Impact of naringenin on the inhibition of protein glycation in diabetic rats as a protection mechanism against nephropathy

Taha A. Kumosani,[1,2,3] Abeer Alnefayee,[1] Elie Barbour,[2,3] Mohammed Qari,[4] Soonham S. Yaghmoor,[2,3] Tarek Ahmed,[5,6] Turkyah J. Albogami,[1] Said S. Moselhy[7]

[1]Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia; [2]Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia; [3]Production of Bio-products for Industrial Application Research Group, King Abdulaziz University, Jeddah, Saudi Arabia; [4]Hematology Department, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia; [5]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia; [6]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt; [7]Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

Abstract

Protein glycation is one of the most serious issues in diabetes playing a critical role in the development of many cellular dysfunction as aging, cardiovascular diseases and neural disorders. This study investigated the impact of naringenin on the glycation rate of glomerular basement membrane protein in diabetic rats to prevent nephropathy as a complication of diabetes. Fifty male Wistar rats were sorted into 2 main groups, Gr I (normal; n=10) and Gr II (Diabetic, n=40). Diabetes was induced in rats by injection of a single dose of 60 mg/kg, *i.p.* streptozocin (STZ). Diabetic rats were allocated to Gr IIa: (not treated), Gr IIb: treated with naringenin (50 mg/kg body weight - bw), Gr IIc: Diabetic treated with naringenin (100 mg/kg bw), Gr IId: Diabetic treated with metformin (100 mg/kg bw). Serum was utilized for the determination of malondialdehyde (MDA), glycated hemoglobin (HA1c), fructosamine, and total antioxidant activity. Kidney tissue was utilized for the determination of oxidative stress and Advanced Glycated End products (AGEs). Obtained data showed that naringenin supplementation in diabetic rats reduced the levels of serum MDA (p=0.0111), HA1c (p=0.0112), fructosamine (p=0.011), and improved antioxidant capacity (p=0.032). In addition, it reduced the level of AGEs (p=0.0008), and enhanced antioxidant enzymes (p=0.01134) compared with untreated. Diabetic rats showed a significant elevation in inflammatory markers tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), AGEs and decreased reduced glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) activity in kidney tissue versus control. The effect of naringenin was dose dependent, and improved these alterations versus untreated (p=0.0111). It was concluded that the inhibition of the formation of AGEs by naringenin in diabetics contributed to the protection against complications. Therefore, naringenin and its mechanisms of action is promising for the development of safety and effective new antidiabetic agent.

Correspondence: Said Moselhy, Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt.
E-mail: Moselhy6@hotmail.com

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Ethics approval: the Ethics Committee of King Abdulaziz University approved this study (KAU-SCi-Bioc-7/140/1443). The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights.

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Availability of data and materials: all data generated or analyzed during this study are included in this published article.

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Introduction

High blood glucose in diabetic patients increased rate of protein glycation in different tissues to form nonfunctional Advanced Glycated end Products (AGEs) that affect tissue functions.[1] Protein glycation begins with binding of glucose or fructose or their derivatives to tissue proteins. AGEs can lead to nephropathy, neuropathy, retinopathy and Cardiovascular Diseases (CVD).[2]

These glycated proteins cannot be turned over to be utilized again. Diabetic complications can be overcome and abrogated by agents having higher affinity to blood glucose than tissue protein. Thus, they can avoid retinopathy, micro and macrovascular implications.[3] Aldose reductase contributes to the formation of sorbitol that leads to a hyperosmotic effect, resulting in degeneration and cataract formation.[4] Long term consumption of functional foods rich in bioactive molecules, such as polyphenols, can attenuate

glycation mechanism improving health status. These biomolecules have the ability to bind with blood glucose with higher affinity than tissue proteins.[5] Therefore, most researchers are looking for the development of novel molecules with lowest side effects to inhibit protein glycation.

