were fed equally and food was removed 12 hrs before testing the fasting blood glucose levels. Blood glucose levels and the body weights were tested at the begging of the experiment and every week for all groups. At the end of the experiment (after one month), all blood was collected from rats by sacrificing and the pancreases were prepared for histology. The blood serum was used to measure the insulin levels, then the data were recorded and analyzed.

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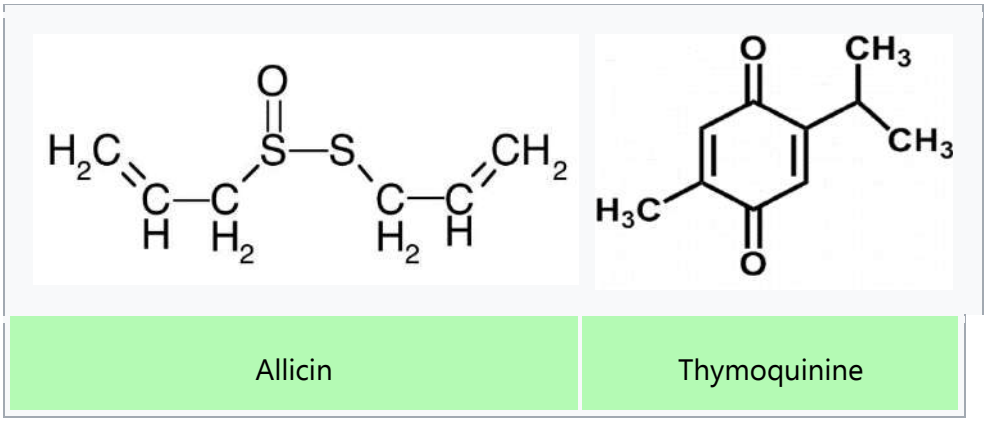


Figure1: The Allicin and Thymoquinine chemical structures.

## Results

The effect of Allicin and TQ on body weights:

The body weights of all the rats in all groups were recorded at the begging of the experiment, after one week and at the end of the experiment (after one month). The body weights were then expressed as the mean (for each group) of the percentage of body weight changes and compared to the negative control group as they have been illustrated in Figure (1). The mean of the body weight of the negative control group showed an increase by 7% by the end of the first week and this increase continued to reach 31% by the end of the four weeks. The diabetic (STZ only) control group showed a decrease of 7% in the mean body weight by the end of the first week and this decrease in the mean body weight reached 10% at the end of the four weeks. In diabetic group treated with daily dose of 8mg/kg of Allicin the body weight was decreased by 1.7% at the end of the first week, but this group showed an increase in body weight by 19.4% at the end of the four weeks. The diabetic group treated with daily dose of 16mg/kg of Allicin had showed a 6.4% decrease in body weight at the end of the first week and this decrease was reduced to 2.2% by the end of the month. In diabetic group treated with daily dose of 5mg/kg TQ, the body weight was decreased by 17.4% at the end of the first week and by the end of month the body weight was improved to reduce the decrease to 3.1%. The diabetic groups treated with a daily dose of 10mg/kg TQ the body weight had decreased in the body weight by 5% but there was slight improvement in the body wight to reduce the decrease to 3% by the end of the month. The unpaired t-test did not show significant differences when the STZ group was compared to the allicin treated groups in the first week, however at the end of the four weeks only the 8mg allicin treated group showed a significant difference with a p-value less than 0.001. The unpaired t-test also did not show significant differences when the STZ group was compared to the TQ treated groups in the first week or at the end of the four weeks.

