Signal Transduction of Fucoxanthin Nanoparticles as Anti-Oxidative Stress and Anti-Inflammatory Protecting Aortic Damagein Diabetic Rats

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Keywords: Fucoxanthin nanoparticle; Antioxidant; Anti-inflammatory; Aorta; Streptozotocin; Diabetic rat

Article

Signal Transduction of Fucoxanthin Nanoparticles as Anti-Oxidative Stress and Anti-Inflammatory Protecting Aortic Damage in Diabetic Rats

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Abstract: The anti-oxidative stress and anti-inflammatory effects of natural products can prevent diabetic complications such as retinopathy, nephropathy, neuropathy, and blood vessel damage. Fucoxanthin has potent antioxidant and anti-inflammatory properties. This research aimed to examine the preventative effects of fucoxanthin nanoparticles against aortic damage in diabetic rats. Dynamic Light Scattering (DLS) was utilized to identify the size of fucoxanthin nanoparticles. The experiment consisted of five groups (n=8) namely: rats only received streptozotocin (STZ) solvent and fucoxanthin nanoparticles solvent as a control group; rats only received STZ and solvent of fucoxanthin nanoparticles solvent as the diabetic group; and rats received STZ and fucoxanthin nanoparticles at a dose of 75, 150 and 300 mg/kg BW as the fucoxanthin nanoparticle group. The fucoxanthin nanoparticle sizes were 217.2 ± 42.8 nm in DLS. The dose-dependent administration of fucoxanthin nanoparticles elevated significant superoxide dismutase (SOD), glutathione peroxidase (GPx), nitric oxide (NO), endothelial nitric oxide synthase (eNOS), and insulin. However, blood glucose, malondialdehyde (MDA), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and endothelial cell necrosis decreased compared to those in the streptozotocin group. Our findings suggest that fucoxanthin nanoparticles decrease malondialdehyde levels and increase superoxide dismutase and glutathione peroxidase levels to inhibit oxidative stress, consequently preventing diabetes-induced aortic damage. Furthermore, fucoxanthin nanoparticles also may inhibit inflammation by reducing IL-6 and TNF-α levels. These mechanisms reduce endothelial cell necrosis, which can increase the expression of eNOS and NO levels in the aorta of diabetic rats.

Keywords: fucoxanthin nanoparticle; antioxidant; anti-inflammatory; aorta; streptozotocin; diabetic rat

1. Introduction

Hyperglycemia is one marker of Diabetes Mellitus (DM) due to the inhibition of the secretion of insulin or function of insulin. This results in several complications, including retinopathy, atherosclerosis, neuropathy, cardiomyopathy, nephropathy and aortic vascular damage [1–3]. The development of diabetic complications is substantially affected by oxidative stress and inflammation [4–6]. Furthermore, oxidative stress and inflammation are closely associated. Hyperglycemia causes oxidative stress, which may lead to inflammation. In addition, it plays an important role in the progress of diabetic complications, such as aortic vascular injury [7–9]. Oxidative stress is a state in which the body is unable to counteract or repair cell damage brought on by free radicals due to an increase in ROS production and a decrease in antioxidants. Free radicals produced during oxidative

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stress can damage tissues and trigger the release of pro-inflammatory cytokines such as IL-6 and TNF-α.[10,11]. Increased ROS can be caused by hyperglycemia, which activates enzymes such as NADPH oxidase and protein kinase C, causing mitochondrial dysfunction and increasing the formation of advanced glycation end products (AGEs), which can damage cells and tissues, increasing oxidative stress and lead to cardiovascular injury [12,13]. Hyperglycemia also induces the reduction of nuclear factor erythroid 2- related factor 2 (Nrf2), which is linked to inhibiting antioxidant enzymes such as SOD, GPx, and Catalase formation [14–16].

ROS influence processes like cell development, differentiation, and apoptosis (planned cell death) by acting as signaling molecules in a variety of biological pathways. However, the overproduction of ROS is a sign of oxidative stress-reduced levels of NO and eNOS in endothelial cells indicating weakened aortic function in rats . Excess ROS may damage the lipids, proteins, and deoxyribonucleic acid (DNA) of aortic cells in pathological circumstances such as diabetes. Lipid peroxidation may result in the generation of malondialdehyde (MDA) due to the oxidation of polyunsaturated fatty acids (PUFAs) in cellular membranes, which are particularly susceptible to ROS-induced damage. Thus, MDA may be a biomarker for increased ROS generation and free radical-induced aortic cell damage [17–19]. Therefore, controlling and preventing vascular damage in diabetes requires drugs with antioxidant and anti-inflammatory effects [20–22].

Fucoxanthin, an aquatic carotenoid found in various brown algae, exhibits potent anti-inflammatory and antioxidant properties. Its pharmacological activities include anti-inflammatory, anti-cancer, antibacterial, immunostimulant, antidiabetic, anti-atherosclerotic, and antioxidant properties [23–25].