Naringenin (4′,5,7-trihydroxyflavonone) is the active ingredient found in citrus fruits[6] and grapefruit.[7] Naringenin, found as solid in nature, can be easily dissolved in organic solvents.[8-9] Due to its low water solubility, its bioavailability is low.[10] Naringenin showed different biological activities such as antidiabetic, anticancer, antimicrobial, anti-obesity, nephron-protective, and neuroprotective due to its oxygen scavenger and analgesic effect.[11,12] Complementary or alternative therapy are used to treat or prevent diseases without side effects. This increases the interest in dietary bioactive components that prevent or reduce the severity of chronic diseases.[13] The rationale of the current study was to investigate the protecting effect of naringenin as nephron-protective agent in diabetic rats. The health-promoting effect of naringenin suggested that it exerts antidiabetic effects. The target of the current study was to investigate the impact of naringenin on the protein glycation in kidney in order to prevent nephropathy.

Materials and Methods

Handling of animals was done according to ethical committee of King Abdulaziz University, Jeddah, Saudi Arabia under approval # (KAU-SCi-Bioc-7/140/1443). Naringenin was purchased from Merck , product number (W530098, purity 98%), and was dissolved in Dimethyl Sulfoxide (DMSO).

Animals

Nine weeks-old (120±20 g) male Wistar rats (n=50) were obtained from animal house, King Abdulaziz University (KAU). Ten rats were considered as control (n=10). Diabetes was induced in forty rats by injecting a single dose of streptozocin (STZ) (60 mg/kg, *i.p*). After 3 days, if blood sugar resulted ≥ 250 mg/dL , they were considered as diabetics. Diabetic rats were sub-grouped into four groups: untreated, treated with either naringenin (50 or 100 mg/kg body weight - bw) or metformin (100 mg/kg bw) for 8 weeks. At the end of experiment, animals were fasted overnight and anesthetized by using thiopental. Blood was collected directly from the heart. Serum was separated after centrifugation at 5,000 rpm for 10 minutes, and was used for the determination of fasting