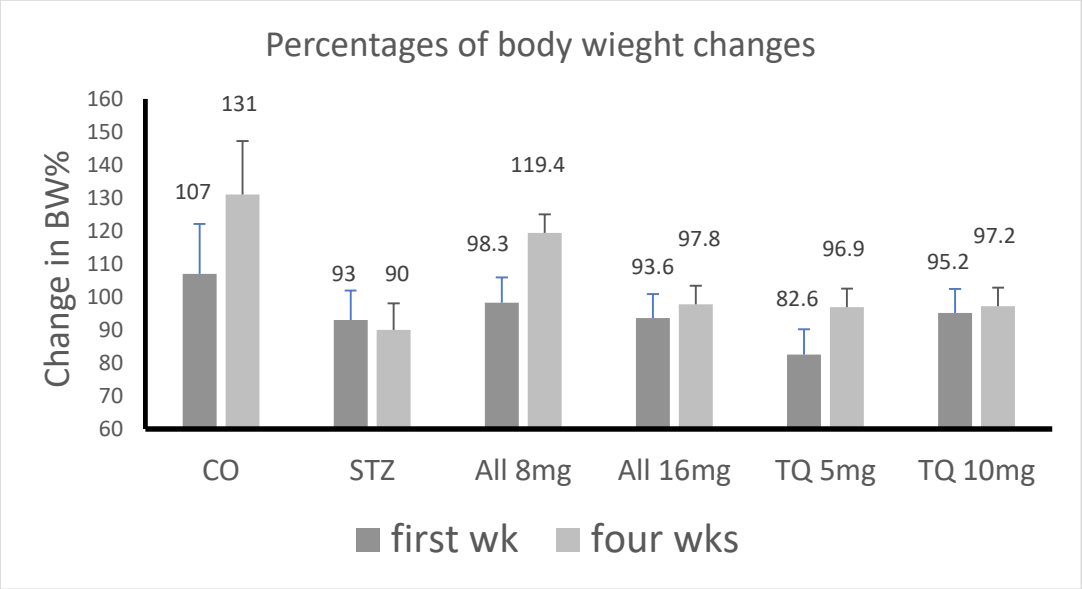


Figure 1. The effects of Allicin and Thymoquinone on body weights. ata are expressed as M+SD. This figure showed that the body weight in the control group increased in the first week by 7% and 31% by the end of the four weeks. By the end of the first week, the group treated with 8mg Allicin were the only group had body weight increased. By the end of the month the 8mg/kg Allicin treated group showed the best result with 19% increase in body weight. There were no significant differences in the first week when STZ group was compared to other treated groups, however after four weeks only the group treated with 8mg allicin showed significant difference to STZ group ($P < 0.001$).

The effect of Allicin and TQ on FBG levels:

Blood glucose levels were examined for each animal when fasting at the end of the first week and at the end of the month, and then expressed as mmol/l. The mean for each group was calculated and compared among the groups as shown in Figure (2). In the negative control group, the fasting blood sugar (FBG) levels were within normal range with averages of 5.5 mmol/l and 5.3 mmol/l at the end of the first week and the end of the month respectively. The STZ induced diabetic positive control group showed very high FBG levels with means that are very close reached 23.5 mmol/l at the end of the first week and 24 mmol/l at the end of the four weeks. The diabetic group treated with daily dose of 8mg/kg of Allicin showed high FBG levels with mean of 11.6 mmol/l at the end of the first week, however by the end of the four weeks the mean of FBS levels was at the normal range with a value of 5.3mmol/l. The diabetic group that was treated with daily dose of 16 mg/kg of Allicin showed an initial high level of FBG with mean reached 11.6 mmol/l at the end of the first week, while these levels showed considerable decrease at the end of the month with a mean reached 8.3 mmol/l. Similar results were observed in the diabetic group treated with daily dose of 5mg/kg TQ. The means of FBG levels of this group were14 mmol/l at the first week and 11.1mmol/l at the end of the month. The diabetic group that was treated with daily dose of 10mg/kg TQ showed higher levels of FBG with mean reach 18.1 mmol/l at the end of the first week and these levels were decreased at the end of the month with a mean of 10.5

mmol/l. The unpaired t-test showed significant differences when the STZ group was compared to the allicin treated groups and the TQ treated groups in the first week and also at the end of the four weeks with p-values less than 0.001.