Nanobiotechnology is crucial for creating nanoparticles from natural products. Therefore, natural-product nanoparticles may be used to prevent and cure illnesses in both humans and animals. Natural product-based nanoparticles can markedly improve the stability, absorption, distribution, and therapeutic efficacy of medication [26–28]. Fucoxanthin nanoparticles may increase the solubility, bioavailability, and stability of fucoxanthin, as well as its delivery to target cells. Furthermore, this research examined whether the anti-oxidative stress and anti-inflammatory effect of fucoxanthin nanoparticles could inhibit aortic cell injury in diabetic rats.

2. Results

2.1. Characterization of Fucoxanthin Nanoparticle

Figure 1 shows dynamic light scattering (Figure 1) revealed the particle sizes. The size of fucoxanthin particles was 217.2 ± 42.8 nm.

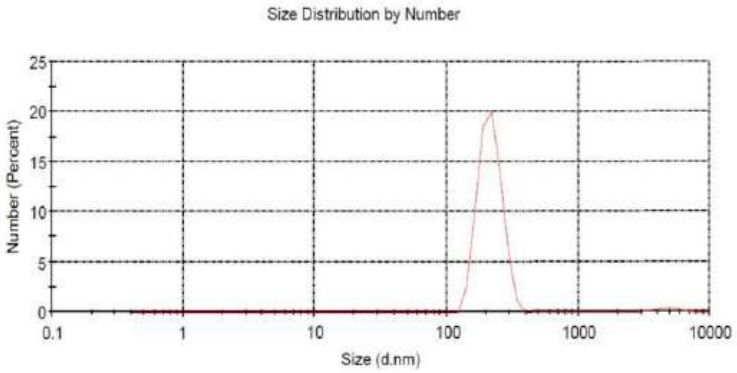

Figure 1. Size distribution of fucoxanthin nanoparticles by Dynamic Light Scattering.

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2.2. Effects of Fucoxanthin Nanoparticles on Serum Insulin and Blood Glucose Levels in Diabetic Rats

STZ administration dose-dependently caused diabetes in rats compared to the control group, as evidenced by an increase in the levels of blood glucose (Figure 2A) and a decrease in levels of insulin (Figure 2B). Only at a dose of 300 mg/kg BW, fucoxanthin nanoparticles showed a significant decrease in blood glucose levels and increased insulin levels compared to the streptozotocin group ($P < 0.05$).

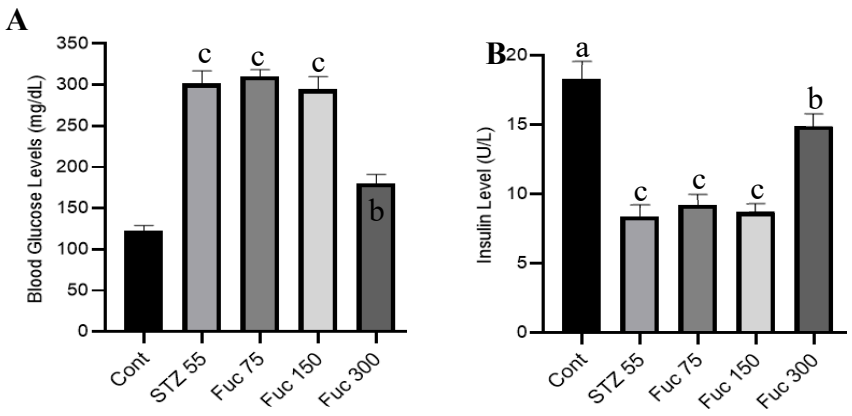

Figure 2. Effect of fucoxanthin nanoparticles on Blood glucose (Figure 2A) and insulin levels (Figure 2B). Cont (control); STZ 55 (streptozotocin with a dose of 55 mg/kg BW); and Fuc 75,150, 300 (fucoxanthin nanoparticle at a dose of 75 mg/kg BW, 150 mg/kg BW, and 300 mg/kg BW). $^{a\text{-}c}$Bar charts that have different letters are statistically different ($p<0.05$).

2.3. Effects of Fucoxanthin Nanoparticles on MDA Levels in Diabetic Rats Aorta Tissue

The MDA levels in aortic tissues are shown in Figure 3. MDA, a lipid peroxidation product, accumulates as an effect of oxidative stress. MDA levels in aortic tissue increased significantly after streptozotocin administration compared to the control group ($p<0.05$). However, fucoxanthin nanoparticle administration was dose-dependent and only a dose of 300 mg/Kg BW could significantly reduce MDA levels compared to the diabetic group ($p<0.05$), which tended to be toward the control group.