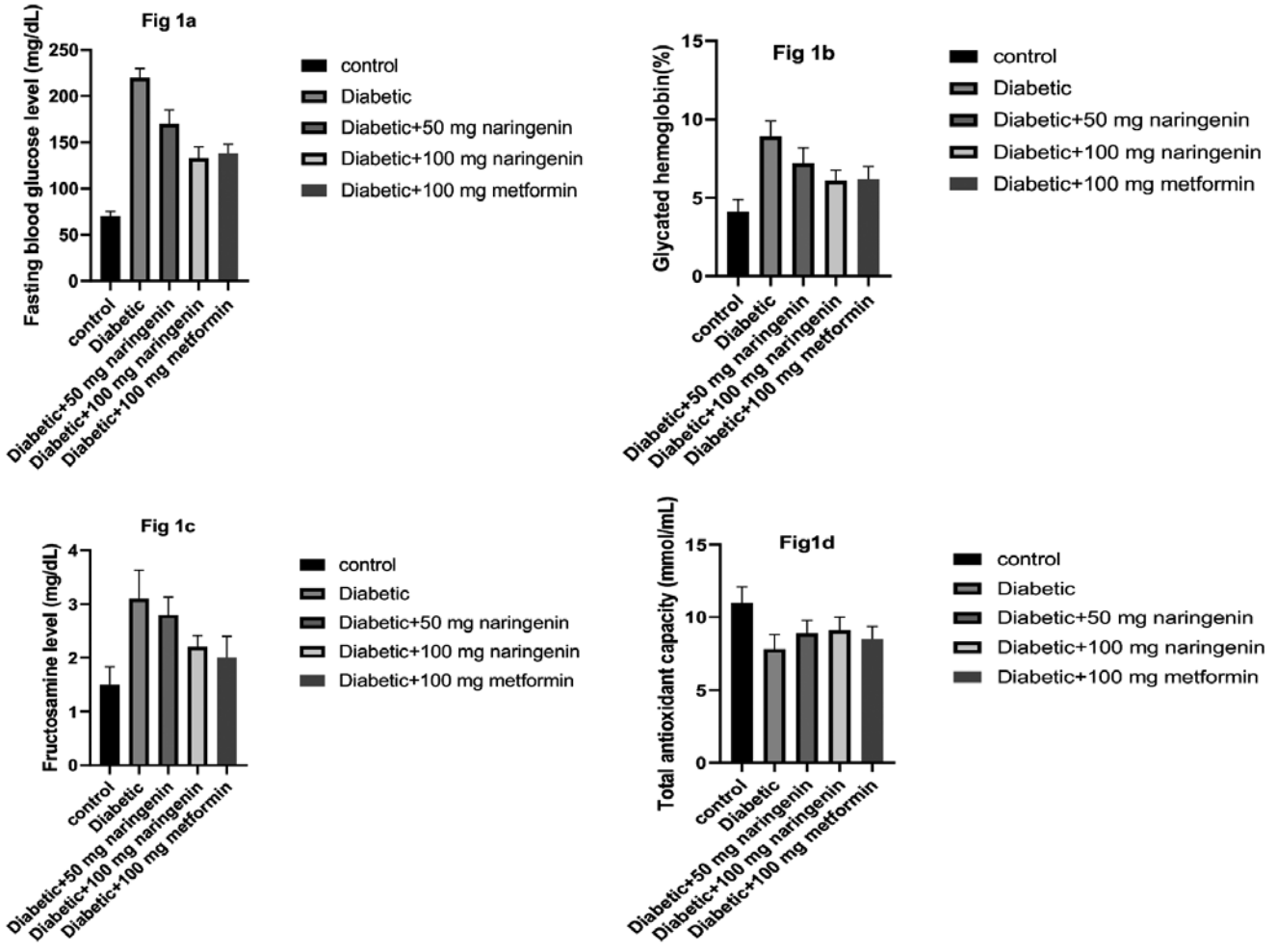

Figure 1. a) Fasting blood glucose levels in all groups; b) Glycated hemoglobin (%) in all studied groups; c) Serum frusctosamine level in all studied groups; d) Serum total antioxidant capacity in all studied groups.

glucose, glycated hemoglobin (HA1c), and fructosamine by kits from My BioSource, Southern California, San Diego (USA). Kidney tissue was used for the determination of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase assays using available commercial kits (Biodiagnostic, Jeddah, Saudi Arabia). The levels of interleukin-6 (IL-6), Tumor Necrosis Factor α (TNF-α), and advanced glycated end products (AGEs) were determined by enzyme linked immunoassay (ELISA) using the commercially available kits (Biodiagnostic, Jeddah, Saudi Arabia), according to the instructions of the manufacturer.

Statistical analysis

Statistical analysis of data was done using SPSS version 18. Data are expressed as mean ± Standard Deviation (SD). Data were analyzed utilizing Analysis of Variance (ANOVA) and comparisons were performed employing the t test. A p value < 0.05 was considered as significant.

Results

Results in Figure 1a, b, and c show that rats injected with a single dose of STZ showed a significant elevation in the levels of fasting blood glucose, HA1c, and fructosamine compared with control (p= 0.0135, 0.0112, 0.011), respectively. However, diabetic rats treated with naringenin at doses of 50 or 100 mg/kg bw showed a dose-dependent reduction in the levels of fasting blood glucose, HA1c and fructosamine compared with untreated rats. The effect of naringenin showed no differences with metformin as positive control. Serum total antioxidant (Figure 1d) activity was significantly reduced in diabetic rats compared with control (p=0.032). However, the activity was restored with treatment with naringenin at doses 50 and 100 mg/kg bw, showing a dose-dependent reduction in the levels of MDA compared with untreated. The oxidative stress markers showed that the levels of serum and kidney MDA (Figure 2a, 2b) were significantly elevated in diabetic rats versus control (p=0.0111 and p=0.0013) respectively. Treatment with naringenin at doses of 50 or 100 mg/kg bw showed a dose-dependent reduction in the levels of MDA compared with untreated. A significant reduction in the level of GSH (p=0.0009) and in the activities of catalase (p=0.011) and SOD (p=0.0134) (Figure 2c, 2d, 3a) was observed in diabetic rats compared with control. Treatment with naringenin at doses of 50 or 100 mg/kg bw showed an increase in the level of GSH and in the activities of catalase and SOD versus diabetics. The impact of naringenin is better than that of metformin. The inflammatory markers (TNF-α, IL-6) (Figure 3b, 3c) were significantly elevated in diabetic rats versus control (p=0.0001). On the other hand, treatment with naringenin at doses 50 or 100 mg/kg bw showed a dose-dependent reduction in the levels of TNF-α and IL-6 compared with untreated. AGEs (Figure 3d) were significantly elevated in diabetic rats versus control (p=0.0008). Treatment with naringenin at doses of 50 or 100 mg/kg bw showed a dose-dependent reduction in the level of AGEs compared with untreated.