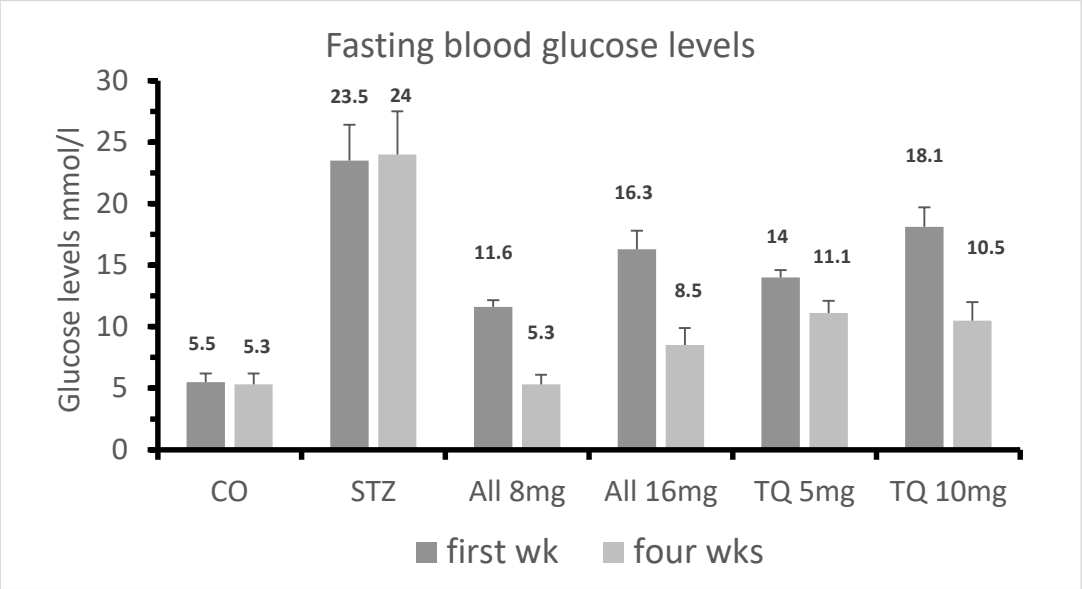


Figure 2. The effects of Allicin and Thymoquinone on FBS levels. Data was expressed as M+SD. This figure showed that all treated groups with both Allicin and TQ caused decreased in the FBG level as compared by STZ group. The group treated with 8mg/kg Allicin showed the best result where the FBS level reach to the normal level by the end of the month. All the treated groups showed significant differences compared to STZ group ($P<0.001$).

The effects of Allicin and TQ on blood insulin level:

The blood insulin levels were calculated at the end of the experiment for all the animals in all groups and the means were expressed as percentages of the negative control mean as shown in Figure 3. The mean of blood insulin levels in the negative control group was 100%. The blood insulin levels of the positive diabetic STZ control group were remarkably reduced to less than half with a mean of 46.25% when compared to the control. The blood insulin levels in diabetic group treated with daily dose of 8mg/kg Allicin had a higher mean than the positive diabetic group reached 64.06% when compared to the control. The blood insulin levels in diabetic group treated with daily dose of 16mg/kg of Allicin showed the highest levels among all the diabetic groups with a mean reached 79.26 % when compared to the control group. The blood insulin levels in diabetic group treated with 5mg/kg of TQ were also higher than the positive control group with a mean of 70.21 % when compared to the control group. The blood insulin levels in the diabetic group treated with daily dose of 10 mg/kg of TQ were also improved comparing to the positive diabetic control group with a mean reached 72.74 % when compared to the control group. The unpaired t-test showed significant

differences when the STZ group was compared to the 16 mg allicin treated group and the 5 mg TQ treated group with p-values less than 0.05 but did not show significant differences when the STZ group was compared to the 8 mg allicin treated group and the 10 mg TQ treated group.

