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

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Abstract

Background and Objective: 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 shown evidence of multiple therapeutic effects towards diabetic subjects. 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. **Materials and Methods:** About 48 mice were randomly divided into 6 groups including control (C), diabetic (D), glibenclamide 5 mg kg^{-1} (D+GLC), melatonin 10 mg kg^{-1} (D+M), garlic extract 100 mg kg^{-1} (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-β, were estimated. **Results:** Combined melatonin and garlic resulted in a 37.2% reduction in fasting blood glucose levels with amelioration in OGTT ($p<0.05$). Also, M+G increased serum insulin levels (25.3%), improved lipid parameters and reduced serum levels of IL-6 and TNF-β ($p<0.05$). **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 of them as adjuncts to the traditional diabetes drugs to reduce their doses.

Key words: Diabetes, garlic, melatonin, NA/STZ, lipid profile, fasting blood glucose, oral glucose tolerance test

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of the endocrine system[1]. The rate of diabetes is increasing, during 2019 around 463 million adults are considered to living with this metabolic disease 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 the 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 is mediated via melatonin receptors (MT1 and MT2)[6]. It works by activating the phospholipase C/IP3 pathway that mobilizes Ca^{2+} 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 by cardiovascular diseases and diabetes[14]. Garlic is shown to have multiple therapeutic effects. Garlic's strong odour is largely due to sulphur-containing compounds that account for most of its medicinal efficacies[14]. Moreover, garlic contains many compounds that show anticoagulants, anti-antioxidant, antibiotics, anti-inflammatory, hypocholesterolemia, hypoglycemic and hypotensive activities[14-18]. Previous studies have investigated the anti-diabetic effects of garlic both in animals as well as humans and indicated that garlic extract had ameliorative action on indicators of diabetes[19,20].

To our knowledge, no previous research if any used the combination of garlic and melatonin in diabetic subjects. Therefore, due to the frequent use of antidiabetic drugs with their associated side effects, we hypothesized that a combination of low doses of garlic and melatonin might have beneficial effects on glycemic and lipid profiles in diabetes mellitus. Also, this combination can be used with traditional antidiabetic drugs to reduce their doses and potential side effects. This study aimed to investigate the effects of low doses of melatonin and garlic extract both individually and combined on blood glucose levels as well as lipid profile in Nicotinamide-Streptozotocin (NA/STZ) induced diabetic mice.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Pharmacology Laboratory, College of Pharmacy, From January to May, 2021.

Reagents: Reagents and chemicals including were purchased from Sigma-Aldrich (MO, USA), unless otherwise stated.

Preparation of garlic extract: Black garlic was supplied from Ueisung-Nongsan (Korea) and extracted by heating with water twice under reflux at 80°C (yield, 12.8%). The resulting solution was freeze-dried after evaporation and kept at 4°C until use. The BG was fermented with edible Saccharomyces cerevisiae (KCTC 7910) by 2-stage cultivation. At the first stage of cultivation, the microorganism was cultivated in a medium containing 3% (wt/vol) malt extract for 36 hrs at 28°C to enhance cell growth. The cell mass was obtained by centrifugation and recultivated in a medium containing 5% (wt/vol) of the BG extracts under the same conditions to increase the concentration of physiologically active substances, such as polyphenol and allycystein, which play a key role in antioxidation. After cultivation, the culture solutions were extracted by heating after filtration to remove the cells. The solution was then freeze-dried after evaporation and kept at 4°C until needed. Then it was dissolved directly in distilled water and administered orally at a dose of 100 mg kg^{-1}, once per day for 8 weeks after induction of diabetes[19,21].

Melatonin: Melatonin from (Sigma-Aldrich Co., St., Louis, MO) 10 mg kg^{-1} daily dissolved in 0.04% ethanol was added to the drinking water at night[22].

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

Animals: All procedures concerning animal care and treatment were approved by Umm Al-Qura University's Biomedical and Research Ethics Committee (HAPO-02-K-012-2020-12-344). For this study total number of 48 males of C57BL/6J (B6) mice were purchased from Harlan (Charles River Laboratories, Wilmington, Massachusetts, USA). Mice were

maintained in a temperature-controlled room (23±1°C) under a 12-12 hrs light to dark cycle. Mice were individually housed in standard cages with *ad libitum* water and standard chow (CRM pellets, SDS diets, UK).

