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Role of antioxidative stress activity of Fucoxanthin nanoparticle as hepatoprotective in diabetic rats

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Abstract: This study attempts to prove that the antioxidant effect of fucoxanthin nanoparticles can prevent streptozotocin-induced rat liver damage. Fucoxanthin nanoparticles are synthesized using the high-energy ball milling method. Dynamic Light Scattering (DLS) was then used to describe the sizes of the fucoxanthin nanoparticles. Each of the five groups involved in the research had an average of eight rats. Distilled water was given to the rats in the control group whereas Streptozotocin (STZ) was given to the diabetic group. Additionally, streptozotocin and fucoxanthin administration were performed as the fucoxanthin group. The administration of fucoxanthin nanoparticles caused a significant decline in the levels of the enzymes ALT, AST and ALP in the blood and MDA in the liver tissue of diabetic rats. Furthermore, as compared to the group of diabetic rats, the fucoxanthin nanoparticles treatment produced a significant rise in SOD and GPx levels. These effects directly can prevent histological abnormalities, notably fatty degeneration, and necrosis, in diabetic rats. The findings of this research suggest that fucoxanthin nanoparticles exhibited significant antioxidant activity in STZ-induced diabetic rats. The antioxidant activity of fucoxanthin nanoparticles potential to prevent diabetes complications such as hepatopathy

Keywords: Fucoxanthin nanoparticle, antioxidant, hepatoprotective, diabetes.

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INTRODUCTION

Diabetes Mellitus (DM) has emerged as a significant global public health issue in modern society, affecting over 537 million people, which accounts for 9.3% of adults worldwide. Alarming projections indicate that its prevalence will increase by 25% in 2030 and 50% in 2045, with an estimated 783 million individuals affected (Sanchez *et al*., 2024) The impact of diabetes on the general population's quality of life can cause serious complications such as cardiomyopathy, retinopathy, neuropathy, nephropathy, hypertension and hepatopathy (Volpe *et al*., 2018; Pasupuleti *et al*., 2020; Darenskaya *et al*., 2021).

Research indicates that DM is associated with several liver abnormalities, such as abnormal glycogen deposition, non-alcoholic fatty liver disease (NAFLD), fibrosis, cirrhosis, hepatocellular carcinomas (HCCs), abnormal elevated hepatic enzymes, acute liver disease, and viral hepatitis (Trivedi *et al*., 2023).

Many researchers have reported that hyperglycemia in diabetes, through activation protein kinase C, hexamine metabolism, sorbitol formation and glucose autooxidation lead to disruption of the balance of the generation of ROS such as O_2^-, OH^- and H_2O_2 and the antioxidant defense system such as SOD, GPx and Cat. Excessive production of ROS and lowering of antioxidant enzyme activity induce oxidative stress, essential in diabetes complications, including diabetic hepatopathy (Rajendiran *et al*., 2018; Ighodaro, 2019). Under regular physiological circumstances, reactive oxygen species (ROS) are generated as part of essential cellular processes, contributing to cell signaling and maintaining tissue equilibrium. Nevertheless, excessive ROS production can result in lipids, proteins and DNA oxidation. This oxidative damage harms cellular components and can lead to necrosis and apoptosis (Oguntibeju, 2019; Tomic *et al.,* 2022). When ROS production surpasses normal levels, it triggers the initiation of lipid peroxidation in the cell membrane's polyunsaturated fats (PUFA), yielding lipid peroxides, or MDA. Elevated MDA levels signify an escalation in ROS concentration, leading to the necrosis and apoptosis of liver cells (Wardani *et al*., 2017; Bigagli and Lodovici, 2019). MDA is a biomarker to assess oxidative stress in diabetic rats induced with STZ. The use of STZ in a diabetic rat model causes liver cell damage, which can release AST, ALT and ALP from the liver tissue into the bloodstream so that AST, ALT and ALP levels in the serum elevate. Therefore, AST, ALT and ALP can be used as markers of liver function disorder (Altindag F *et al*., 2021; Talaat *et al*., 2022).

Many experiments show that antioxidants from natural products help prevent liver injury due to ROS in diabetic rats. Natural antioxidants derived from various sources are frequently employed as an economical and low-risk option for exogenous antioxidants. Their negligible

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adverse effects are what motivated this decision. Fucoxanthin, a natural substance, has demonstrated potent antioxidant capabilities, effectively neutralizing ROS and thus impeding oxidative stress. Beyond its antioxidant properties, fucoxanthin also exhibits various beneficial effects, including *antidiabetic, anti-inflammatory, anticancer, antibacterial and immunostimulant effects (Oliyaei et al.,* 2021; Mumu *et al*., 2022).