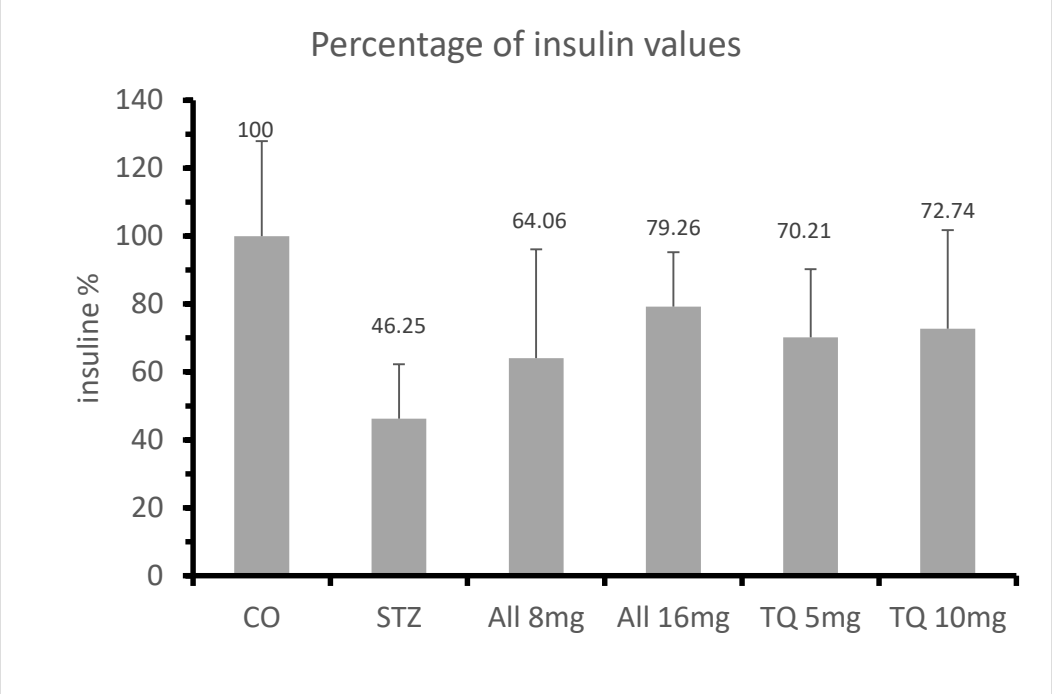


Figure 3. The effects of Allicin and TQ on blood insulin level. Data Expressed as M+SD. In this figure the level of insulin in all treated groups where higher compared to the STZ group. The group treated with 16mg/kg Allicin showed the best result were the insulin level restored by 79.26% compared to the control group. however, the other treated groups showed very close results. The groups treated with 16 mg allicin and 5 mg TQ showed significant differences ($p<0.05$) while the other two groups did not show significant differences when compared to the STZ group.

Effects of Allicin and TQ on pancreatic islet

The histological sections of all the groups were compared as shown in figure 4. The histological sections of the negative control group showed normal pancreatic structure with normal cells polygonal cells that have regular nuclei. The histological sections of the positive diabetic STZ control group showed degenerative and necrotic changes and shrinkage in the islets of Langerhans; the islets were relatively small, atrophied with reduction in the number of polygonal islet cells. The diabetic group treated with daily dose of 8mg/kg of Allicin histological sections showed that the pancreatic islets were relatively smaller in size and have irregular shapes when compared to the negative control croup, but they had less hydropic degeneration, degranulation and necrosis in the islet cells when compared to the positive diabetic STZ control group. The histological sections of the diabetic group treated with daily dose of 16mg/kg of Allicin showed similar

appearance as in the previous group, but there were more viable cells present and less hydropic degeneration, degranulation and necrosis when compared to the positive diabetic STZ control group. The histological sections of the diabetic group treated with daily dose of 5mg/kg of TQ showed that the islets were relatively small in size and irregular in shape compared with normal control group, and there were less hydropic degeneration, degranulation and necrosis in the islet cells when compared to the positive diabetic STZ control group. The histological sections of the diabetic group treated with daily dose of 10mg/kg of TQ showed that the islet of Langerhans appeared regular in shape, and the islets consisted of polygonal cells with regular nuclei, however, the number of cells was reduced and smaller cytoplasmic vacuoles were observed.