Measurements then started at the age of 10 weeks and were taken for 8 weeks. Body weight was monitored, just before lights off, 3 times a week (Mondays, Wednesdays and Fridays) throughout the experimental protocol. After 8 weeks mice were fasted overnight and euthanized by CO_2 and blood samples were taken by cardiac puncture.

Induction and assessment of diabetes: A method of inducing type 2 DM with hyperglycemia and relatively low insulin levels can be produced by combining STZ and nicotinamide [NA]. Mice were intraperitoneally (i.p.) injected with STZ (50 mg kg^{-1} of BW) in 0.1 M citrate buffer (pH 4.2) on two consecutive days. NA (120 mg kg^{-1} of BW) in saline was i.p., injected 30 min before the STZ injection on the 1st day after overnight fasting. Seven days after the second i.p., injection, mice that exhibited an 8 hrs fasting blood glucose (FBG) level of 200 mg dL^{-1} were recognized as being hyperglycemic. The others that exhibited an FBG level of <200 mg dL^{-1} were injected with STZ and monitored until the FBG level reached 200 mg dL^{-1}. The FBG was monitored with a glucometer (Dragon Pharmaceutical Co, New Taipei, Taiwan)[22,23].

Experimental design: Mice were randomly classified into 6 groups (n = 8 each), one group served as a control non-diabetic group and 5 groups were injected with NA/STZ to induce diabetes, then four groups received their respective drug treatments daily for 8 consecutive weeks according to the following design:

- **Group 1 (C):** Control non-diabetic mice treated with saline orally and citrate buffer intraperitoneally
- **Group 2 (D):** Nontreated induced diabetic mice
- **Group 3 (D+GLC):** Induced diabetic mice treated with the standard antidiabetic drug, glibenclamide (5 mg/kg/day)[24]
- **Group 4 (D+M):** Induced diabetic mice treated with melatonin (10 mg/kg/day in drinking water)[22]
- **Group 5 (D+G):** Induced diabetic mice treated with prepared garlic extract (100 mg/kg/day in drinking water)[19]
- **Group 6 (D+M+G):** Induced diabetic mice treated with melatonin plus garlic extract

The doses of melatonin and garlic were chosen based on previous experimental studies that examined a wide range of doses in diabetes. The lowest doses were selected for both melatonin and garlic due to the potential side effects of large doses[25-27].

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

Assessment

Bodyweight: 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: At weeks 2 and 8 all mice fasted overnight and blood was taken from their tails to measure their FBG and insulin levels per the manufacturer's instructions. Fasting blood glucose was measured with a One Touch II glucose meter (Lifescan, Inc., Johnson and Johnson, Milpitas, CA). Fasting insulin concentrations were measured in duplicates 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, UK).

Oral Glucose Tolerance Test (OGTT): One day before the end of the experiment, all rats were fasted overnight and infused intragastrical with 2 g glucose per kilogram of body weight. Rattail blood samples were taken at 0, 30, 60 and 120 min to evaluate FBG, 30 min, 1 hr Postprandial Blood Glucose (PBG1) and 2 hrs Postprandial Blood Glucose (PBG2), respectively[28].

Plasma lipids and inflammatory cytokines (IL-6 and TNF-α): Blood samples were taken from mice's tails and hearts. This was done by puncturing the heart and collecting blood samples in 2 mL containers and these samples were placed in a centrifuge at 1500 g at 4 for around 10 min (5702/R, Eppendorf, Hauppauge, USA). The supernatant was immediately separated from the pellet to prepare serum samples to determine the level of triglyceride (TG) and total cholesterol (TC) directly, while, Very Low-Density Lipoprotein (VLDL) was calculated from TG (VLDL = TG/5)[29]. The volume/unit of IL6 and TNF-α protein complex were measured in the blood serum by using ELISA kits (Biosource International Inc., Camarillo, CA) following the manufacturer's guidelines. The IL-6 and TNF-α were determined from a standard curve and their levels were expressed in pg mL^{-1}.

Statistical analysis: All results are expressed as group Means±SEM. Results were analyzed by one-way analysis of variance, followed by Tukey's *post hoc* Test to assess significance, using a criterion of the p>0.05. The statistical analysis was carried out using GraphPad Prism version 5 (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 33.10, 34.10, 35.90 and 36.20 g at the end of the experiment, respectively. They were significant in the combined and glibenclamide group (p<0.05, Fig. 1).