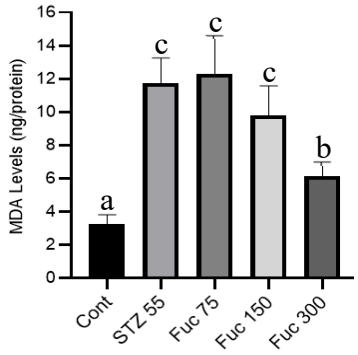

Figure 3. Effect of fucoxanthin nanoparticles on MDA levels in rat aorta tissue. Cont (control); STZ 55 (streptozotocin with a dose of 55 mg/kg BW); and Fuc 75,150, 300 (fucoxanthin nanoparticle with a

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dose of 75, 150, and 300 mg/kg BW). [a-c]Bar charts that have different letters are statistically different ($p<0.05$).

2.4. Effect of Fucoxanthin Nanoparticles on SOD and GPx Levels in Diabetic Rat Aorta Tissue

Antioxidant enzymes, such as SOD, are required to stop ROS production, which causes oxidative cell damage. The levels of SOD significantly decrease after being treated with streptozotocin if compared to the control group ($p<0.05$). However, treatment of fucoxanthin nanoparticles in a dose-dependent manner elevated the levels of SOD if compared with the streptozotocin group, and only at a dose of 300 mg/kg BW, which could significantly increase the levels of SOD in the diabetic rat aorta tissue ($p<0.05$) (Figure 4A). Meanwhile, Figure 4B shows GPx levels in the aortic tissue. Streptozotocin treatment of aorta tissue in a dose-dependent manner reduced the levels of GPx if compared with the control group ($p<0.05$), only at a dose of 300 mg/kg BW of fucoxanthin nanoparticles significantly increased the levels of GPx compared to the streptozotocin group.

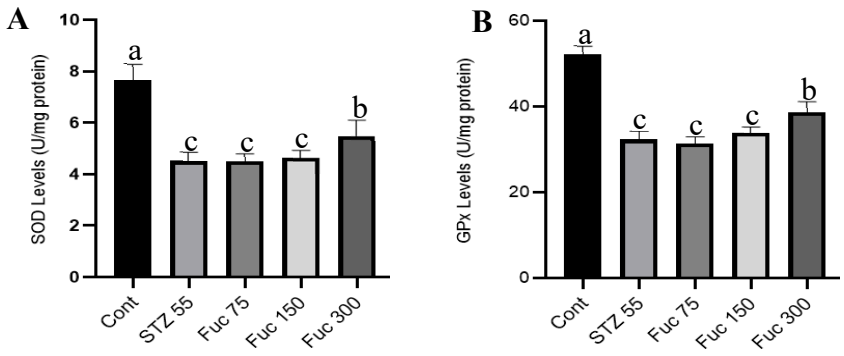

Figure 4. Effect of fucoxanthin nanoparticles on SOD levels (Figure 4A) and GPx levels (Figure 4B) in rat aorta tissue. Cont (control); STZ 55 (streptozotocin with a dose of 55 mg/kg BW); and Fuc 75,150, 300 (fucoxanthin nanoparticle with a dose of 75, 150, and 300 mg/kg BW). [a-c]Bar charts that have different letters are statistically different ($p<0.05$).

2.5. Effect of Fucoxanthin Nanoparticles IL-6 and TN-α Levels in Diabetic Rat Aorta Tissue

Cytokine inflammatory IL-6 and TNF-∝ have important roles in causing aortic cell damage in diabetic rats. As shown in Figure 5A,B, streptozotocin administration significantly elevated the levels of IL-6 and TNF-α if compared with the control group ($p<0.05$). Whereas treatment of fucoxanthin nanoparticles in a dose-dependent manner reduced the levels of IL-6 and TNF-α compared to the streptozotocin group, and only at a dose of 300 mg/kg BW of fucoxanthin nanoparticles could significantly decrease the levels of IL-6 and TNF-∝ if compared with the streptozotocin group ($p<0.05$).

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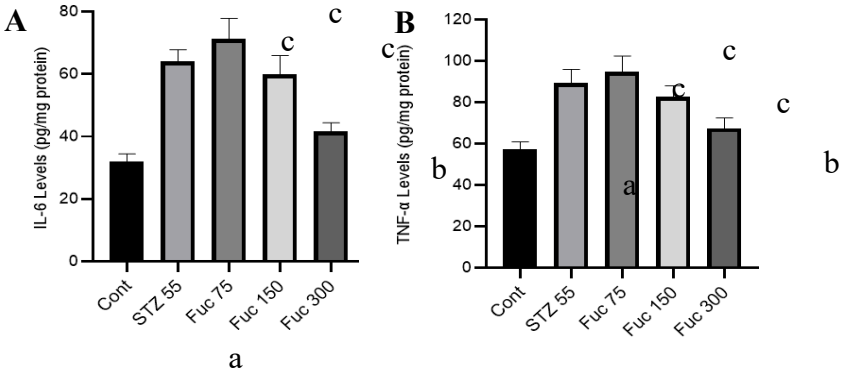

Figure 5. Effects of fucoxanthin nanoparticles on IL-6 (A) and TNF-α (B) levels in rats. Cont (control); STZ 55 (55 mg/kg BW streptozotocin); and Fuc 75,150 and 300 (fucoxanthin nanoparticles at doses of 75, 150, and 300 mg/kg BW). [a-c]Bar charts that have different letters are statistically different ($p < 0.05$).

