# Anti-Diabetic and Antihyperlipidemic Potential of Combined Melatonin and Garlic in Nicotinamide-Streptozotocin Induced Diabetic Mice

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**Research Article**

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## Abstract

# Background

The traditional antidiabetic medications have limitations in terms of safety, efficacy and cost. So, it is critical to investigate natural or herbal remedies that can overcome these obstacles. Melatonin and garlic separately have showed evidence of multiple therapeutic effects towards diabetic subjects.

# Objective

To investigate the anti-diabetic and hypolipidemic effects of the combination of low doses of melatonin and garlic in nicotinamide-streptozotocin (NA/STZ)-induced diabetic mice.

# Methods

48 mice were randomly divided into six groups including control (C), diabetic (D), glibenclamide 5 mg/kg (D + GLC), melatonin 10 mg/kg (D + M), garlic extract 100 mg/kg (D + G) and combined melatonin and garlic (D + M + G). All treatments were given orally daily for 8 weeks after induction of hyperglycemia by STZ/NA. Food intake, body weight, fasting blood glucose, oral glucose tolerance test (OGTT), serum insulin, lipid profile as well as serum IL-6 and TNF-ß were estimated.

# Results

Combined melatonin and garlic resulted in 37.2% reduction in fasting blood-glucose levels with amelioration in OGTT ($p < 0.05$). Also, M + G increased serum insulin level (25.3%), improved lipid parameters and reduced serum levels of IL-6 and TNF-ß ($p < 0.05$).

# Conclusion

These results indicate that the combination of melatonin and garlic possessed a beneficial potential impact in diabetic mice. The effect of the two drugs together was comparable to that of glibenclamide, which enhances the possibility of using one or both as adjuncts to the traditional diabetes drugs to reduce their doses.

## Introduction

Diabetes mellitus is a chronic metabolic disorder of endocrine system [1]. In 2019, approximately 463 million adults (20–79 years) are living with diabetes and this figure is expected to rise to 700 million by 2045 [2]. In the gulf and Arab countries, diabetes mellitus is considered an epidemic disease [3] with highest prevalence in Saudi Arabia as it affects around 30% of adults [4].

Melatonin, sometimes known as the "dark hormone," is primarily produced by the pineal gland, with higher levels at night and lower levels throughout the day [5]. The effect of melatonin on insulin secretion are mediated via melatonin receptors (MT1 and MT2) [6]. It works by activating the phospholipase C/IP3 pathway that mobilizes $Ca2+$ from organelles causing increases in insulin secretion. Insulin production by pancreatic islets in a circadian manner is caused by melatonin action on melatonin receptors generating a phase shift in the cells, both in vivo and in vitro [6, 7]. Melatonin may have a role in the development of diabetes, as diabetic patients have lower melatonin levels [7]. Moreover, a functional link between melatonin and insulin was indicated in patients with diabetes [8]. According to evidence from experimental research, melatonin stimulates insulin growth factor synthesis and promotes insulin receptor tyrosine phosphorylation [9, 10]. Glucose intolerance and insulin resistance are caused by a disruption in the endogenous circadian clock, which can be corrected with melatonin administration [11]. Experimental studies found that melatonin treatment ameliorates metabolic changes associated with obesity and diabetes [12, 13].

Garlic (Allium sativum) is common and one of the most popular herbs used worldwide to decrease multiple risk factors accompanied with cardiovascular diseases and diabetes [14]. Garlic is showed to have multiple therapeutic effects. Garlic's strong odor is largely due to Sulphur-containing compounds that account for most of its medicinal efficacies [14]. Moreover, garlic contains many compounds that shows anticoagulant, anti-antioxidant, antibiotic, anti-inflammatory, hypocholesterolemia, hypoglycemic, and hypotensive activities [14, 15, 16, 17, 18]. Previous studies have investigated the anti-diabetic effects of garlic both in animals as well as human and indicated that garlic extract had ameliorative action on indicators of diabetes [19]

Previously we investigated the combination of garlic and melatonin in diabetic mice to examine the oxidative effect and we found that this combination can enhance the oxidative status to diabetics [20]. However, we need to further check thus we hypothesized that combination of low doses of garlic and melatonin might have beneficial effects on blood glucose and lipid profile in diabetes mellitus. Therefore, our main aim of this study was to investigate the effects of combining melatonin and garlic extracts on levels of the plasma blood glucose and lipid profile in nicotinamide-streptozotocin (NA/STZ) induced diabetic mice.

# Materials And Methods

### Materials

All materials and solutions were purchased from Fisher Scientific (Mumbai, India) unless otherwise stated.

### Garlic extract

Black garlic was extracted by refluxing water twice at 80 ° C (yield 12.8%). The resulting solution was evaporated, then lyophilized and stored at 4 ° C until use. Then it was fermented in edible Saccharomyces cerevisiae (KCTC 7910) by two-step culture. In the first stage culture, the microorganisms were cultured in a medium containing 3% (w / v) malt extract at 28 ° C for 36 hours to promote cell proliferation. Cell clusters were collected by centrifugation and recultured in a medium containing 5% (wt / vol) of garlic extract under the same conditions to increase the concentration of bioactive substances such as polyphenols and alicysteine. After culturing, the cells were removed by extracting the culture broth by filtering and heating. The solution was then lyophilized after evaporation and kept at 4 ° C until use. After that, it was dissolved directly in distilled water and orally administered at 100 milligrams / kilograms one time daily for eight weeks [19,21].

### Melatonin

Melatonin from (Thermofisher, Mumbai, India) ten milligrams per kilograms every day /kg mixed in 0.05% ethanol then given to the mice in the water they drink [22].