By weeks 4, 6 and 8 the untreated diabetic mice showed a significant decrease in their food intake (2.0±0.01, 1.6±0.02 and 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 3.90, 4.20, 5.44 and 5.99 g 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: 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, p>0.05) when compared to diabetic controls at weeks 2 and 8 with a percentage reduction of 20.1 and 21.3%, respectively at weeks 2 and 21.4 and 21.5% at week 8. However, the combination of M and G resulted in a significant decrease in FBG levels compared to the 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) (Fig. 3).

Effects of melatonin and garlic on oral glucose tolerance in diabetic mice: The antihyperglycemic activity of melatonin and garlic was studied in STZ/NA-induced diabetic mice after 8 weeks. As shown in Fig. 4, between and within-group comparisons were performed to analyze Blood Glucose Level (BGL) differences across the various groups and time points, respectively. Administration of glucose (2.5 g kg^{-1} orally) to overnight-fasted mice caused no significant difference in

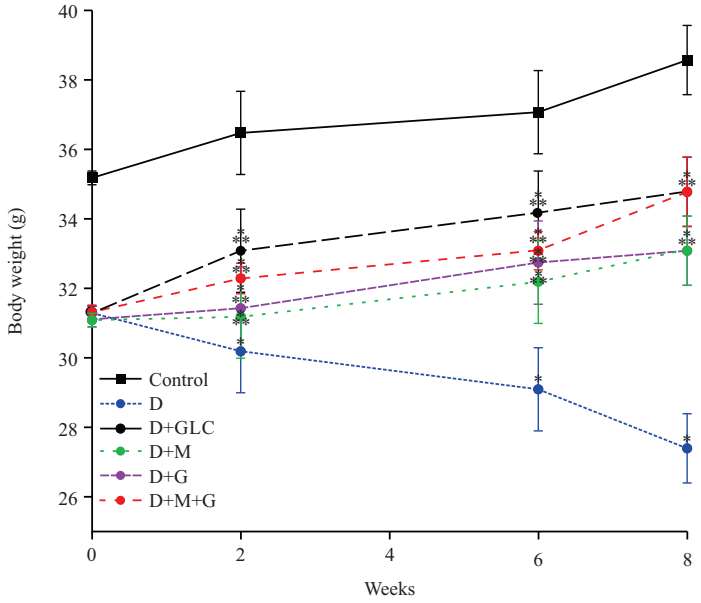

Fig. 1: Bodyweight measurements in mice for 8 weeks

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 a combination of melatonin and garlic, *p<0.05 compared to normal control mice and **p<0.05 compared to control diabetic mice

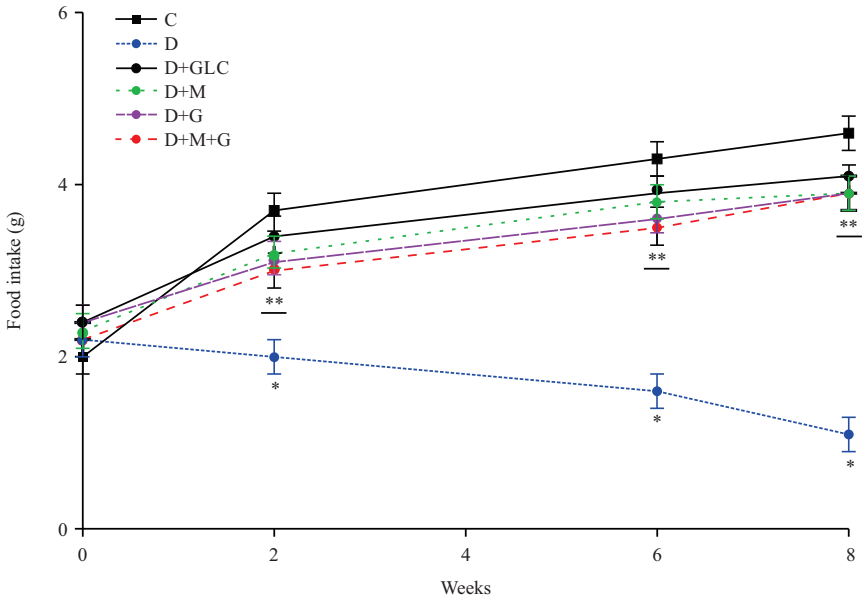

Fig. 2: Food intake measurements for male mice in all groups for 8 weeks

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 the combination of melatonin and garlic, *p<0.05 compared to normal control mice and **p<0.05 compared to control diabetic mice

