Original Research Article

Oxidative effects in streptozotocin-induced male and female mice: the effect of garlic oil and melatonin

Abstract

Background

Recent studies have revealed that a hyperglycemia-induced overproduction of superoxide can be the first event in the activation of all pathways involved in the pathogenesis of complications of diabetes. Supplementation of garlic was found to decrease diabetes-induced oxidative stress complications. Studies shown also that melatonin attenuates diabetes- induced oxidative stress in diabetic induced rabbits and rats**.**

Objective

In this present study, oxidative stress in diabetic model and the effect of garlic oil or melatonin treatment were examined in both genders' male and females' mice.

Methods

96 mice were randomly divided into 4 groups including control (C), diabetic (D), 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 16 weeks after induction of hyperglycemia by streptozocin (STZ). Fasting blood glucose and antioxidant levels were estimated.

Results

Streptozotocin induced diabetic mice, showed a significant increase of plasma glucose, lipid peroxide and uric acid. Accordingly, significant decreases in the levels of antioxidants ceruloplasmin were found in the plasma of diabetic mice. Treatment of diabetic mice with garlic oil or melatonin for 16 weeks significantly increased plasma levels of ceruloplasmin activities. Lipid peroxides, uric acid, blood glucose was decreased significantly after treatment with garlic oil or melatonin.

Conclusion

The results suggest that garlic oil or melatonin may effectively normalize the impaired antioxidants status in streptozotocin induced diabetes in both males and females mice.

Keywords: Diabetes, Garlic, Melatonin, STZ, mice, antioxidants.

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Introduction

Nowadays, researches are focused on the relation of antioxidants activity and diabetes [1].The oxygen species like hydrogen peroxide, superoxide anions, singlet oxygen and hydroxyl radicals could be developed through ionizing radiation and aerobic metabolism of either endogenous or exogenous materials [2]. Evidence have shown that diabetes has been related to an increased free radical production [3]. The mechanisms of this state could be contributed to the free radical development in diabetes that can increase the non-enzymatic and auto-oxidative glycosylation, and moreover metabolic stress due to differences in the metabolism of energy, inflammatory processes, and antioxidant activation [4].

A lot of focus has been developed in using herbal medicine and other natural products in treating diseases that have a state of oxidative stress like diabetes and metabolic syndrome [5]. Garlic is one of the elements that were used as herbal therapy for long time and have been seen to help in eliminating cardiovascular risks and diabetes [6]. Moreover, melatonin was found to have a great antioxidant ability towards diseases [7].

To our knowledge, no previous researches if any used the combination of garlic and melatonin in diabetic subjects to measure antioxidants activity. Therefore, due to the frequent use of antidiabetic drugs with their associated side effects, we hypothesized that combination of low doses of garlic and melatonin might have beneficial effects on glycemic and antioxidants in diabetes mellitus. Also, this combination can be used with traditional antidiabetic drugs to reduce their doses and potential side effects. The aim of this study was to investigate the effects of low doses of melatonin and garlic

extract both individually and combined on blood glucose levels as well as antioxidants in streptozotocin induced diabetic male and female mice.

Materials and methods

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 cultivation, the microorganism was cultivated in a medium containing 3% (wt/vol) malt extract for 36 hours 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, once per day for 8 weeks after induction of diabetes [8].

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Melatonin

Melatonin from (Sigma-Aldrich Co., St. Louis, MO) 10 mg/kg daily dissolved in 0.04% ethanol added to the drinking water at night [9].

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-

2021-10-788). For this study total number of 96 males and females 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-h to 12-h light to dark cycle. Mice were individually housed in standard cages with ad libitum water and standard chow (CRM pellets, SDS diets, U.K.).

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Measurements then started at the age of 10 weeks and were taken over a period of 16 weeks. After 16 weeks mice were fasted overnight and euthanized by CO2 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 STZ (Thomson et al., 2016). Mice were intraperitoneally (i.p.) injected with STZ (50 mg/kg of BW) in 0.1 M citrate buffer (pH 4.2) on two consecutive days. NA (120 mg/kg of BW) in saline was i.p. injected 30 min before the STZ injection on the first day after overnight fasting. Seven days after the second i.p. injection, mice that exhibited an 8-h fasting blood glucose (FBG) level of 200 mg/dL were recognized as being hyperglycemic. The others that exhibited an FBG level of <200 mg/dL were injected with STZ and monitored until the FBG level reached 200 mg/dL. FBG was monitored with a glucometer (Dragon Pharmaceutical Co, New Taipei, Taiwan).