Challenges commonly encountered with natural product antioxidants encompass issues related to their bioavailability, absorption, solubility and distribution. To surmount these challenges, the field has turned to the advancement of nanotechnology, which involves crafting nanoparticles of natural antioxidants. Nanobiotechnology, a technology employed for generating particles sized within the 10-1000 nm range, is utilized for this purpose (Sim and Wong. 2021; Sahu *et al*., 2021). Nanotechnology is anticipated to enhance the therapeutic effects of these antioxidants while mitigating their toxicity. Given fucoxanthin's nanoparticles established anti-diabetic and anti-ROS impact, the current research aimed to substantiate the antioxidant efficacy of fucoxanthin nanoparticles in safeguarding liver cells from damage induced by STZ in rats.

MATERIALS AND METHODS

Synthesis of fucoxanthin nanoparticles

Fucoxanthin nanoparticles are synthesized using the high-energy ball milling technique, following the guidelines provided by the nanomachine manufacturer. Subsequently, the size of the chitosan nanoparticles is assessed using Dynamic Light Scattering.

Animal

Wistar rats weighing 250-300g were purchased from the LPPT at the Universitas Gajah Mada in Indonesia. Before the trial began, the rodents were acclimated for one week in plastic enclosures under controlled conditions. The temperature was maintained at 26±2ºC and the rats were subjected to a 12-hour light/dark cycle. All rats had unrestricted access to drinking water and commercial pellets during this period.

Approval for using rats was obtained from The University of Airlangga Animal Care and Ethics Committee No: 1.KEH.115.09.2023. All procedures of research were conducted according to the Association for Assessment and Accreditation of Laboratory Animal Care International, which was recommended by the Declaration of Helsinki.

Induction of diabetes in animals

After a 12-hour fast, the rodents received an intraperitoneal injection of 55mg/kg body weight of streptozotocin. This substance was dissolved in a citrate buffer (0.1M, pH4.5). On the third day after the streptozotocin injection, Roche Diagnostic's Accu-Check glucometer was used to measure the rats' blood sugar levels. Rats with blood sugar levels greater than 200 mg/dl were chosen to participate in the study (Farrag *et al*., 2019; El-Haskoury *et al*., 2019).

Experimental design

The study utilized rats randomly divided into five groups with eight rats in each group. Control rats (rats received aqua dest), Diabetic rats (rats received streptozotocin 55mg/kg by ip), Fucoxanthin rats (rats received fucoxanthin nanoparticles orally at doses of 75mg/kg, 150mg/kg and 300mg/kg and injected with STZ 55mg/kg BW by ip). Fucoxanthin nanoparticles were given orally once a day for 72 days. On day 75, all groups of rats were anesthetized with ketamine and blood was taken intracardially to measure levels of ALT, AST and ALP. Then the liver is taken to investigate the MDA, SOD, GPx and Hematoxylin-eosin staining is another method used for the liver's histopathological investigation.

Biochemical estimation of ALT, AST and ALP

Erba Semi-auto analyzer by Erba Biochemkit was used to determine Alanine Transaminase, Aspartate Aminotransferase and Alkaline Phosphatase in serum.

Assessment of MDA in diabetic rat liver tissues

The Malondialdehyde (MDA) level of the liver tissue is assessed using the Thiobarbituric Acid (TBA) technique. This involves the employment of a TBARS assay kit (supplied by Cayman Chemical, USA) to quantify MDA formation. The MDA-TBA complex is determined by measuring the absorbance at 532 nm using a micro plate reader. This measurement is used to evaluate MDA levels in tissue, and the results are expressed in nanometers per milligram of tissue.

Assessment of SOD and GPx in diabetic rat liver tissue

Protein was extracted from the liver to evaluate the enzymatic activity of Super oxide Dismutase (SOD) in rat liver tissue and assessed using the Bradford method. Using spectrophotometry at 560 nm, the inhibitory impact of SOD on Nitro Blue Tetrazolium (NBT) reduction in each sample was determined. NBT was purchased from Sigma-Aldrich, USA. SOD concentrations are shown as units per milligram (U/mg) of protein**.**

To assess Glutathione Peroxidase (GPx) levels, the samples underwent incubation with NaN3 and H_2O_2. A homogenate comprising 0.1ml of liver tissue was combined with 0.2ml of ethylene diamine tetraacetate, Sodium Azide and a mixture of H_2O_2 in a Phosphate Buffer. This amalgamation was subjected to centrifugation at 200 rpm, followed by the addition of the stopping reagent TCA. Subsequently, the supernatant was blended with Disodium Hydrogen Phosphate and DTNB.