### Glibenclamide

Glibenclamide (GLC; Julphar Pharmaceuticals, Ras Al Khaimah, United Arab Emirates)

### Mice

All Mice care and treatment procedures have been approved by the Institutional Review Board for Biomedicine and Research (HAPO-02-K-012-2021-10-789) at the University of Umm Alqura. A total of 48 male C57BL / 6J (B6) male mice were purchased from Harlan (Charles River Laboratories, Wilmington, Massachusetts, USA) for this study. Mice were housed in a temperature-controlled room (23 ± 1 ° C) under a 12-to-12-hour light-dark cycle. Mice were individually housed in standard cages with free access to water and standard solid diets (CRM pellets, SDS diet, USA).

Measurements were started at ten weeks of age and performed over eight weeks. Weight was monitored three times a week (Monday, Wednesday, and Friday) just before the lights were turned off throughout the experimental protocol. Eight weeks later, mice were fasted overnight, euthanized with $CO_2$, and blood samples were taken by heart puncture.

### Diabetes Induction of mice

The combination of STZ and nicotinamide [NA] can prepare a method for inducing type 2 diabetes with hyperglycemia and relatively low insulin levels. Mice were injected intraperitoneally (i.p.) with STZ (50 mg / kg body weight) in 0.1 M citrate buffer (pH 4.2) for 2 consecutive days. NA (120 mg / kg body weight) in physiological saline was administered intraperitoneally. Injections were made thirty minutes before the STZ injection on the first day after an overnight fast. Mice with an eight-hour fasting blood glucose level of 200 mg / dL, injected 7 days after the second injection, were recognized as hyperglycemia. Other mice that showed fasting blood glucose levels less than FBG 200 mg / dl, were infused with more ATZ and monitored until the FBG level reached 200 mg / dl. Fasting blood glucose was monitored with a glucose meter (Free style, USA). [22, 23]

### Experimental design

The experimental design of our study used mice that were chosen randomly and then we divided them into six groups with eight mice in each group. We placed a group for control non-diabetic group and five groups were injected with NA/STZ to induce diabetes, the four groups received drug treatments daily for eight consecutive weeks:

• 1(C); is the control non-diabetic group that was treated with saline orally and citrate buffer.

• 2 (D); is the non-treated induced diabetic group.

• 3(D+GLC); is the induced diabetic group that was treated with the standard antidiabetic drug, glibenclamide (5 mg/kg/day) [24]

• 4 (D+M); is the induced diabetic group that is treated with melatonin (10 mg/kg/day in drinking water) [22]

• 5 (D+G); is the induced diabetic group that is treated with prepared garlic extract (100 mg/kg/day) [19]

• 6 (D+M+G);is the induced diabetic group that is treated with melatonin and garlic extract.

We choose the doses of melatonin and garlic because of previous research studies [25,26, 27]_

At 2$^{nd}$ and 8$^{th}$ weeks, the levels of FBG and fasting insulin levels were examined. After 8 weeks; oral glucose tolerance test, plasma lipids and inflammatory cytokines (IL-6 and TNF-α Levels) were examined.

### Assessment

### Body weight

Changes in body weight and food intake were recorded every week in the studied groups throughout the experiment.

### Fasting blood glucose and fasting insulin measurements

Mice were fasted overnight at week two and eight then blood were taken out from their tails to measure their fasting blood glucose and insulin levels. Fasting blood glucose was measured with a One Touch II glucose meter (Free style, USA). Fasting insulin were examined by using the enzyme-linked immunosorbent assay (insulin ELISA, # nr 10-1247-01, Mercodia, Sweden) and the spectrophotometric plate reader (Synergy HT Multi-Mode Microplate Reader, BioTek, USA).

### Oral glucose tolerance test (OGTT)

One day before the end of experiment, all rats were fasted overnight and infused intragastrical with 2 g glucose per kilogram of body weight. Rat tail blood samples were taken at 0 min, 30 min, 60 min and 120 min to evaluate FBG, 30 min, 1 h postprandial blood glucose (PBG1) and 2 h postprandial blood glucose (PBG2) respectively [28].

### Plasma lipids and Inflammatory Cytokines (IL-6 and TNF-α)

Blood samples that were taken from mice used in these measurements following manufacturer's guidelines. IL-6 and TNF-α were determined from a standard curve and their levels were expressed in pg/ml [29].

### Statistical Analysis

All results are expressed as group means ± standard deviation. Results were analyzed using one-way analysis of variance ANOVA, followed by Tukey's post-hoc test to assess significance. Any *P* value of less than 0.05 was considered significant. We used GraphPad Prism version 6 (GraphPad Software Inc., California, USA).

## Results

# Effects of Melatonin and Garlic on Body Weight and food intake in Diabetic Mice

After the induction of diabetes, significant differences in body weight between normal and untreated diabetic mice were observed ($P < 0.05$, Fig. 1). Melatonin, garlic, and their combinations improved weight gain when compared to diabetic controls ($P < 0.05$).

The weight of normal control mice had significantly increased ($P < 0.05$), while that of diabetic control mice had significantly decreased ($P < 0.05$). Mice treated with M, G, G + M and GLC had gained weight of 7.77%, 12.13%, 19.33%, and 22.39% at the end of the experiment, respectively. They were significant in combined and glibenclamide group ($P < 0.05$, Fig. 1).

By week 4, 6 and 8 the untreated diabetic mice showed a significance decrease in their food intake (2.0 ± 0.01g, 1.6 ± 0.02g, 1.1 ± 0.01g, $P < 0.05$) compared with that of normal control mice. The treated ones showed a significant increase in their food intake by 6.22%, 11.09%, 15.23%, and 19.06% at the end of the experiment in M, G, G + M and GLC respectively ($P < 0.05$, Fig. 2).