Experimental design

Mice were randomly classified into 4 groups (n=16 each); one group served as a control non-diabetic group and 3 groups were injected with STZ to induce diabetes, then they received their respective drug treatments daily for 16 consecutive weeks according to the following design (Figure 1):

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• 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+M); induced diabetic mice treated with melatonin (10 mg/kg/day in drinking water)

• Group 4 (D+G); induced diabetic mice treated with prepared garlic extract (100 mg/kg/day in drinking water)

The doses of melatonin and garlic were chosen based on previous experimental studies that examined 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.

At 2nd and 16th weeks, the levels of FBG were examined. After 16 weeks antioxidants were examined.

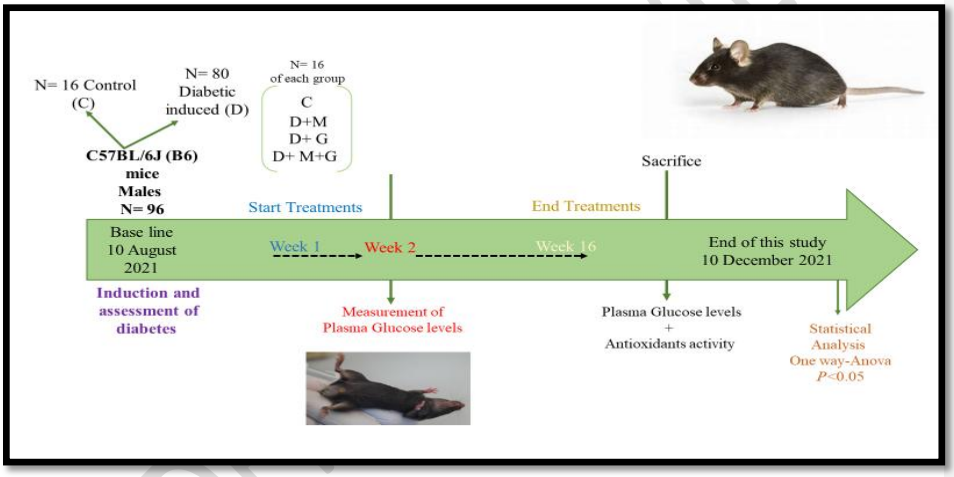

Figure 1: Experimental design of the study

Assessment

Fasting blood glucose measurements

At week 2 and 16 all mice were fasted overnight and blood were taken from their tails in order 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 & Johnson, Milpitas, CA).

Antioxidant's measurements

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Lipid peroxide levels were measured in plasma, hemolysate and tissue homogenates as thiobarbituric acid reactivity (TBARS). The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described by Thayer. Ceruloplasmin activity was determined using a para-phenylenediamine dihydrochloride method. Uric acid was determined by enzymatic colorimetric method using commercial kit (Biocon, BurbachyGermany) [10].

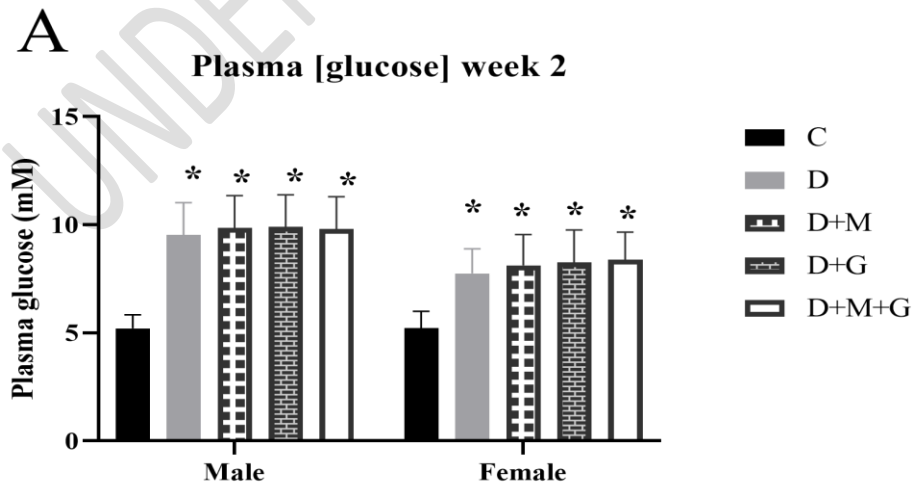
Statistical Analysis

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 *P* value of less than 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 Fasting Blood Glucose in Diabetic Mice