2.6. Effects of Fucoxanthin Nanoparticles on the Expression of eNOS in Diabetic Rat Aorta Tissue

Endothelial nitric oxide synthase (eNOS) is an enzyme that plays a critical role in vascular biology by producing nitric oxide from L-arginine. Figure 6 shows the administration of streptozotocin could significantly decrease the expression of eNOS in the aortic tissue if compared with the control group ($p<0.05$). Contrastingly, treatment of fucoxanthin nanoparticles in a dose-dependent manner increases the expression of eNOS, and only at a dose of 300 mg/kg BW could increase significantly the expression of eNOS in diabetic rat aorta tissue.

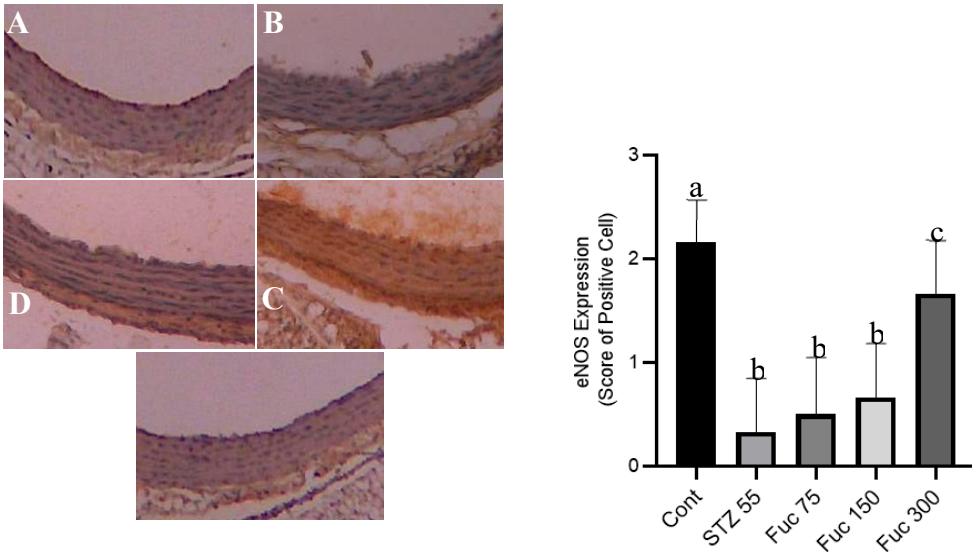

Figure 6. Photomicrographs of immunohistochemical staining of eNOS expression in aortic tissues of rats (left). (400×). Score of the immunoreactive cell of eNOS expression (right). Cont (control); STZ 55

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(55 mg/kg BW streptozotocin); and Fuc 75,150 and 300 (fucoxanthin nanoparticles at doses of 75, 150, and 300 mg/kg BW). [a-c]Bar charts that have different letters are statistically different ($p < 0.05$).

2.7. Effects of Fucoxanthin Nanoparticles on the Levels of NO on Diabetic Rat Aorta Tissue.

NO relaxes the smooth muscles in blood vessels, leading to increased blood flow and reduced blood pressure. This is crucial for regulating cardiovascular health. As shown in Figure 7, NO levels in the aortic tissue decreased significantly after streptozotocin administration ($p < 0.05$). In contrast, NO levels increased after fucoxanthin nanoparticle administration, especially at a dose of 300 mg/kg BW.

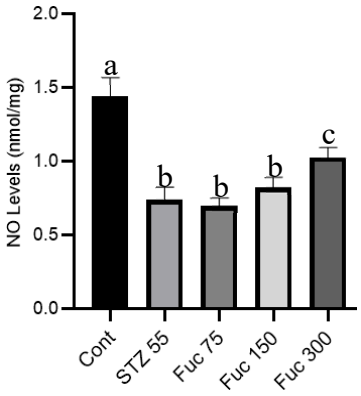

Figure 7. Effect of fucoxanthin nanoparticles on NO levels in rat aorta tissue. Cont (control); STZ 55 (55 mg/kg BW streptozotocin); and Fuc 75,150 and 300 (fucoxanthin nanoparticles at doses of 75 mg/kg BW, 150 mg/kg BW and 300 mg/kg BW). [a-c]Bar charts that have different letters are statistically different ($p < 0.05$).