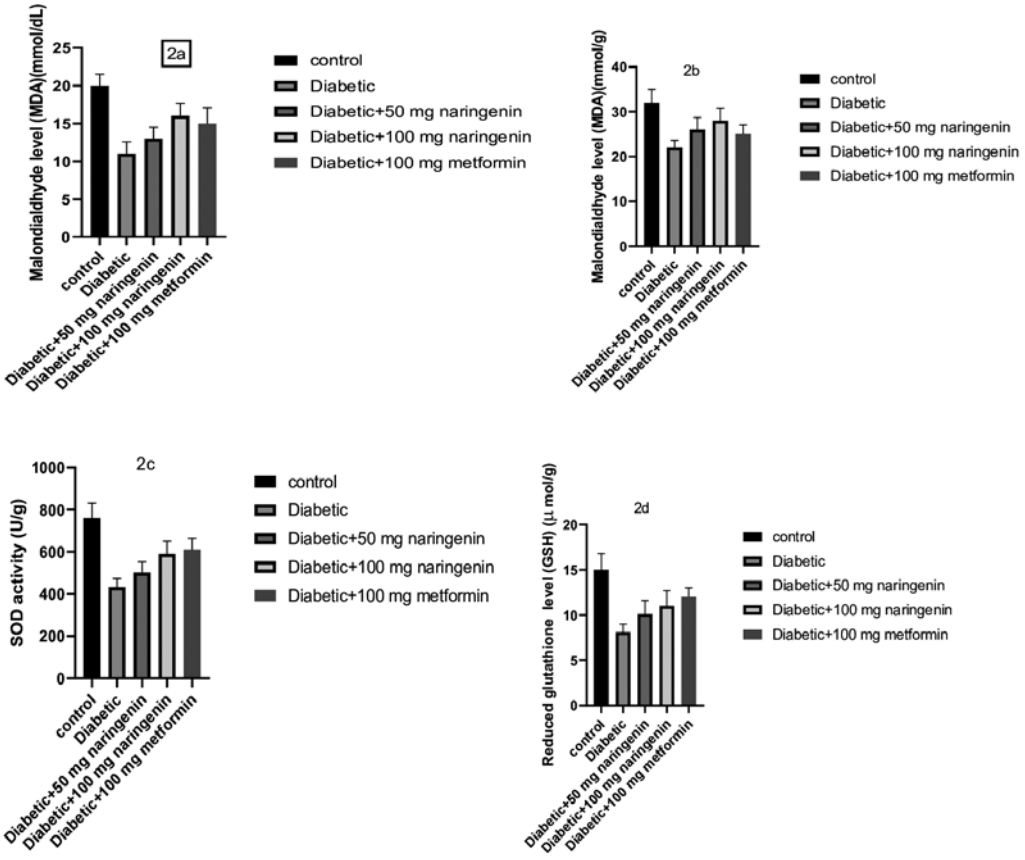

Figure 2. a) Serum malondlaldhyde (MDA) level in all studied groups; b) Kidney level of malondlaldhyde (MDA) in all studied groups; c) Kidney superoxide (SOD) activity in all studied groups; d) Kidney reduced glutathione (GSH) level in all studied groups.