# Effects of Melatonin and Garlic on FBG Levels in Diabetic Mice (Fig. 3)

FBG levels of diabetic control mice were significantly higher than normal control mice at week 2 and week 8 ($P < 0.05$). Those administered M and G individually showed insignificant reduction (9.5 ± 0.34, 9.9 ± 0.33, p0.05) when compared to diabetic controls at weeks 2 and 8 with percentage reduction of 20.1% & 21.3% respectively at week 2 and 21.4% & 21.5% respectively at week 8. However, the combination of M & G resulted in significant decrease in FBG levels compared to diabetic control group [percentage reduction of 37.2%] (8.1 ± 0.33 $P < 0.05$), an effect that was comparable to that of GLC [percentage reduction of 45.6%] (6.2 ± 0.14, $P$  0.05)

# Effects of Melatonin and Garlic on Oral Glucose Tolerance in Diabetic Mice (Fig. 4)

Antihyperglycemic activity of melatonin and garlic was studied in STZ/NA-induced diabetic mice after eight weeks. As shown in Fig. 4, we performed intergroup and intragroup comparisons to analyze differences in blood glucose levels (BGL) between groups and time points. Glucose (2.5 g / kg oral) administration to mice fasted overnight did not cause a significant difference in baseline BGL across all groups, but showed the greatest change in all groups after a 30-minute glucose challenge. Brought. A significant decrease in BGL was observed at 2 hours in the M and G treatment groups compared to diabetes control. And it was treated in half, one, one and a half hours and two hours in GLC group (D + M:110 mg/dl, D + G:107 mg/dl, D + GLC:145 mg/dl, 128 mg/dl, 109 mg/dl, 90 mg/dl $P < 0.05$). There was no statistically significant difference in BGL at all time points when groups treated with the combination of M + G compared to the GLC treated group (D + M + G: 155 mg/dl, 135 mg/dl, 119 mg/dl, 98 mg/dl; D + GLG: 145 mg/dl, 128 mg/dl, 109 mg/dl, 90mg/dl $P$ 0.05).

# Effects of Melatonin and Garlic on Blood insulin Levels in Diabetic Mice (Fig. 5)

As shown in Fig. 5, induction of diabetes with STZ/NA resulted in approximately 75% reduction in serum insulin level (0.99 ± 0.02 ng/ml) compared to normal control rats (3.6 ± 0.13 ng/ml). M and G treatment individually resulted in a higher serum insulin with increases of 18% (1.7 ± 0.2 ng/ml) and 16.8% (1.5 ± 0.1 ng/ml), respectively. However, these effects were not significant when compared to diabetic control group ($P$ 0.05). While, administration of GLC to diabetic mice caused significant increase in serum insulin (2.2 ± 0.13 ng/ml, 25.3%) compared to diabetic control mice ($P < 0.05$). An effect that was comparable to that of combined M + G group (2.1 ± 0.11, 21.2% $P$ 0.05). Insulin levels were slightly elevated after 8 weeks treatments compared to 2 weeks in all treated groups; GLC, M, G and M + G combination. However, these elevations were not significant (2.4 ± 0.11, 1.8 ± 0.12, 1.6 ± 0.03, 2.2 ± 0.12, respectively, P 0.05).

# Effects of Melatonin and Garlic on Serum Proinflammatory Cytokines in Diabetic Mice

Figure 6A showed that STZ caused significant elevation in IL-6 (24.3 ± 0.12) ($P < 0.05$) compared to that in normal control mice (13.5 ± 0.55). M and G individually caused non-significant decrease in IL-6 (19.1 ± 0.45, 19.3 ± 0.34 respectively) compared to diabetic control group (*P* [illegible]0.05, Fig. 6A). However, their combination caused significant amelioration in IL-6. This effect was comparable to that of normal control group as well as GLC group (15.1 ± 0.25, *P* [illegible]0.05, Fig. 6A). Concerning serum level of TNF-α, there was significant higher level in diabetic control group (223 ± 22.4) compared to that in normal control mice (100.1 ± 0.16, $P < 0.05$, Fig. 6B). All treatments caused significant reduction in serum level of TNF-α compared to diabetic untreated group: GLC, M, G and M + G combination (110.1 ± 0.14, 122.1 ± 0.19, 123.4 ± 0.17,115 ± 0.23 respectively; $P < 0.05$, Fig. 6B).

# Effects of Melatonin and Garlic on Lipid Profile in Diabetic Mice (Table 1)

TC, TG, and VLDL cholesterol increased significantly ($P<0.05$) in diabetic control mice compared to normal control ones. Melatonin and garlic both individually and in combination significantly decreased ($p<0.05$) these parameters compared to diabetic controls. The combined M+G as well as glibenclamide (5 mg/kg) reduced the three lipid parameters significantly ($p<0.05$) when compared to each drug alone. Moreover, they reduced TC, and VLDL near to values of normal controls (Table 1).

**Table (1): The Effect of Treatments on Lipid Profile in Diabetic Mice**

| Groups (n= 8) | TG (mg/dl), Mean± SD | TC (mg/dl), Mean± SD | VLDL (mg/dl), Mean± SD |
|---|---|---|---|
| Control | 56.91±4.23 | 69.45±1.34 | **12.2±0.32** |
| Diabetic | 190±1.45* | 181±2.81* | **35.23±0.23*** |
| Diabetic treated with glibenclamide 5 mg/kg | 60.12±0.87$^{\#}$ | 79.41±1.24* $^{\#}$ | **12.8±1.23$^{\#}$** |
| Diabetic treated with Melatonin | 71.34±1.55*$^{\#\ @}$ | 91.18±1.23*$^{\#@}$ | **13.91±0.33*$^{\#}$** |
| Diabetic treated with garlic | 78.23±1.55*$^{\#\ @}$ | 96.21±1.34*$^{\#@}$ | **14.5±0.14*$^{\#}$** |
| Diabetic treated with (Melatonin + Garlic) | 62.17±1.35$^{\#}$ | 83±2.11*$^{\#}$ | **12.1±0.21$^{\#}$** |

*$P<0.05$ compared with normal control, $^{\#}$ $p<0.05$ compared with diabetic control, $^{@}$ $p<0.05$ compared with diabetic treated with glibenclamide 5 mg/kg. TC, total cholesterol; TG, triglyceride; VLDL, very low-density lipoproteins; SD, standard deviation

# Discussion

Diabetes is among the most common contemporary human afflictions and is often accompanied by serious acute and chronic complications [30]. For the treatment of diabetes, many synthetic medicines have been produced. However, these medications have limitations in terms of efficacy and side effects [31]. As a result, there is a lot of interest in learning about natural or herbal remedies that don't have much bad side effects and can help in decreasing the severity of the diabetic complications. In this research we examined low doses of melatonin and garlic extract [19, 22] both individually and combined on glycemic and lipid indices of NA/STZ diabetic mice and compared their effects with glibenclamide, a standard antidiabetic drug.