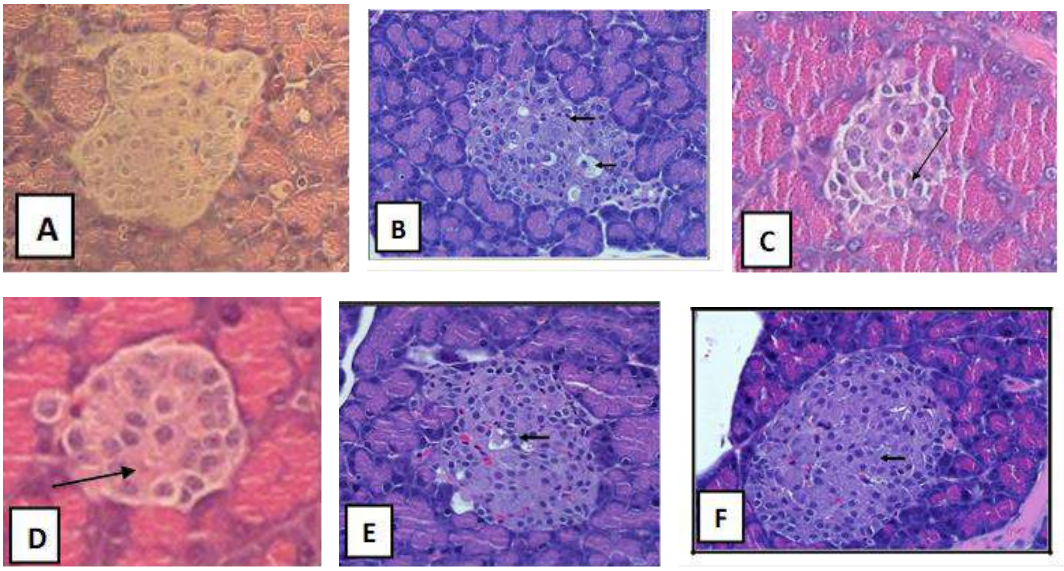


Figure 4. The effect of Allicin an Thymoquinone on the pancreatic islet. In this figure the histological sections for all the groups were compared. The negative control group show normal structure. The Pancreatic islet of the positive STZ diabetic rats showed reduction in number of cells, as well as necrotic changes and hydropic degeneration. Both of the two groups treated with 8 and16 mg/kg Allicin showed less hydropic degeneration, degranulation and necrosis in the islet cells. The other two groups treated with 5 and10 mg/kg TQ showed also less hydropic degeneration, degranulation and necrosis in the islet cells.

## Discussions

The present study conducted to evaluated the effects of Allicin and Thymoquinone on Type 1 diabetes. The effects of these plant active extracts on the level of FBG, body weight and insulin level were evaluated. The negative untreated control group was used as a standard for normal functions, whereas the positive diabetic STZ only group was used as an uncontrolled diabetic positive control.

The effect of the treatments on body weight

The negative control group showed a normal increase in body wight and the difference between body wight at the end of the first week and the end of the month was 25% increase (from 107% to 131%). The untreated diabetic group had a continuous loss in body

wight and the difference between body wight at the end of the first week and the end of the month was 3% decrease (from 93% to 90%). Both of allicin treated groups and also both of TQ treated groups showed an initial decrease of body weight at the end of the first week; By the end of the month All of these groups had an improvement in body weight. The 8 mg allicin treated group showed the highest difference between body wight at the end of the first week and the end of the month among all the treated groups and this difference was 21.1% increase (from 98.3% to 119.4%). The 16 mg allicin treated group showed a difference of body wight between the end of the first week and the end of the month of 4.2% increase (from 93.6% to 97.8%). The 5 mg TQ treated group showed a difference of body wight between the end of the first week and the end of the month of 14.3% increase (from 82.6% to 96.9%). The 10 mg TQ treated group showed the lowest difference between body wight at the end of the first week and the end of the month among all the treated groups and this difference was 2% increase (from 95.2% to 97.2%). The only treated group that the body weight by the end of the month was higher than the body weight at the biggening of the experiment was the group treated with a daily dose of 8mg/kg Allicin. The only group that the decrease in the body weight by the end of the first week was higher than the decrease of the body weight of the diabetic untreated group was the group treated with 5mg/kg TQ. These findings regarding the moderate increase in body weight when high dose of allicin was used agreed with other researches findings such as Elkayam et al, Dhanarasu, and Shi X et al where they found that allicin prevents weight gain 10–12. Chuanhai Zhang et al also found that allicin inhibits weight gain 13. TQ treatments also shown to have similar results on body weight by Alduwish and his group where they found that TQ significantly reduced body weight in diabetic rats compared with normal non treated animals 14.