2.8. Effects of Fucoxanthin Nanoparticles on Structural Changes in Diabetic Rat Aorta Tissues

Histological examination of rats with STZ-induced damage to the aortic vasculature demonstrated the protective effects of fucoxanthin nanoparticles. Light microscopy revealed a normal histopathology of the aorta in the control group. In contrast, STZ treatment resulted in morphological abnormalities and endothelial cell necrosis (Figure 8). Administration of fucoxanthin nanoparticles prevented the necrosis of endothelial cells and preserved the normal vascular structure of the aorta.

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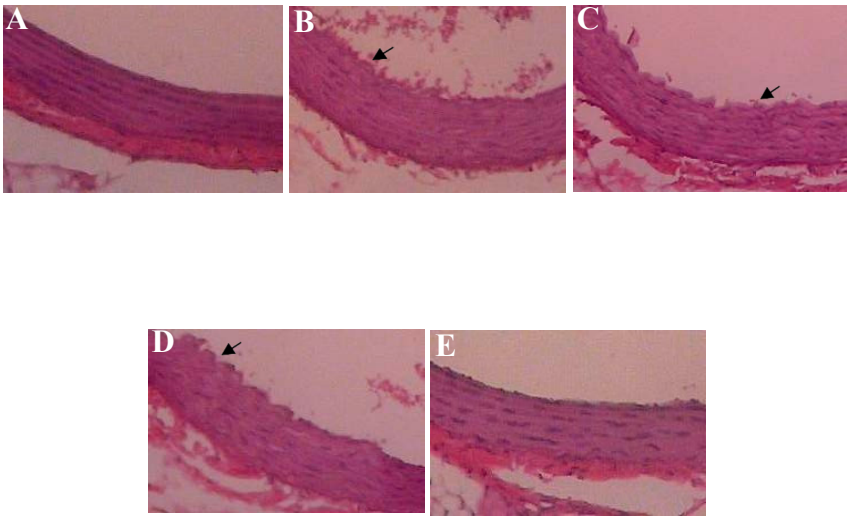

Figure 8. Photomicrographs of H&E staining of rat aortic tissue. The control group showed normal morphology in the aorta (A). Endothelial cell necrosis (black arrows) is found in the streptozotocin group (B). Mild necrosis remained after treatment with fucoxanthin nanoparticles (75 mg/kg BW and 150 mg/kg BW) (C and D). In contrast, 300 mg/kg of fucoxanthin nanoparticles inhibits necrosis in the diabetic rat aorta (E). H&E, 400×.

3. Discussion

Recent years have seen a significant advancement in the field of medication nanotechnology development. Drug nanotechnology can reduce pharmacological toxicity and increase drug biodistribution, specificity, and sensitivity [24,26]. Fucoxanthin nanoparticles were generated through ball milling in the present study. Our findings indicate that fucoxanthin nanoparticles are 217.2 ± 42.8 nm in size. This size may enhance drug absorption, specificity, biodistribution, and sensitivity, and reduce pharmacological toxicity. This suggests that fucoxanthin nanoparticles may increase the solubility, bioavailability, and stability of fucoxanthin, as well as its delivery to target cells. These improvements can increase the efficacy of fucoxanthin in preventing aortic vascular damage associated with hyperglycemia.

Excessive ROS (O_2^-, OH^- and H_2O_2) production in hyperglycemia may impair antioxidant defenses (SOD and GPx) and induce the oxidation of proteins, DNA, and lipids resulting in MDA formation [2,29]. Additionally, an excess of ROS may result in the production of inflammatory cytokines such as IL-6 and TNF-α that can exacerbate and accelerate vascular damage in diabetes. The onset and progression of angiopathy, vascular cell damage in diabetic complications, is attributed to the relation between oxidative stress and inflammation [10,30]. In a diabetic rat model, the administration of streptozotocin increases multiple oxidative stress parameters, including MDA, SOD, and GPx levels. Streptozotocin also has a crucial role in increasing inflammatory cytokine parameters such as IL-6 and TNF-α. These factors considerably affect the occurrence of diabetic vascular disorders [13,20].

Our findings suggest that streptozotocin causes damage to pancreatic beta cells leading to an inhibition of secretion and production of insulin, resulting in high blood glucose levels. Fucoxanthin nanoparticles increased insulin levels and lowered blood glucose levels in the present study. Fucoxanthin modulates the expression of monocyte chemoattractant protein-1 mRNA in white adipose tissue and reduces the secretion of adipocytokines such as TNF-α and IL-6 which may help

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improve insulin sensitivity. Fucoxanthin also influences the expression of glucose transporters, such as Glut 4, which are critical for glucose uptake in cells, especially in muscle and adipose tissue [31].