After the development of color, the absorbance was measured at 412 nm. GPx levels are quantified in units per milligram (U/mg) of protein.

Histopathological observations

After the trial, the liver samples from every rat were stored in a 10% formalin buffer solution before being paraffin-embedded. Hematoxylin and eosin were used to stain liver tissue slices four µm thick. Using a light microscope, histological analysis of the liver was performed to look for signs of liver cell injury, particularly diseases such as fatty degeneration and necrosis.

STATISTICAL ANALYSIS

All data were expressed as means ± standard deviation. One-way ANOVA was used for the analysis, and Duncan's multiple comparison test was performed to compare the groups (SPSS version 19.0).

RESULTS

The size distribution of fucoxanthin nanoparticles

The current study was designed to investigate the protective effect of fucoxanthin nanoparticles against STZ-induced liver cell damage in rats. Examination by DLS showed that the distribution of the fucoxanthin nanoparticles size is 79.02±22.98 nm, as seen in fig. 1. Nanotechnology can be used to increase the therapeutic value by reducing toxicity and increasing bioavailability.

Fucoxanthin nanoparticles effect on ALT, AST and ALP level in diabetic rat serum

ALP, AST and ALP levels in the blood are used to detect liver disease and liver cell deterioration. table 1 details how fucoxanthin nanoparticles affected the serum ALT, AST and ALP concentrations of diabetic rats. The serum concentrations of ALT, AST and ALP are increased after intraperitoneal injection of STZ and this increase is significantly more than in the control group ($p<0.05$). The blood levels of ALT, AST and ALP do, however, decrease after receiving fucoxanthin nanoparticles in advance in a dose-dependent manner. This outcome implies that fucoxanthin nanoparticles possess the potential to mitigate liver dysfunction and protect liver cells from injury in diabetic rats.

Fucoxanthin nanoparticles effect on MDA levels of diabetic rat liver tissue

The measurement of MDA levels serves as an indicative measure of liver impairment arising from oxidative stress stemming from heightened production of ROS. table 2 illustrates the impact of fucoxanthin nanoparticles on MDA levels within liver tissue. Rats subjected to streptozotocin injection displayed notably elevated MDA levels in liver tissue compared to the control group ($p<0.05$). However, a dose-dependent reduction in liver tissue MDA levels was observed upon administering fucoxanthin nanoparticles, signifying a significant mitigation of MDA concentrations.

Fucoxanthin nanoparticles effect on SOD and GPx levels in diabetic rat liver

As the primary antioxidant in the body, SOD assists in converting super oxide anion (O^{-}_{2}) to hydrogen peroxide (H_2O_2), which GPx then degrades into oxygen and water. The levels of GPx and SOD in the liver tissue are displayed in table 3. The levels of SOD and GPx in the liver tissue of rats administered an intraperitoneal dose of STZ were significantly lower than those of the control animals ($p<0.05$). In contrast, pretreatment with fucoxanthin nanoparticles increased SOD and GPx levels dose-dependently. Notably, a statistically significant increase in SOD and GPx levels in liver tissue was observed at 300mg/kg compared to diabetic rats ($p<0.05$).

Fucoxanthin nanoparticles effect on the structural change of diabetic rats liver tissue

Histopathological examinations were employed to explore alterations in the structural integrity of liver cells in diabetic rats, as depicted in fig. 2. Under light microscopy examination, control rats exhibited a normal liver cell structure. Conversely, rats administered STZ displayed irregular morphology and indicated fatty degeneration and necrosis in liver cells. However, the administration of fucoxanthin nanoparticles showed its ability to inhibit cell fatty degeneration and suppress liver cell necrosis.

DISCUSSION

Elevated blood sugar levels, known as hyperglycemia, are a hallmark of diabetes mellitus (DM). Hyperglycemia contributes to an increased production of reactive oxygen species (ROS), thereby expediting damage to liver cells in diabetic mellitus (Volpe *et al*., 2018; Pasupuleti *et al*., 2020).