Figure 2 describes the effect of garlic, melatonin and their combination on fasting blood glucose on both male and female mice. It is significant that after 16 week there were a vast improvement in all treatments in decreasing blood glucose levels in diabetic mice (P<0.005).



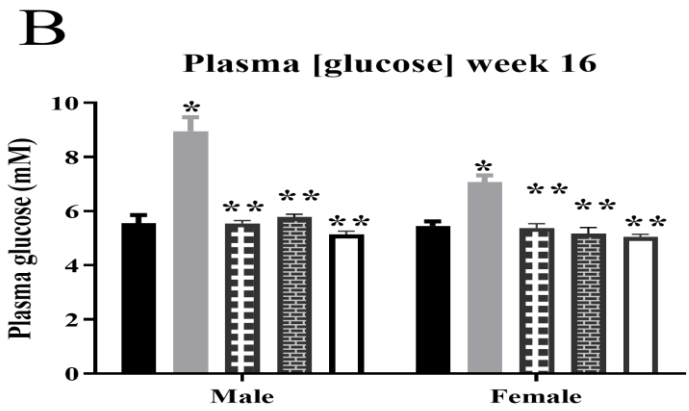


Figure 2: A) Plasma Glucose measurements in week 2 of the study. B) Plasma Glucose measurements in week 16 of the study C; control, D; diabetic induced mice, D+M; diabetic mice treated with melatonin, D+G; diabetic mice treated with garlic, D+M+G; diabetic mice treated with combination of melatonin and garlic.

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

Effects of Melatonin and Garlic on antioxidant levels in Diabetic Mice

Figure 3 describes the effect of garlic, melatonin and their combination on antioxidants levels on both male and female mice. It is significant that after 16 weeks of treatments there was an increased plasma level of ceruloplasmin activities. Lipid peroxides and uric acid were decreased significantly after treatment with garlic oil or melatonin or their combinations (P<0.005).

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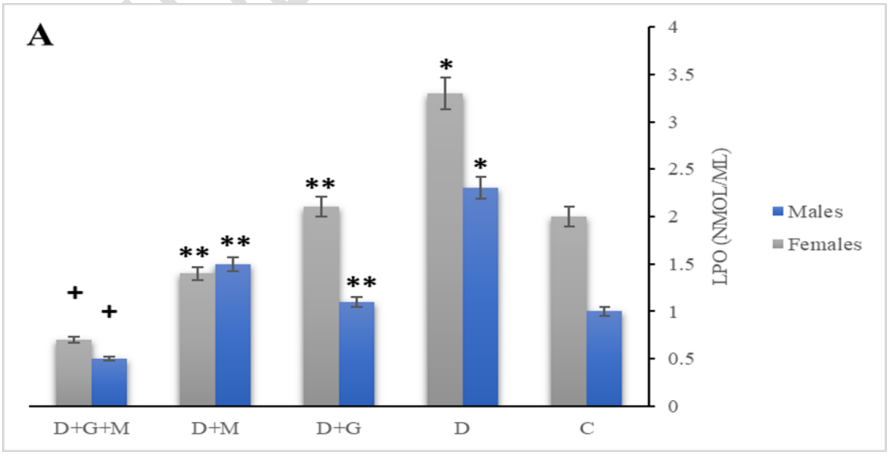