In this study, MDA levels were higher in rats with streptozotocin-induced diabetes. The levels of MDA in the treatment of fucoxanthin nanoparticles were considerably lower than in the streptozotocin group. These findings are consistent with those reported in previous studies, which show that streptozotocin increases MDA levels in several tissue types, including the aorta, via lipid peroxidation. MDA levels are an indicator of elevated ROS production during tissue injury [22,32]. Additionally, streptozotocin may reduce the antioxidant enzyme activity such as SOD and GPx by glycating scavenging enzymes. This is because advanced glycation end products are formed when glucose interacts with proteins. Advanced glycation end products subsequently enhance ROS formation and deactivate antioxidant enzymes. The enhanced ROS production increases oxidative stress and may damage the vasculature of the aorta [7,11,32]. In streptozotocin-induced diabetic rats, fucoxanthin nanoparticles significantly decreased MDA levels and elevated expression of SOD and GPx at a dose of 300 mg/kg BW. Fucoxanthin nanoparticles reduced oxidative stress in a dose-dependent manner, thereby decreasing the development of aortic vascular damage in diabetic rats. These findings corroborate a previous finding that the reduction of ROS production by fucoxanthin may be partly attributed to improved antioxidant enzyme activity. Fucoxanthin possesses antioxidant activity; it prevents alcohol-induced liver injury by increasing glutathione, superoxide dismutase, and glutathione peroxide production [33]. Moreover, Wardani et al (2022) also reported that the administration of fucoxanthin nanoparticles can decrease MDA and increase SOD and GPx levels in streptozotocin-induced kidney cell damage.

Cells have a sophisticated defense system against excessive ROS production, including antioxidant enzymes such as SOD, GPx, and Catalase [34,35]. ROS is a significant factor in the development of diabetic angiopathy-associated vascular cell damage. Owing to the imbalance between antioxidant defenses and ROS generation, elevated blood glucose levels induce oxidative stress. Exogenous antioxidant therapy may prevent ROS formation by scavenging intracellular ROS [4,9,36]. Therefore, the administration of antioxidants, such as fucoxanthin nanoparticles, might enhance aortic function by reducing the oxidative vascular damage caused by diabetes. A crucial regulator of antioxidant enzymes, including SOD, GPx, and catalase, is NFE2-related factor 2 (Nrf2). Antioxidant response elements (AREs) in the promoter regions of these genes are bound by the transcription factor Nrf2, which increases the production of antioxidant enzymes when activated. Balancing the redox potential of cells and neutralizing ROS helps shield cells from oxidative stress. Because of its function in cellular defense systems, Nrf2 is essential to many physiological and pathological processes [8,14,15]. The activation of Nrf2 using natural or synthetic therapies or antioxidants effectively prevents and treats oxidative stress-related toxicities and diseases. Thus, small molecule Nrf2 activators have been extensively investigated to address the unmet therapeutic demand for oxidative stress reduction in illnesses such as diabetes-related complications. This is crucial for preventing cell damage caused by free radicals and lowering the prevalence of radical-induced degenerative diseases, such as diabetes [14,37]. Fucoxanthin inhibits apoptosis by enhancing the expression of natural antioxidant enzymes that increase Nrf2 levels. In vivo analyses have shown that fucoxanthin pretreatment decreases ROS production, cell death, and lipid peroxidation [33,34]. This suggests that Nrf2 activation by fucoxanthin nanoparticles in diabetic rats may increase SOD and GPx levels.

Hyperglycaemia may increase the production of inflammatory cytokines, accelerate vascular cell damage in diabetes, and cause excess ROS accumulation. The current investigation shows that the aorta tissue of streptozotocin-induced diabetic rats has significantly higher levels of IL-6 and TNF-α, indicating vascular injury due to inflammation. Consistent with previous studies, streptozotocin may encourage diabetic vascular damage by activating ROS, which then increases cytokine of inflammatory (IL-6 and TNF-α) production, leading to vascular damage in diabetes [10,15,38]. ROS and antioxidant production are unbalanced in diabetes, resulting in the development of oxidative stress disorders. Thus, ROS may contribute to systemic inflammation and complications [4,32]. In the results of this research, fucoxanthin nanoparticle administration only at a dose of 300 mg/kg BW significantly reduced the

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levels of IL-6 and TNF-α in diabetic rat aorta tissue. This study showed that fucoxanthin nanoparticles may protect the vascular cells of diabetic rats from damage by reducing the effects of inflammation. A commonly reported mechanism for fucoxanthin anti-inflammatory effects is the inhibition of the MAPK and NF-kB signaling pathways, which leads to the decreased generation of inflammatory cytokines including IL-6 and TNF-α [9,38]. Nrf2 is also an anti-inflammatory cytokine. Therefore, inflammation is often observed in chemically induced diseases associated with Nrf2 deficiency. Numerous antioxidant response element inducers are effective anti-inflammatory drugs, and their ability to induce antioxidant response element genes is highly correlated with their ability to prevent inflammation. Nrf2 inhibits inflammation by inhibiting the NF-kB pathway and cytokine of inflammatory production [15,37].