This study aims to validate the capability of fucoxanthin nanoparticles to mitigate ROS-related damage to liver cells in rats induced with STZ. Numerous research efforts have employed STZ to establish rat models of diabetes-associated liver disease. Within this rat model, diabetic hepatopathy manifests with elevated ROS levels, evidenced by increased MDA concentrations and reduced antioxidants like SOD and GPx, leading to liver cell injury (El-Haskoury *et al*., 2019 and Kumar V and Sahai, 2020).

The outcomes of our investigation have demonstrated that intraperitoneal administration of STZ leads to a noteworthy elevation in MDA levels, coupled with a considerable reduction in SOD and GPx levels within liver tissue, as compared to the control rats ($p<0.05$).

Table 1: Fucoxanthin nanoparticles effect on ALT, AST and ALP in diabetic rats serum

Group	Mean ± SD		
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control Rats	$168.2^{a} \pm 8.3$	$38.6^{a} \pm 4.3$	$111.4^{a} \pm 7.2$
Diabetic Rats	$204.1^{b} \pm 10.1$	$59.5^{b} \pm 6.1$	$133.3^{b} \pm 11.1$
Fucoxanthin Nano 75mg/kg	$210.6^{b} \pm 11.8$	$64.1^{b} \pm 5.8$	$132.8^{b} \pm 8.7$
Fucoxanthin Nano 150mg/kg	$198.4^{b} \pm 7.6$	$55.3^{b} \pm 4.1$	$126.5^{b} \pm 6.1$
Fucoxanthin Nano 300mg/kg	$186.2^{c} \pm 6.7$	$47.2^{c} \pm 3.1$	$117.3^{c} \pm 5.7$

[a-c] Significant differences between the means were indicated by a distinct superscript in each column ($p < 0.05$).

Table 2: Effect of fucoxanthin nanoparticle on MDA levels of diabetic rat liver

Group	Means ± SD MDA (nmol/mg)
Control Rats	$4.32^{a} \pm 0.24$
Diabetic Rats	$8.10^{b} \pm 0.46$
Fucoxanthin Nano 75 mg/kg	$7.87^{b} \pm 0.57$
Fucoxanthin Nano 150 mg/kg	$6.70^{b} \pm 0.32$
Fucoxanthin Nano 300 mg/kg	$5.91^{c} \pm 0.37$

[a-c] Significant differences between the means were seen in each column's various superscripts ($p < 0.05$).

Table 3: Fucoxanthin Nanoparticles Effect on SOD and GPx Levels in Diabetic Rat Liver

Group	Mean ±SD	
	SOD U/mg protein	GPx U/mg protein
Control Rats	$34.3^{a} \pm 3.81$	$24.3^{a} \pm 2.92$
Diabetic Rats	$20.2^{b} \pm 2.17$	$14.5^{b} \pm 1.22$
Fucoxanthin Nano 75 mg/kg	$19.4^{b} \pm 1.35$	$15.8^{b} \pm 1.87$
Fucoxanthin Nano 150 mg/kg	$22.5^{b} \pm 1.96$	$16.9^{b} \pm 2.01$
Fucoxanthin Nano 300 mg/kg	$27.1^{c} \pm 2.53$	$19.4^{c} \pm 1.92$

[a-c] A significant difference between the means is indicated by a distinct superscript in each column ($p < 0.05$).