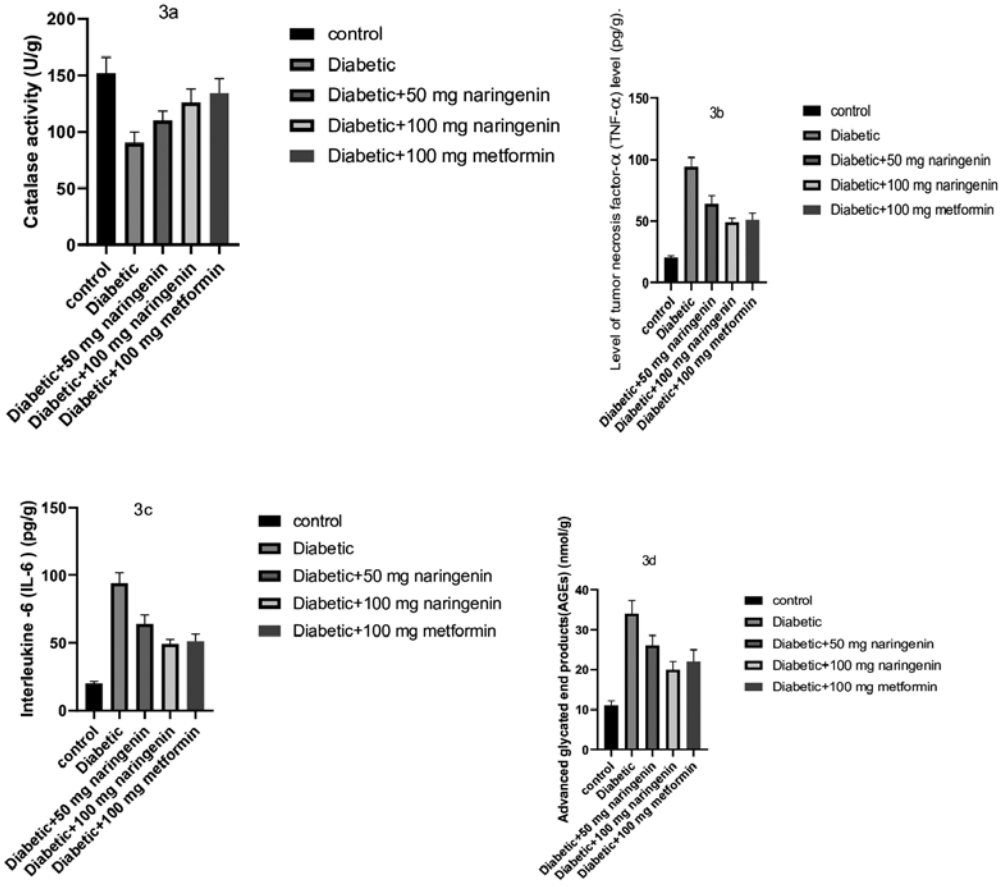

Figure 3. a) Kidney Catalase activity in all studied groups; b) Kidney level of tumor necrosis factor-α (TNF-α)in all studied groups; c) Kidney Interleukin-6 (IL-6) level in all studied groups; d) Kidney advanced glycated end products (AGEs) level in all studied groups.