In our experiment, treatment of diabetic mice with melatonin and garlic individually for 8 weeks showed insignificant reduction in FBG levels. However, the combination of both resulted in 37.2% reduction in FBG levels compared to diabetic control group, an effect that was significant and similar to that of glibenclamide. Moreover, OGTT in the present study proved that there was no statistically significant difference in blood glucose level at all time points when groups treated with the combination of melatonin & garlic compared to the glibenclamide treated group. These results were associated with significant increase in serum insulin level (25.3%) in mice administered both drugs. In many organisms, pineal melatonin is the primary endogenous synchronizer of circadian rhythms [32]. It is a well-known endogenous antioxidant, with potent anti-inflammatory, anti-hyperlipidemic, and anti-hypertensive properties, as well as modulating insulin secretion and action [32, 33, 34]. A previous experimental study indicated that animals which received a pinealectomy exhibited impaired glucose tolerance, insulin resistance, and diabetes, which were improved by treatment with melatonin [35]. Another study found that the treatment of melatonin enhanced a better glycemic control, improving insulin level and sensitivity in white adipose tissues of diabetic mice [36]. Similarly, garlic is said to have several therapeutic characteristics, including hypoglycemic, hypolipidemic as well as anti-inflammatory effects [37, 38]. These effects are associated with different bioactive compounds such as phenolic compounds, flavonoids, and organosulfur compounds [39]. Multiple research examined the effect of garlic with multiple safe doses and found that garlic had a significant effect in reducing blood glucose, cholesterol and triglyceride levels in diabetic animal models [19, 40, 41]. Concerning garlic's effect on blood glucose level, it could be attributed to an increase in pancreatic insulin secretion from beta-cells, the release of bound insulin, or an improvement in insulin sensitivity. Garlic has previously been suggested to improve serum insulin by effectively interacting with chemicals like cysteine, sparing insulin from SH group interactions, which are a common source of insulin inactivation [19]. These findings were coincided with our results that proved increase in insulin level by administration of both melatonin and garlic to mice with diabetes. However, each drug alone caused insignificant effects in most of glycemic parameters which may be due to the low doses used.

In the present study, mice with NA/STZ induced hyperglycemia were associated with significant body weight loss in the diabetic control mice. This effect was similar to previous research [24, 42, 43] who indicated that hyperglycemia induced by STZ caused loss of large percentage of mice body weight which may be due to increased wasting of fat stores, muscle and tissue proteins [44, 45]. In the current research, melatonin, garlic, and their combinations improved weight gain and food intake when compared to diabetic controls with the significant effect in the combination group that was comparable to that of glibenclamide. Hence, the weight gain after treatment of melatonin and garlic in diabetic mice might suggest the antihyperglycemic activity of both.

In the present study, NA/STZ caused significant elevation in IL-6 as well as TNF-α in diabetic control mice. The inflammatory cytokines are associated with the development of insulin resistance and diabetes [46]. Indeed, TNF α and interleukins can activate proteins of the inflammatory pathway causing attenuation of insulin signaling with impairment of blood glucose [47]. Like glibenclamide, the combination of melatonin and garlic in the current experiment resulted in significant reduction in serum levels of IL-6 and TNF-α. These effects were in accordance with previous research that proved the ameliorating action of melatonin and garlic on inflammatory cytokines [34, 38]. Herein, NA/STZ caused significant elevation in TC, TG, and VLDL cholesterol in diabetic control mice. Dyslipidemia is a major symptom and risk factor for the development of cardiovascular diseases in DM [48]. The results of the present study revealed that melatonin and garlic significantly improved lipid parameters. Moreover, their combination reduced TC, and VLDL near to values of normal controls. Several previous studies in both humans and animals have reported that garlic has a lipid-lowering property [19, 49, 50]. In contrast, treatment of type 2 diabetic patients with garlic daily had no effect on serum cholesterol but did slightly, although not significantly, lower serum triglycerides after 3 months of treatment [51]. Regarding melatonin, it could decrease plasma total cholesterol and triglyceride in different animal models of diabetes and obesity [22, 52, 53]. It is well known that there is a close link between cholesterol accumulation with oxidative stress and inflammatory mediators. Increased lipoperoxidation because of oxidative stress can trigger activation and proliferation of cytokines, such as TNF-α and interleukins. In addition, lipoperoxidation reduces the activity of the mitochondrial respiratory chain and producing more reactive oxygen species [54]. Taken together these data, one might speculate that the beneficial effects of melatonin and garlic on diabetes could be in part through their anti-inflammatory and hypolipidemic effects with their documented antioxidant activity [19, 22, 32, 34, 41].

To our knowledge, the potential anti-diabetic effects of combined melatonin and garlic have not been tested before. Here, we demonstrated that the combined therapy was more effective than individual low dose therapy. The better glycemic control observed could be in part due to an improvement of the metabolic capacity of adipose tissue or though amelioration of inflammatory cytokines.

In conclusion, the present data demonstrated that the combined low doses of melatonin and garlic promoted advantageous effects in ameliorating blood sugar and lipid profile in diabetic mice. An effect that was comparable to that of glibenclamide, which enhances the possibility of using these extracts as an alternative to conventional antidiabetics or add-on therapy to reduce their doses and possible side effects. However, clinical studies are recommended to test their combination with conventional antidiabetics as well as the best safe and effective doses for use.

# Declarations

### Authors' contributions

This work was carried out in collaboration among all authors. Authors YA and SE designed the study, conducted experimental work and data analyses, wrote the protocol, and drafted the initial manuscript. Authors AF, YA, SE, AB help in literature searches and experimental work, AB prepares the garlic extract. YA and SE critically reviewed the manuscript. All authors read and approved the final manuscript.