Reduced eNOS and NO levels in endothelial cells indicate altered aortic function in diabetic rats. Similarly, elevated ROS production under oxidative stress indicates vascular cell injury in diabetic rats [9,12,20]. The results of this investigation indicate that functional impairment of endothelial cells was caused by STZ-induced diabetic vascular injury, as evidenced by the significantly lower levels of NO and eNOS expression compared to the control group. Fucoxanthin nanoparticles were administered only at a dose of 300 mg/kg BW significantly elevated eNOS expression and NO levels in the diabetic rat aorta tissue.

The ability of antioxidants to eliminate ROS significantly affects their efficacy against vascular damage [15,39]. Fucoxanthin nanoparticles markedly reduced STZ-induced endothelial cell damage brought on by streptozotocin. This suggests that they may mitigate endothelial cell damage caused by STZ, as this effect is associated with increased eNOS expression and NO levels. These findings align with earlier studies showing that antioxidant enzymes can prevent STZ-induced endothelial cell damage by upregulating eNOS and NO levels. To our knowledge, this study is the first to demonstrate that fucoxanthin nanoparticles markedly reduce streptozotocin-induced damage to aortic endothelial cells. A drop in aortic MDA levels, an increase in the levels of antioxidants such as SOD and GPx, a decrease in the levels of inflammatory cytokines such as IL-6 and TNF-α, as well as an increase in eNOS expression and NO levels indicated the prevention of aortic endothelial cell damage. In addition, endothelial function improved in this study because nanoparticles reduced streptozotocin-induced endothelial cell necrosis, as shown by histological observations..

4. Materials and Methods

4.1. Ball Milling Methods to Make Fucoxanthin Nanoparticles

The powder of fucoxanthin powder was placed in a stainless-steel ball mill jar. The intense kinetic energy produced by the ball bearings further ground the powder to fine particles. To obtain these fine particles, the powders were processed in a ball mill (PM100; Retsch, Haan, Germany) for one hour at a constant speed of 500 rpm for five hours. The fine particles were further processed in a ball mill for 10 h using 10 mm balls at 300 rpm, as described by Mihailović et al. (2021). Dynamic light scattering was used to determine the particle size.

4.2. Experimental Animal

Wistar rats were purchased from Gadjah Mada University in Yogyakarta Indonesia, weighing approximately 175-200 g. The animals were acclimatized for 15 days with 12-hour light and dark cycles, with ambient temperature of 25 ^{o}C and 45-55 % humidity. Rats were fed a pelleted diet and water ad libitum. The Ethical Clearance Committee approved all procedures for Preclinical Research, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia (Ethical clearance No. 88/FKH.UA/6/2023).

4.3. Induction of Diabetes

The rats were fasted overnight before injection. Streptozotocin was dissolved in citrate buffer at a pH of 4.5. Then streptozotocin a single dose of 55 mg/kg BW was administered intraperitoneal injection. Blood was collected from the lateral tail vein three days after streptozotocin administration.

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Blood glucose levels were subsequently evaluated using an Accu check glucometer (Roche, Mannheim, Germany). The level of blood glucose of > 250 mg/dL was used in this study [32].

4.4. Experimental Design

The research consists of five groups (n=8) namely: Rats only received STZ solvent and fucoxanthin nanoparticle solvent as a control group; Rats received STZ injection at a dose of 55 mg/kg BW intraperitoneally and fucoxanthin nanoparticles solvent as a diabetic group, Rat received STZ and after 3 days, rats were given fucoxanthin nanoparticle at a dose of 75, 150 and 300 mg/Kg BW orally for 72 days. On day 75th, all rats were anesthetized by ketamine at a dose of 60 mg/Kg BW and Xylazine at a dose of 7.5 mg/Kg BW intraperitoneally. Blood and aortic tissue samples were collected from rats in each group. The levels of glucose and insulin were measured in the blood. Furthermore, aortic tissue was used to investigate eNOS, NO, SOD, GPx, MDA, IL-6, and TNF-α. Additionally, the aortic tissue was histologically analyzed using Haematoxylin & Eosin staining.

4.5. Measurement of Serum Insulin and Blood Glucose Levels in Diabetic Rats

Blood samples were centrifuged for 5 min at 25 °C at 1200 rpm to extract serum from blood. The samples were then stored at -70 °C. The levels of serum insulin were measured by an ELISA kit, which was purchased from Cayman Chemical (Ann Arbor, MI, USA). While the levels of blood glucose were measured by Accu Check glucometer.

4.6. Measurement of MDA in the Diabetic Rats Aorta Tissue

The levels of MDA in the supernatant of homogenized aortic tissue were measured by the thiobarbituric acid method. The MDA concentration was subsequently determined at 532 nm using the absorbance coefficient of the MDA-thiobarbituric acid combination. MDA was quantified in nanomoles per milligram of tissue.