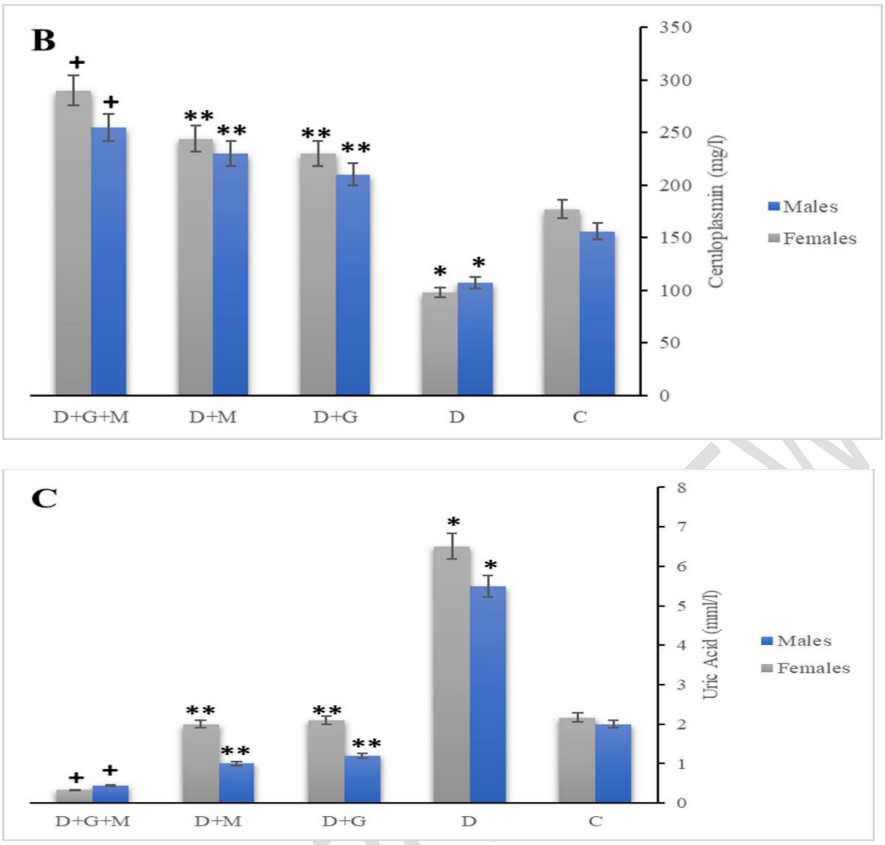

Figure 3: A) Levels of lipids peroxidase; B) Ceruloplasmin and C) Uric acids after 16 weeks of treatments .

*P<0.05 compared to control mice, **P<0.05 compared to diabetic mice, +P<0.001 compared to D+M+G.

Discussion

Diabetes can cause multiple changes systemically and also upon the cellular levels [10, 11]. In our present study, diabetic mice both genders induced by streptozotocin showed the expected raise in their plasma glucose levels and the decrease in antioxidant levels. However, treatment of diabetic mice with garlic oil or melatonin for 16 weeks significantly increased plasma levels of ceruloplasmin activities. Lipid peroxides, uric acid, blood glucose was decreased significantly after treatment with garlic oil or melatonin. Our results were in agreement of other researchers whom used garlic or melatonin alone, they showed a lower levels of blood glucoses and other

parameters as cholesterol and lipid profile [11,12,13,14]. Researches tried to explain the possible mechanism of this blood glucose lowering, for garlic they approved multiple pathways of anti-inflammatory and antihyperlipidemic and antioxidant effects. On the other hand, for the melatonin they believed it works on brain site and can achieves this action through direct detoxification of reactive oxygen and reactive nitrogen species and indirectly by stimulating antioxidant enzymes at the same time inhibiting the activity of enzymes as prooxidant's. Moreover, melatonin shown to chelates transition metals, that have an involvement in the Fenton/Haber–Weiss reactions; therefore, melatonin can decrease toxic hydroxyl radical that results in the inhibition of oxidative stress. However, more investigation for a clearer explanation is in need [15]. Evidence showed also that when given melatonin to rats taking cytotoxic drugs that there state of antioxidants were improved [16,17,18, 19].

From the benefits shown in our study of using the combination of garlic and melatonin together in diabetic subjects, we recommend starting a trail with diabetic patients using the combination of melatonin and garlic to investigate in depth their beneficial effect.

Conclusion

Our results from this present study suggest that garlic oil or melatonin may effectively normalize the impaired antioxidants status in streptozotocin induced diabetes in both males and females mice. The effects of these antioxidants of both agents may be useful in delaying the complications of diabetes such as retinopathy, nephropathy and neuropathy due to imbalance between free radicals and antioxidant systems.

Data Availability

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

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-012-2021-10-788).

The study highlights the efficacy of " Herbal " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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