### Conflict of interest

Authors state no conflict of interest.

### Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.


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This study was supported by the supervisor of this study, YA.


### Authors' Relationships and Activities

The authors declare that no relationships or activities might bias, or be perceived to bias, their work.

### Animal Research Ethics

All procedures concerning animal care and treatment were approved by Umm Al-Qura university's Biomedical and research Ethics Committee (HAPO-02-K-012-2021-10-787).

### HUMAN AND ANIMAL RIGHTS

Not applicable

### CONSENT FOR PUBLICATION

Not applicable


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# Figures

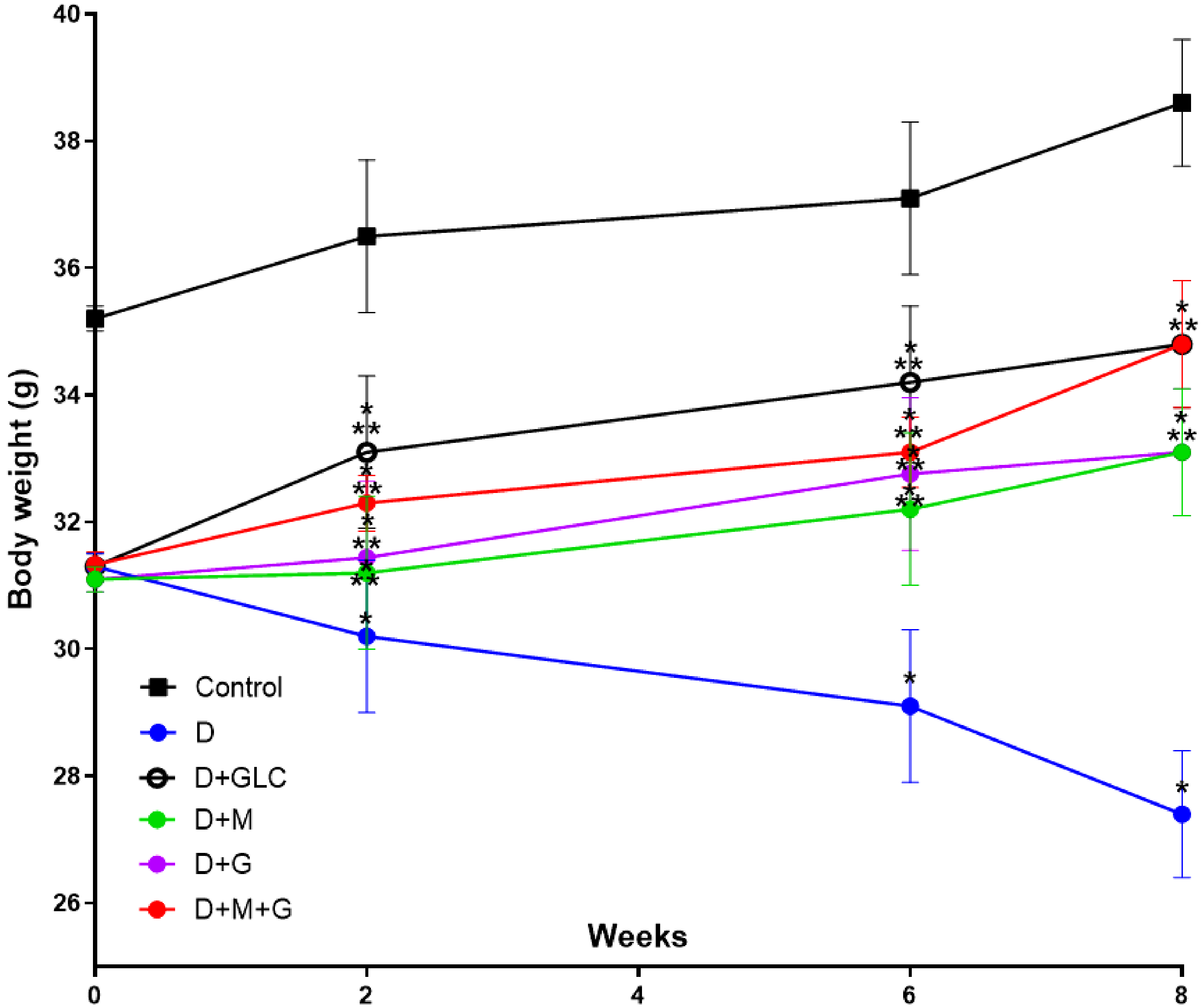
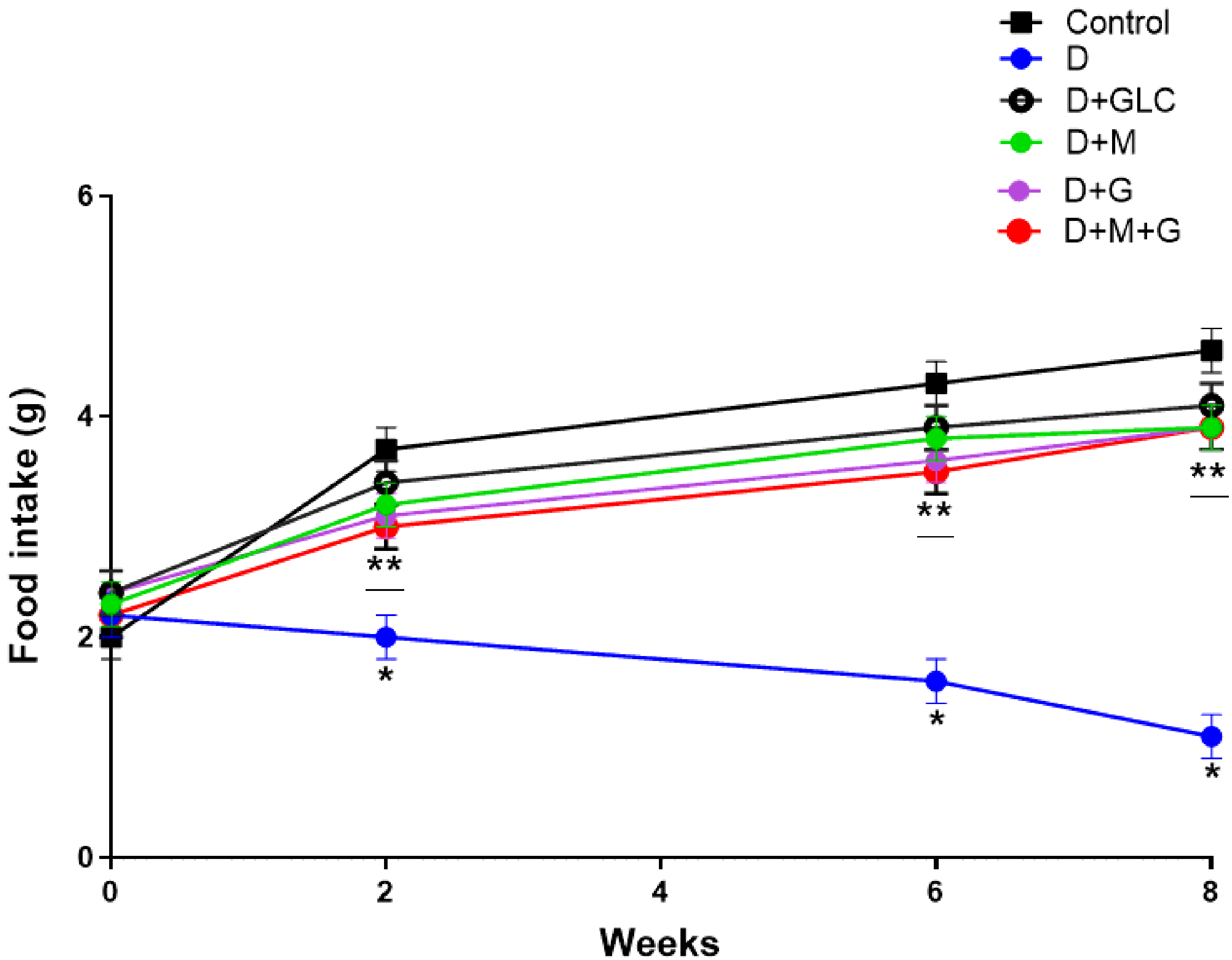