The effect of the treatments on fasting blood glucose levels (FBG)

The effects of Allicin and TQ on the levels of FBG were also investigated in all groups. The negative control group showed a normal FBG levels of 5.5 mmol/l at the end of the first week and 5.3 mmol/l at the end of the month. The diabetic untreated positive control group showed a very high glucose levels that is more than four times the normal levels where the FBG levels reached 23.5 mmol/l at the end of the first week and 24 mmol/l at the end of the month. All the treated groups showed markedly lower FBG levels than the STZ group in the end of the first week and FBG levels were lower by the end of the month; however, only the group treated with 8mg/kg Allicin had a FBG levels equal to the negative control group (5.3 mmol/l). The other three groups showed a FBG levels at the end of the month about double the concentration of the negative control group (8.5 mmol/l, 11.1 mmol/l, and 10.5 mmol/l four 16 mg/kg allicin, 5 mg/kg TQ, and 10 mg/kg TQ groups respectively).

These results regarding TQ effect on lowering blood glucose levels in agreement with Fararh KM et al and Alshahrani et al where they found that TQ lowers glucose levels in diabetic rats 15,16. These results also found by Abdelmeguid NE et al and Lutfi et al where TQ significantly reduced glucose levels 17,18. Karandrea et al and Alduwish et al also found reduction of blood glucose levels after TQ treatment 14,19. The results regarding allicin

reducing FBG also supported by the results found by Huang et al, Dhanarasu, and Arellano-Buendía et al where they both observed FBG levels reduction after allicin administration to diabetic rats 12,20,21.

The effect of the treatments on blood insulin levels

The effect of Allicin and TQ on blood insulin level were also investigated by the end of the month since it needed a quantity of blood that the animals have to be killed to obtain. The mean of insulin levels of the negative control group was considered as the standard and the mean of insulin levels of the other groups were expressed as a percentage of the standard. The insulin level of the diabetic positive control group was less than half of the control group (46.25%). The allicin treated groups showed an improvement of about 18 % increase of insulin level for 8 mg/kg and 33% increase of insulin level for 16 mg/kg with a difference of about 15% increase when the dose of allicin was doubled. The TQ treated groups showed an improvement of about 24% increase of insulin level for 5 mg/kg and 26% increase of insulin level for 10 mg/kg with a difference of about 2% increase when the dose of TQ was doubled. These data revealed that the best improvement in insulin levels was achieved when 16mg/kg of Allicin was used, and the lowest improvement in insulin levels was achieved when 8mg/kg of Allicin was used. These results regarding that the insulin levels in TQ treated animals were lower than the negative control group agreed with Bule and his team where they suggested in their meta- analysis article of 18 studies that TQ has significant effect on reducing insulin levels 22. Allicin also was found by Arellano-Buendía and his group to be effective in enhancing insulin levels in diabetic rats 21.

The effect of the treatments on Pancreatic islets

The histological result from the current study indicated that the treatments by Allicin and TQ ameliorated most of the pathological changes caused by STZ on pancreatic islets but did not reach to the full recovery, and there were no obvious differences in histology among these treated groups. Similar results were shown by Sangi et al and Abdelmeguid et al where they both found that TQ significantly cured pancreatic islets after STZ damage 17,23

## Conclusion

The data showed that 8 mg/kg allicin treatment gave the best results for body weight increase and FBG levels but for insulin levels it was the lowest. The treatment with 16 mg/kg allicin gave the best result for insulin levels but the effect on body wight and FBG were mild. Using the 5mg/kg TQ and 10 mg/kg TQ had mild effects on all the three parameters used, and there isn't much difference between them.