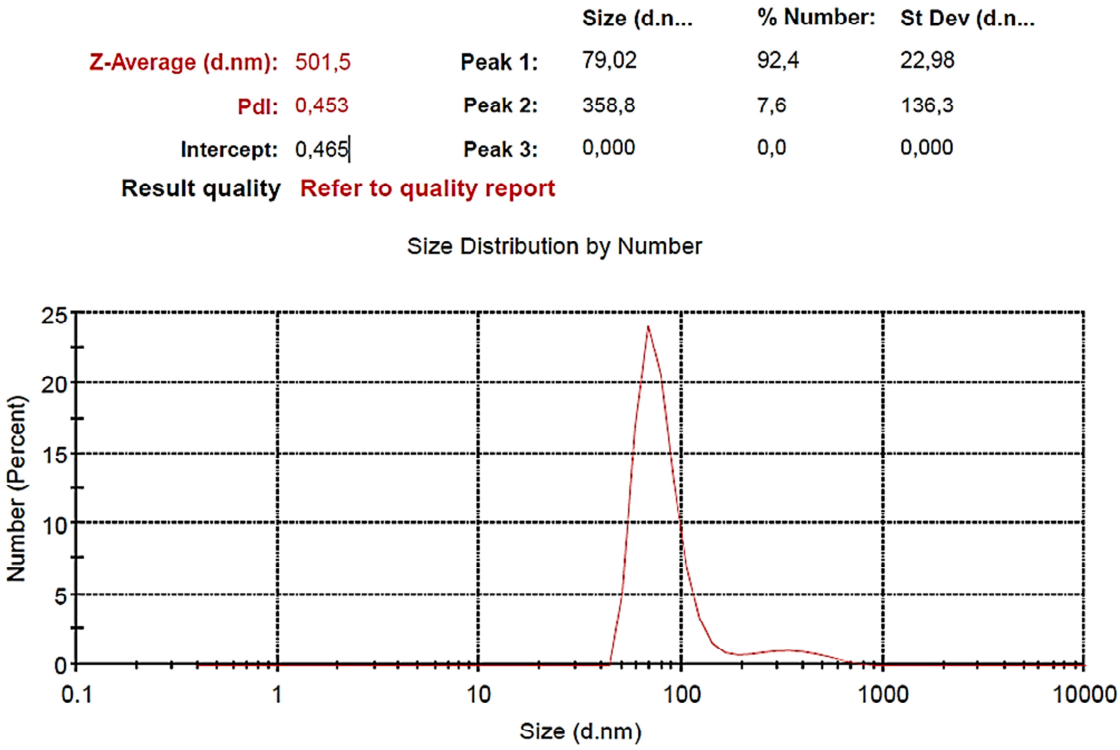

Fig. 1: Size distribution of Fucoxanthin nanoparticles

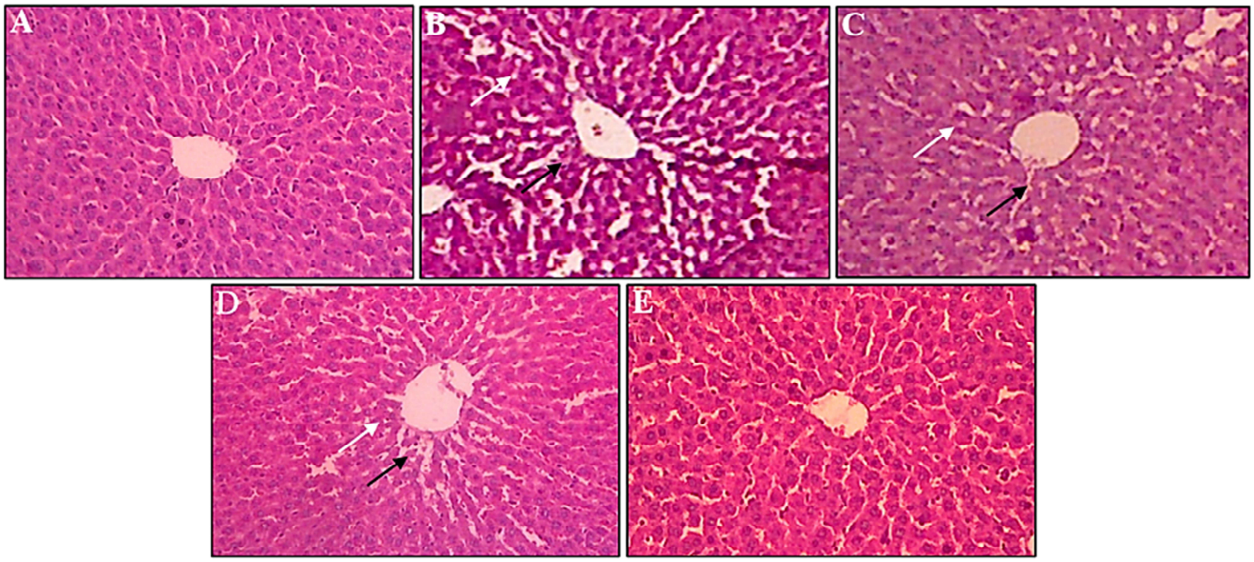

Fig. 2: Liver Tissue Histology in Rats. The liver cells of control rats displayed a regular morphology (a). Diabetic rats exhibited signs of fatty degeneration (indicated by the white arrow) and necrosis (indicated by the black arrow) (b). Administration of Fucoxanthin nanoparticles at 75mg/kg and 150mg/kg BW to diabetic rats revealed mild necrosis (c and d). On the other hand, at a dose of 300mg/kg BW, fatty degeneration and necrosis were inhibited in the liver of diabetic rats (e). Magnification: 400x, H&E staining.