**Figure 1**

Body weight measurements in mice for 8 weeks. C; control, D; diabetic induced mice, D+GLC; diabetic mice treated with glibenclamide; D+M; diabetic mice treated with melatonin, D+G; diabetic mice treated with garlic, D+M+G; diabetic mice treated with combination of melatonin and garlic. *$P$<0.05 compared to normal control mice, **$P$<0.05 compared to control diabetic mice.




## Figure 2

Food intake measurements for male mice in all groups for 8 weeks. C; control, D; diabetic induced mice, D+GLC; diabetic mice treated with glibenclamide, D+M; diabetic mice treated with melatonin, D+G; diabetic mice treated with garlic, D+M+G; diabetic mice treated with combination of melatonin and garlic. *$P$<0.05 compared to normal control mice, **$P$<0.05 compared to control diabetic mice.

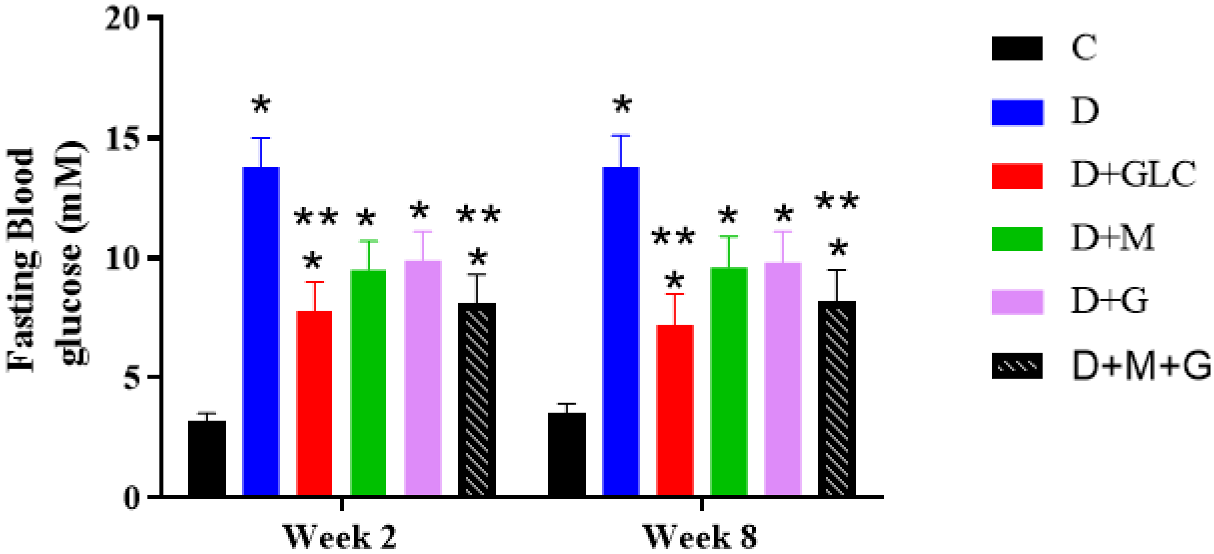
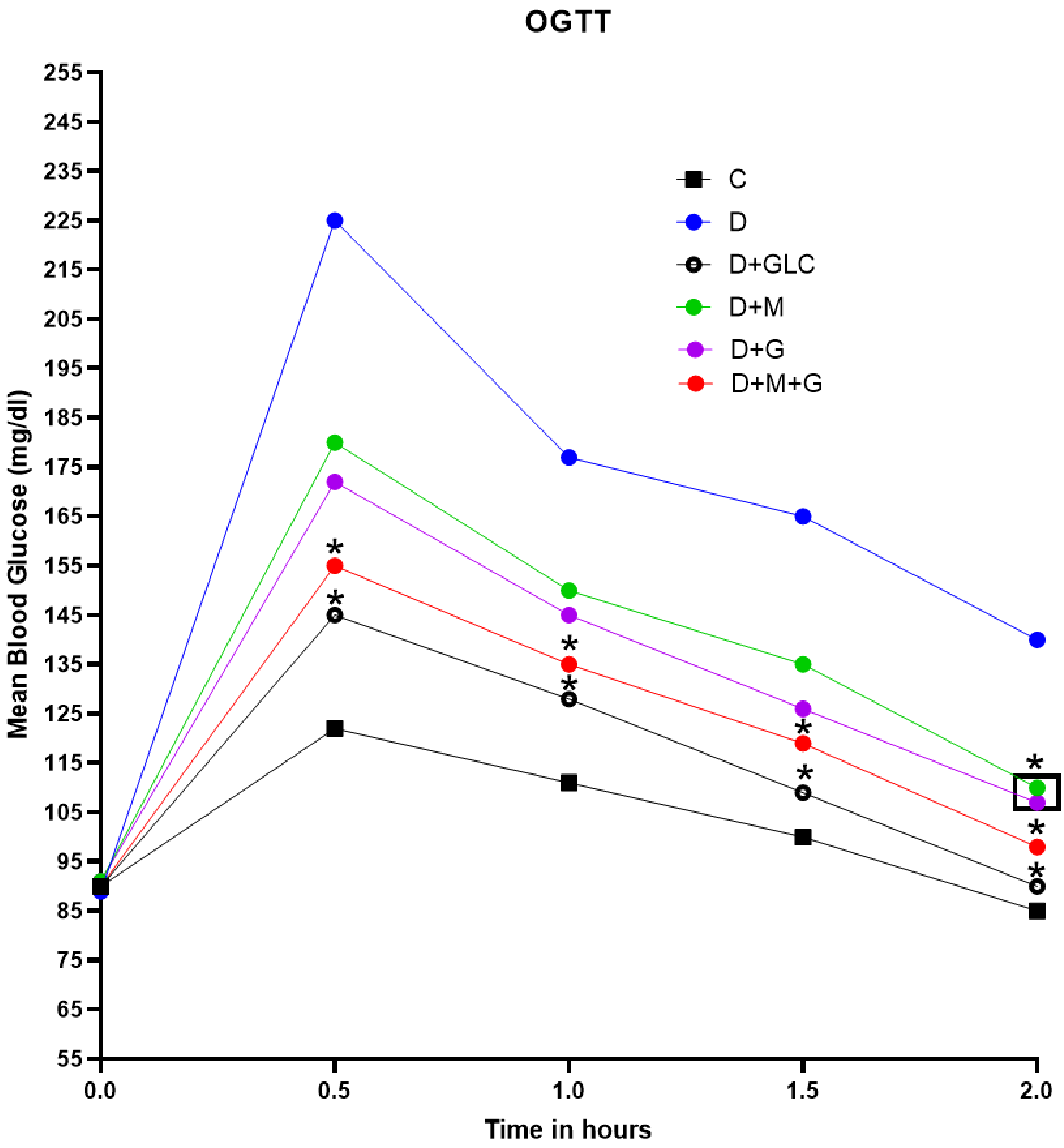



**Figure 3**

Fasting Blood glucose measurements for mice in all groups at week 2 and 8. C; control, D; diabetic induced mice, D+GLC; diabetic mice treated with glibenclamide, D+M; diabetic mice treated with melatonin, D+G; diabetic mice treated with garlic, D+M+G; diabetic mice treated with combination of melatonin and garlic.

*$P$<0.05 compared to normal control mice, **$P$<0.05 compared to control diabetic mice.




## Figure 4

Oral glucose tolerance test in all groups after 8 weeks. C; control, D; diabetic induced mice, D+GLC; diabetic mice treated with glibenclamide, D+M; diabetic mice treated with melatonin, D+G; diabetic mice treated with garlic, D+M+G; diabetic mice treated with combination of melatonin and garlic. *$P$<0.05 compared to control diabetic mice.