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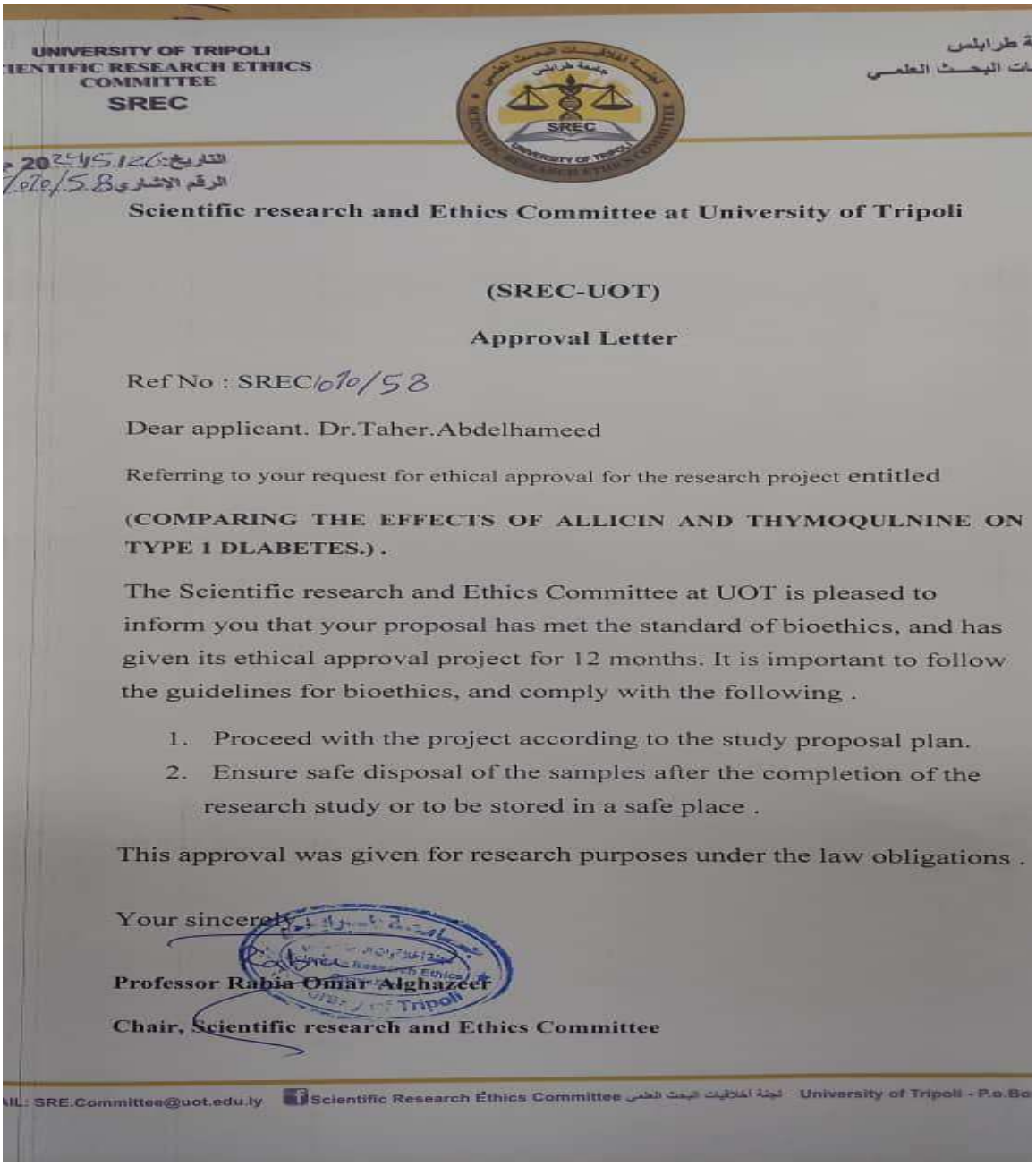

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جامعة طرابلس
لجنة أخلاقيات البحث العلمي

التاريخ: 2024/5/26
الرقم الإشاري 1010/58

**Scientific research and Ethics Committee at University of Tripoli**

**(SREC-UOT)**

**Approval Letter**

Ref No : SREC1010/58

Dear applicant. Dr.Taher.Abdelhameed

Referring to your request for ethical approval for the research project entitled

**(COMPARING THE EFFECTS OF ALLICIN AND THYMOQULNINE ON TYPE 1 DLABETES.) .**

The Scientific research and Ethics Committee at UOT is pleased to inform you that your proposal has met the standard of bioethics, and has given its ethical approval project for 12 months. It is important to follow the guidelines for bioethics, and comply with the following .

1. Proceed with the project according to the study proposal plan.
2. Ensure safe disposal of the samples after the completion of the research study or to be stored in a safe place .

This approval was given for research purposes under the law obligations .

Your sincerely,

**Professor Rabia Omar Alghazeer**

**Chair, Scientific research and Ethics Committee**

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