4.7. Measurement of SOD and GPX in the Diabetic Rat Aorta Tissue

The levels of SOD in the aorta tissue were measured by using the Bradford technique. SOD inhibition in nitro blue tetrazolium (Sigma-Aldrich, St. Louis, MO, USA) was assessed by spectrophotometry at 560 nm. The levels of SOD are expressed as U/mg protein. The levels of GPx levels in aortic tissue were determined by incubating samples in NaN3 and H2O2. Aortic tissue homogenates were added with 0.1 ml ethylenediaminetetraacetic acid, 0.2 mL sodium azide, and H_2O_2 and phosphate buffer. The mixture was centrifuged at 200 rpm, and the TCA stopping agent was added. Next, DTNB (Sigma-Aldrich) and disodium hydrogen phosphate were added to the supernatant. Absorbance was measured by spectrophotometry at 412 nm. The levels of GPx was expressed as U/mg protein.

4.8. Measurement of IL-6 and TNF-α in the Diabetic Rat Aorta Tissue

Fifty milligrams of aortic tissue samples were washed 5 times with 1 % phosphate buffer saline. The tissue was ground in a mortar with PBS (0.5 mL) and centrifuged at 10.000 rpm for 10 min to abolish the surfactants. The levels of IL-6 were measured by the PicoKine TM kit according to the manufacturer's instructions. Similarly, TNF-α levels were measured using the Rat TNF-α ELISA kit PicoKineTM according to the manufacturer's instructions. The samples were placed on a microplate and heated to 37 °C for 90 min. Following three thorough washes with 0.01 M PBS, the plate was coated with biotinylated antibody and incubated at 37°C for 60 min. A standard curve that correlates optical density values with concentration was used to determine the concentration of IL-6 or TNF-α (pg/mL). Following a 20-min incubation at 37 °C, the TMB color development agent was applied to the plates after they had been washed with 0.01 M PBS. Optical density was measured at 450 nm after applying TMB stop solution.

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4.9. Immunohistochemical Staining of eNOS in the Diabetic Rats Aorta Tissue

The eNOS expression levels were assessed using immunohistochemistry. Deparaffinization was used to prepare aortic tissue slices of 4 μm. Next, a 10-minute H2O2 treatment at 37 °C was used to suppress endogenous peroxide activity. A Tris-buffered salt solution and 10% regular sheep serum were then added, and the samples were incubated at 37 °C for 30 min. Then, the cells were incubated with monoclonal antibodies against mouse anti-eNOS (1:100; ab5589; Abcam, Cambridge, United Kingdom). The cells were subsequently rinsed thrice with PBS before dye and secondary antibodies were added using the Quanto UltraVision HRP DAB Detection System (Thermo Fisher Scientific, Waltham, MA, USA). The scores for all slides were determined by inspecting ten microscopic viewing fields at 400× magnification. Slides with no immune-positive cells were given a score of 0. Scores of 1, 2, 3, and 4 indicated the presence of 1–25%, 26–50 %, 51–75%, and >75% immune-positive cells, respectively.

4.10. NO Assay in the Diabetic Rat's Aorta Tissue

The Griess reagent was used to measure NO levels in the aorta. The aortic tissue homogenate (100 μL) was added with 100 μL Griess reagent contain of 0.1% naphthyl ethylenediamine dihydrochloride, and 1% sulphanilamide in phosphoric acid and then incubated for 10 min at room temperature. Absorbance was measured using a plate reader (ECL ELISA reader). The levels of NO were determined by a standard curve of sodium nitrite. The NO levels were expressed in nmol equivalence with NaNO2/mg protein.

4.11. Histopathological Evaluation of the Diabetic Rat's Aorta Tissue

The rat aorta tissue was placed in 10 % buffer formalin and then embedded in paraffin. Haematoxylin and Eosin were used to stain a 4 μm slice of the aorta. The aorta histopathological was investigated using a light microscope to identify aortic cell damage.

4.12. Data Analysis

Data were analyzed by SPSS 20.0 (SPSS Inc, Chicago, IL, USA). Data are displayed as statistical metrics, such as standard deviation and mean. The least significant difference (LSD) test and one-way analysis of variance (ANOVA) were used.

5. Conclusions

These results demonstrate that fucoxanthin nanoparticles may prevent diabetes-induced aortic damage by inhibiting oxidative stress. This may occur through reducing the level of MDA and elevate the levels of SOD and GPx. Fucoxanthin nanoparticles also inhibit inflammation by reducing the levels of IL-6 and TNF-∞ IL-6. Inhibition of stress and inflammation decreases endothelial cell necrosis, which can increase eNOS expression and NO levels in diabetic aortic vascular cells.

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