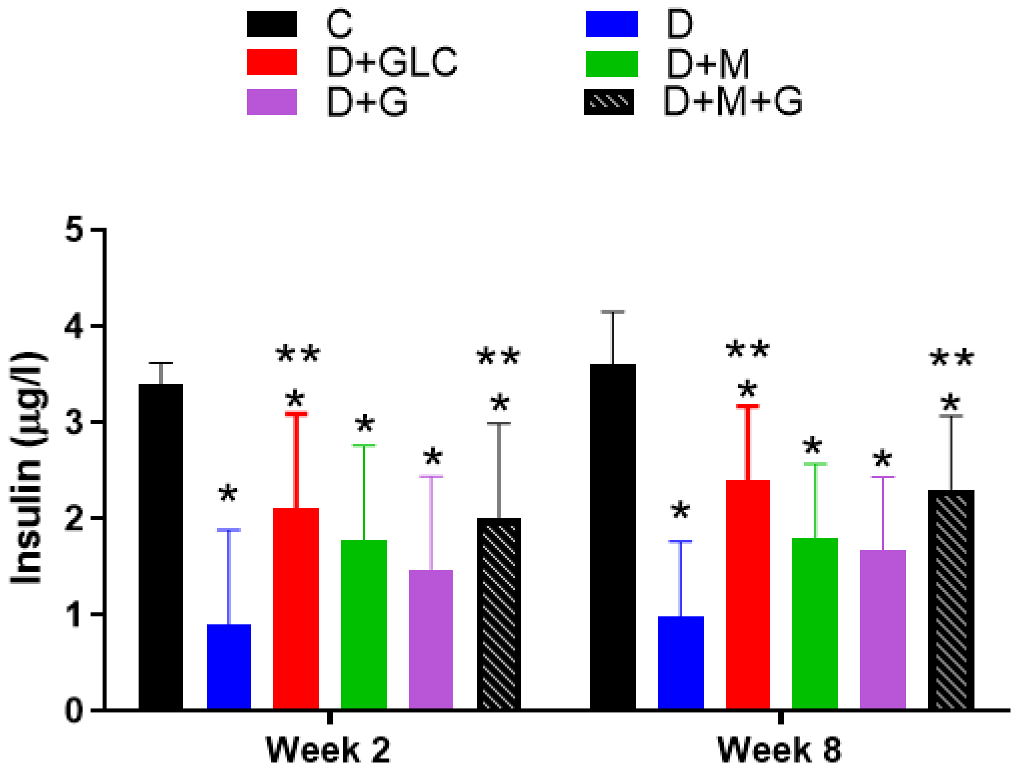
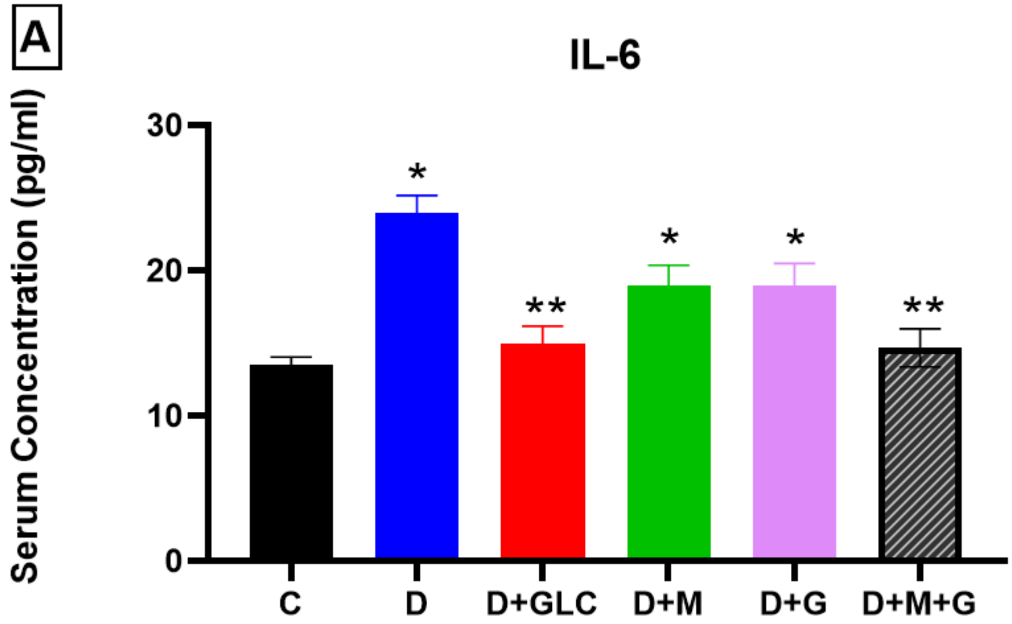
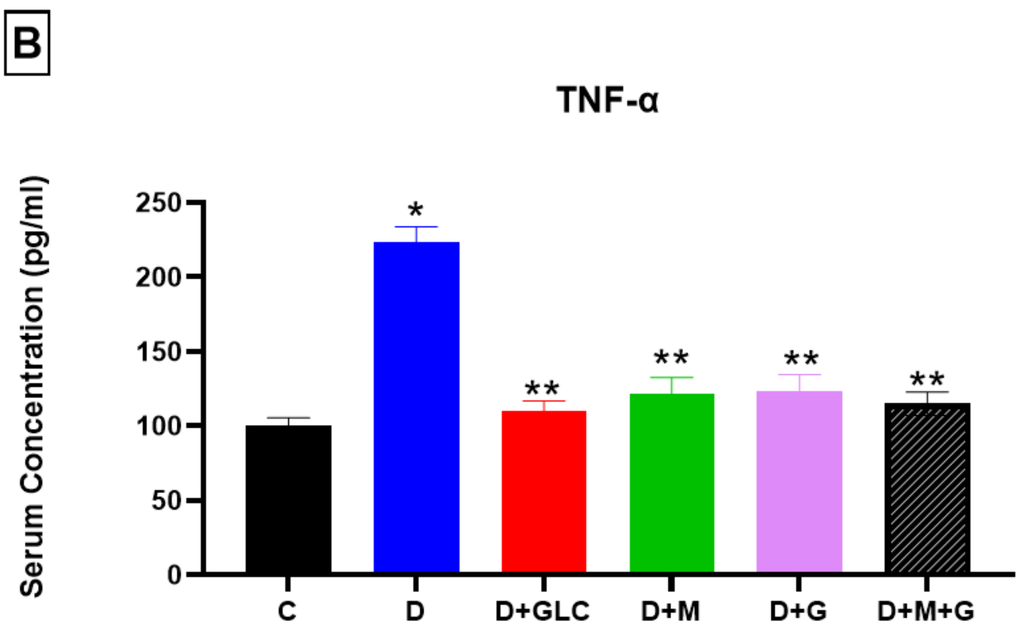



**Figure 5**

Plasma insulin measurements for all groups at week 2 and 8. C; control, D; diabetic induced mice, D+GLC; diabetic mice treated with glibenclamide, D+M; diabetic mice treated with melatonin, D+G; diabetic mice treated with garlic, D+M+G; diabetic mice treated with combination of melatonin and garlic.

**P*<0.05 compared to normal control mice, ***P*<0.05 compared to control diabetic mice.




### Figure 6

Effect of treatments on serum proinflammatory cytokines. [A] The level of IL-6 [B] The level of TNF-α in blood serum of mice. C; control, D; diabetic induced mice, D+GLC; diabetic mice treated with glibenclamide, D+M; diabetic mice treated with melatonin, D+G; diabetic mice treated with garlic, D+M+G; diabetic mice treated with combination of melatonin and garlic.

*$P<0.05$ compared to normal control mice, **$P<0.05$ compared to control diabetic mice.