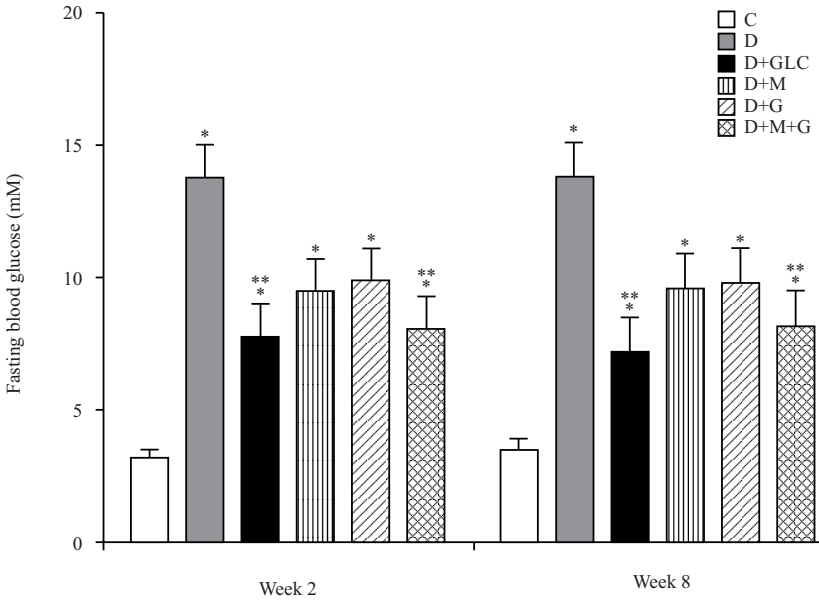

Fig. 3: Fasting Blood glucose measurements for mice in all groups at weeks 2 and 8

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 the combination of melatonin and garlic, *p<0.05 compared to normal control mice, **p<0.05 compared to control diabetic mice and X-axis: Weeks

baseline BGL across all groups while, producing maximum change after 30 min of glucose challenge in all groups. Compared to the diabetic control, a significant BGL reduction was observed at 2 hrs in M and G treated groups and at the half, one, one and a half and 2 hrs in the GLC treated group (D+M:110, D+G:107, D+GLC:145, 128, 109 and 90 mg dL^{-1} $p<0.05$). There was no statistically significant difference in BGL at all time points when groups treated with the

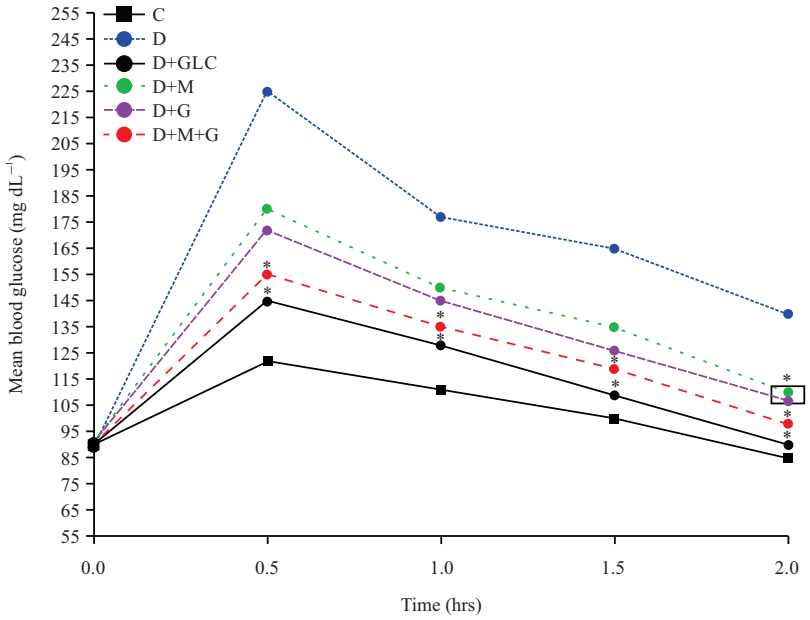

Fig. 4: Oral glucose tolerance test in all groups after 8 weeks

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 a combination of melatonin and garlic, *p<0.05 compared to control diabetic mice