Discussion

The management of diabetes is a critical economic burden, due to the need for glycemic regulation. Uncontrolled diabetes causes serious complications and affects many organs leading to disability and increased mortality rate. Preventing or avoiding diabetic complications requires different protocols, such as lifestyle, pharmaceutical intervention, and natural supplements with functional foods.[14] In the current study, STZ injection in rats caused an elevation in fasting blood glucose, HA1c and fructosamine levels compared with control. It was found that STZ affects insulin production and secretion by pancreatic β-cells[15]. Its toxicity may be due to oxygen radical production or the release of inflammatory mediators. However, the treatment with naringenin showed a significant reduction in fasting blood glucose, HA1c and fructosamine levels compared with untreated. This result is in accordance with a previous study that showed a positive effect of naringenin on glucose level management as pancreatic function, insulin secretion, and glucose uptake in peripheral tissues, via expression of glucose transporter.[16] Another study found that diabetic rats treated with naringenin showed pancreatic β-cell recovery and improved glucose metabolism[17]. The Total Antioxidant Capacity (TAC) refers to the amount of endogenous antioxidants in a biological sample. In this study, TAC was reduced significantly in diabetic rats and restored when rats were treated with naringenin (50 or 100 mg/kg bw). The antioxidant property of naringenin is attributed to its structure with high hydroxyl group and carbonyl group.[18] The antioxidant potential of naringenin contributed in oxygen scavenging and removing free radicals. In the current study, serum and kidney MDA levels were significantly increased in diabetic rats versus control. In addition, the level of GSH and the activities of catalase and SOD were decreased in diabetic rats versus control. SOD is one of the antioxidant enzymes involved in the elimination of Reactive Oxygen Species (ROS). It is responsible for the conversion of the superoxide anion (O^{2-}) into hydrogen peroxide which is finally converted to water by catalase.[19] GSH is an important substrate as hydrogen donor for converting H_2O_2 to water. Supplementation of naringenin was found to enhance the antioxidant enzymes through increased GSH and the activities of catalase and SOD compared with untreated in a dose-dependent manner. A previous study reported that in animal model of diabetes, combining naringenin treatment with an antihypertensive drug, biochemical aligning was improved, with the reduction of oxidative stress and renal damage.[20] In the current study, naringenin treatment in diabetic animals was found to reduce lipid peroxidation and enhance SOD and catalase activities. Naringenin plays a role in protection from diabetic complications; also, it decreased apoptosis and the expression of Tumor Growth Factor β (TGF-β) and Interleukin β (IL-1β).[14] It was reported that naringenin exerts its antioxidant effects directly as a reactive oxygen scavenger,[21] and indirectly via the inhibition of the enzymes that produce ROS, such as NADPH oxidase.[22] It also attenuates the antioxidant enzymes

expression as SOD, and CAT.[23] Naringenin mediated its action via immunomodulatory effects.[24] These effects were observed on TNF-α, CD68, and IL-1β, and in the regulation of phosphorylation mechanism related to inflammation.[25] Naringenin's anti-inflammatory properties might offer therapeutic potential by mitigating oxidative stress.[26] The current study showed that the levels of kidney inflammatory markers such as IL-6 and TNF-α were significantly elevated in diabetic rats versus control. However, diabetic rats treated with naringenin (50 or 100 mg/kg bw) reduced significantly the levels of IL-6 and TNF-α versus untreated diabetics in a dose-dependent manner. A previous study found that in diabetic mice naringenin administration reduced hyperglycemia, increasing insulin through anti-inflammatory mechanisms. Inflammation was mitigated through the modulation of TNF-α, IL-1β, and IL-6 expressions in renal tissue.[27] The mitochondrial ROS production subsequently activates abnormal activation of several kinases that are involved in stress responses, which will trigger inflammation and ROS generation.[28] A plethora of evidence suggests that AGEs/Receptor for Advanced Glycation End products (RAGE) signaling pathway, Nuclear factor kappa B (NF-κB) activation, inflammation, and ROS generation are directly related to the pathogenesis of insulin resistance.[29] The results of this study are in accordance with a previous study that reported flavonoids play an important role in reduction of AGEs formation by covering protein glycation sites, chelating metal ions, eliminating free radicals, and reducing blood sugar levels.[30]

Conclusions

Naringenin caused inhibition of the formation of AGEs in diabetics rats, and it may contribute to the protection against macro and micro vascular complications such as nephropathy, neuropathy and cardiovascular diseases. Therefore, it is promising for the development of potent new antidiabetic agent.

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