This escalation in MDA signifies an amplified production of ROS. In the context of diabetes, heightened ROS levels lead to lipids, proteins and DNA oxidation. This oxidative stress results in detrimental effects such as damage to cell membranes, disruption of protein functionality and fragmentation of DNA. Consequently, these events lead to heightened MDA levels and cellular necrosis and apoptosis (Saroj *et al*., 2020; Altindag, 2021; Wardani *et al.,* 2022). Hyperglycemia caused by STZ also harms antioxidant activity. This happens through several different mechanisms, such as decreased scavenging, interactions between glucose and proteins, the formation of advanced glycation end products (AGE) and the blocking of receptors. This culmination of processes contributes to oxidative damage in cells. Streptozotocin can impact insulin release from the beta cells within the islets of Langerhans. This, in turn, leads to decreased insulin levels, resulting in elevated blood glucose levels. This cascade of events paves the way for the onset of diabetic complications such as hepatopathy (Ighodaro, 2019; Talaat *et al*., 2022).

Nanobiotechnology offers a valuable approach to enhancing the solubility, absorption, distribution, bioavailability, efficacy and toxicity associated with antioxidant substances (Sim and Wong, 2021; Sahu *et al*., 2021). A grinding procedure was implemented for the formulation of chitosan nanoparticles, employing the ball milling technique. The findings of this study indicate that the resultant fucoxanthin nanoparticles possess a nanoscale size measuring 79.02±22.98nm.

Our findings indicate that pre-administration of fucoxanthin nanoparticles, specifically at 300 mg/kg BW, decreases MDA levels and increases SOD and GPx levels in diabetic rats' liver tissue. This shift is notably significant compared to diabetic rats not receiving this treatment. These outcomes suggest that the application of fucoxanthin nanoparticles in a dose-dependent manner is capable of suppressing oxidative stress, thereby providing protective effects against liver impairment in diabetic rats. Past studies in vivo and in vitro studies have documented that fucoxanthin can scavenge ROS, effectively preventing lipid oxidation. This property is attributed to its capacity to decrease MDA levels and elevate SOD and GPx levels. Consequently, this proactive action safeguards cells from oxidative damage (Altindag, 2021; Wardani *et al.,* 2022).

Injected with STZ intraperitoneally in the rats can elevate ALT, AST and ALP levels, which is significant when compared with the control rats ($p<0.05$). The increasing levels of ALT, ASP and ALP in the serum can be used to indicate impaired liver function and cell damage. Whereas the administration of fucoxanthin Nanoparticles was dose-dependent and significantly decreased ALT, AST and ALP levels in the serum of diabetic rats ($p<0.05$). This result demonstrates that pretreatment with antioxidant-capable fucoxanthin nanoparticles may stop liver cell damage in diabetic hepatopathy.

Higher blood sugar levels speed up liver damage, which causes Diabetes Mellitus patients' liver cells to secrete ALT, AST and ALP into the circulation (El-Haskoury *et al*., 2019; Talaat *et al*., 2022). Oxidative stress in hyperglycemia is crucial in developing liver injury associated with increased ALT, AST and ALP levels in

diabetes. Similarly, exogenous antioxidants such as black mulberry fruit, *Heteroxenia ghardaqensis*, *and Actinidia deliciosa* can reduce ROS production. They can prevent liver cell damage by decreasing ALT, AST and ALP in serum (Farrag *et al*., 2019; Abouzeda *et al*., 2020; Altindag, 2021).

Through histological tests, it was clear that STZ caused fatty degeneration and necrosis in the liver cells of rats. On the other hand, the antioxidant activity of fucoxanthin nanoparticles had a hepatoprotective effect by stopping the progression of liver cell degeneration and necrosis when they were given. This pattern of results aligns with findings from various other researchers (Zheng *et al.,* 2019; Chiang *et al*., 2020). It has been established that STZ administration prompts necrosis in liver cells while introducing exogenous antioxidants counteracts this necrotic process. Hence, fucoxanthin Nanoparticles, renowned for their potent antioxidant properties, hold the potential to serve as guardians against diabetes complications, hepatopathy being one of them.

CONCLUSION

In the present study, fucoxanthin nanoparticles exhibited significant antioxidant activity in STZ-induced diabetic rats by inhibiting MDA production and elevated levels of SOD and GPx in the liver tissue. The antioxidant activity of fucoxanthin nanoparticles potential to prevent diabetes complications such as hepatopathy. Next, we will research to prove the anti-inflammatory and anti-apoptotic effects of fucoxanthin nanoparticles on STZ-induced rat liver damage.

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