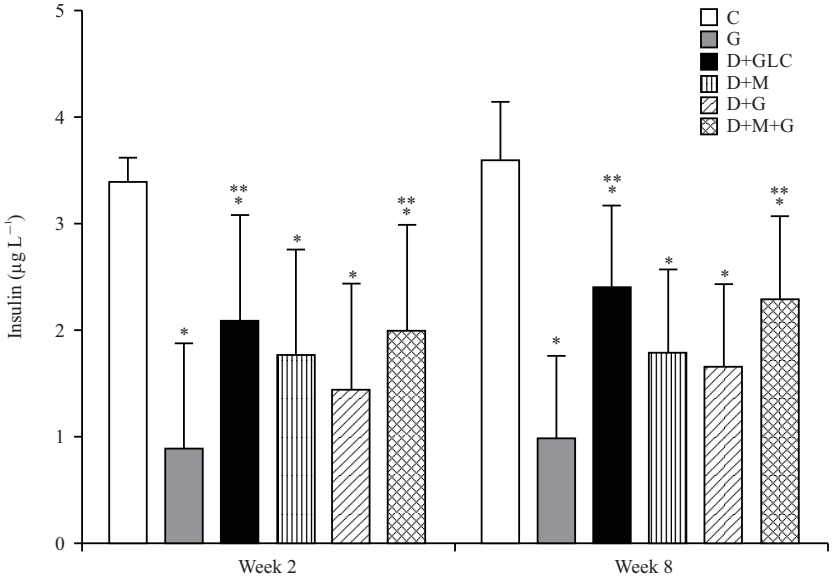

Fig. 5: Plasma insulin measurements for all groups at weeks 2 and 8

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 the combination of melatonin and garlic, *p<0.05 compared to normal control mice, **p<0.05 compared to control diabetic mice and X-axis: Weeks

combination of M+G compared to the GLC-treated group (D+M+G: 155, 135, 119 and 98 mg dL^{-1}, D+GLG: 145, 128, 109 and 90 mg dL^{-1} $p>0.05$).

Effects of melatonin and garlic on blood insulin levels in diabetic mice: 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^{-1}) compared to normal control rats (3.6 ± 0.13 ng mL^{-1}). M and G treatment individually resulted in higher serum insulin with increases of 18% (1.7 ± 0.2 ng mL^{-1}) and 16.8% (1.5 ± 0.1 ng mL^{-1}), respectively. However, these effects were not significant when compared

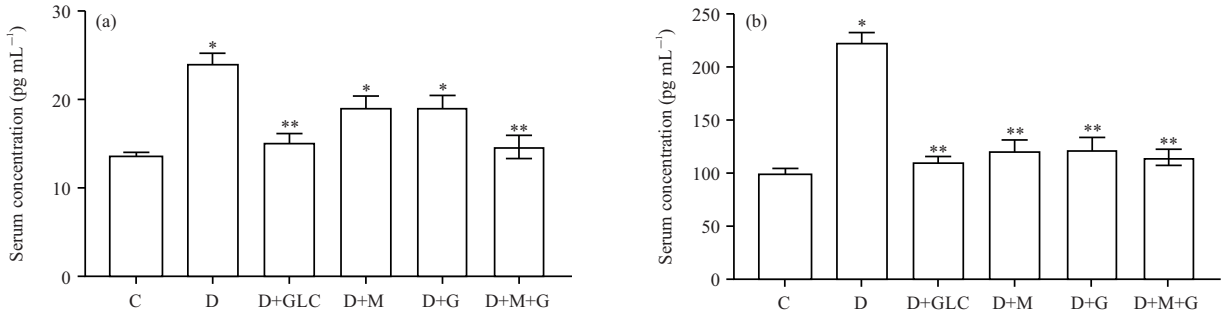

Fig. 6(a-b): Effect of treatments on serum proinflammatory cytokines, (a) Level of IL-6 and (b) Level of TNF-α in the blood serum of mice

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 the combination of melatonin and garlic, *p<0.05 compared to normal control mice, **p<0.05 compared to control diabetic mice and the x-axis of both parts represents groups

Table 1: Effect of treatments on lipid profile in diabetic mice

Groups (n = 8)	TG (mg dL^{-1}), Mean±SD	TC (mg dL^{-1}), Mean±SD	VLDL (mg dL^{-1}), Mean±SD
Control	56.91±4.23	69.45±1.34	12.2±0.32
Diabetic	190.00±1.45*	181.00±2.81*	35.23±0.23*
Diabetic treated with glibenclamide 5 (mg kg^{-1})	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$^{\#}$	83.00±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^{-1}. TC: Total cholesterol, TG: Triglyceride, VLDL: Very-Low-Density Lipoproteins, SD: Standard deviation

to a diabetic control group (p>0.05). While, administration of GLC to diabetic mice caused a significant increase in serum insulin (2.2±0.13 ng mL^{-1}, 25.3%) compared to diabetic control mice (p<0.05). An effect that was comparable to that of the combined M+G group (2.1±0.11, 21.2% p>0.05). Insulin levels were slightly elevated after 8 weeks of 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 and 2.2±0.12, respectively, p>0.05).

Effects of melatonin and garlic on serum proinflammatory cytokines in diabetic mice: The data in Fig. 6a showed that STZ caused a 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 a non-significant decrease in IL-6 (19.1±0.45 and 19.3±0.34, respectively) compared to the diabetic control group (p>0.05, Fig. 6a). However, their combination caused significant amelioration in IL-6. This effect was comparable to that of the normal control group as well as the GLC group (15.1±0.25, p>0.05, Fig. 6a). Concerning serum level of TNF-α, there was a significantly higher level in the 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 a significant reduction in serum level of TNF-α compared to the diabetic untreated group: GLC, M, G and M+G combination (110.1±0.14, 122.1±0.19, 123.4±0.17 and 115±0.23, respectively, p<0.05, Fig. 6b).

Effects of melatonin and garlic on lipid profile in diabetic mice: The 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^{-1}), 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).

DISCUSSION

In this research, we examined low doses of melatonin and garlic extract both individually and combined on glycemic and lipid indices of NA/STZ diabetic mice and compared their effects with glibenclamide, a standard antidiabetic drug. Our results indicated 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 of them as adjuncts to the traditional diabetes drugs to reduce their doses.

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 many bad side effects and can help in decreasing the severity of diabetic complications.

In our experiment, treatment of diabetic mice with melatonin and garlic individually for 8 weeks showed an insignificant reduction in FBG levels. However, the combination of both resulted in a 37.2% reduction in FBG levels compared to the 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 levels at all time points when groups were treated with the combination of melatonin & garlic compared to the glibenclamide-treated group. These results were associated with a 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[32-34]. A previous experimental study indicated that animals that received a pinealectomy exhibited impaired glucose tolerance, insulin resistance and diabetes, which were improved by treatment with melatonin[35]. There is evidence from another study that stated that using melatonin therapy showed an improvement in the glycemic levels and insulin levels in the white adipose tissues of mice with diabetes[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 types of 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 levels, 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 coincided with our results that proved an increase in insulin levels by the administration of both melatonin and garlic to mice with diabetes. However, each drug alone caused insignificant effects in most of the 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 diabetic control mice. This effect was similar to previous research[24,42,43] which indicated that hyperglycemia induced by STZ caused the loss of a 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 a 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 of them.

In the present study, NA/STZ caused a 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 signalling with impairment of blood glucose[47]. Similar to glibenclamide, the combination of melatonin and garlic in the current experiment resulted in a significant reduction in serum levels of IL-6 and TNF-α. These effects were following previous research that proved the ameliorating action of melatonin and garlic on inflammatory cytokines[34,38]. Herein, NA/STZ caused a 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]. On the other hand, when using garlic daily for patients with type 2 diabetes they showed no effect on their cholesterol level, however, their triglycerides showed a better improvement but were not significant[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 as a result 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 produces more reactive oxygen species[54]. Taken together with 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 through amelioration of inflammatory cytokines.

CONCLUSION

In conclusion, the present data demonstrated that the combined low doses of melatonin and garlic promoted advantageous effects in ameliorating blood sugar and lipid profiles 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.

SIGNIFICANCE STATEMENT

This study discovers the combination of garlic and melatonin in low doses can be beneficial for diabetic rats. This study will help the researcher to uncover the critical areas of combining garlic and melatonin that many researchers were not able to explore. Thus a new theory on these drug combinations may be arrived at lowering blood sugars and lipid profiles.

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