Vitamin A Precursor: Beta-Carotene Alleviates the Streptozotocin-Induced Diabetic Retinopathy in Male Adult Zebrafish via the Regulation of the Polyol Pathway

Yamunna Paramaswaran[1], Aswinprakash Subramanian[2], Arunachalam Muthuraman[3]*

[1] PG Research Scholar, Faculty of Pharmacy, AIMST University, Jalan Bedong-Semeling, Bedong, Kedah, Malaysia.
[2] Anatomy Unit, Faculty of Medicine, AIMST University, Jalan Bedong-Semeling, Bedong, Kedah, Malaysia.
[3] Pharmacology, Toxicology and Basic Health Sciences Unit, Faculty of Pharmacy, AIMST University, Jalan Bedong-Semeling, Bedong, Kedah, Malaysia.

ABSTRACT

Diabetic retinopathy (DR) is a progressive neurovascular disorder due to damage to retinal blood vessels. Beta-carotene acts as retinal chromophores and initiates photo-transduction and epithelial maintenance. Beta-carotene (BC) is present in palm oil mill effluent, and it is called palm oil mill effluent-derived beta-carotene (PBC). The present study is designed to evaluate the effect of PBC in streptozotocin-induced DR in zebrafish by measuring the oxidative stress, inflammation, and polyol pathway markers. The five groups of healthy *Danio rerio* were used in this study. The diabetes retinopathy was instigated by intraperitoneal administration of streptozotocin (STZ) followed by intravitreal administration of STZ on the 7th day. The exposure of PBC (50 and 100 mg/L) and dexamethasone (DEX) was administered for 21 continuous days. The DR-associated visual behaviours *i.e.*, optomotor response (OMR) and startle response (SR) were appraised on 0, 7, 14, and 21st days. The biochemical changes *i.e.*, plasma glucose & homocysteine (HCY); retinal tissue lipid peroxidation, reduced glutathione (GSH), tumor necrosis factor-alpha (TNF-α), superoxide dismutase (SOD) and total protein levels were estimated. The lens was used for the evaluation of polyol pathway markers *i.e.*, sorbitol dehydrogenase (SDH) and aldose reductase (AR) activity. The PBC potentially attenuated the DR with the regulation of biochemical abnormalities which is similar to DEX treated group. Hence, PBC can be used for the management of DR due to its anti-hyperglycemia, antioxidant, anti-inflammatory, and polyol pathway regulatory actions.

Keywords: Aldose reductase; free radical; intravitreal injection; optomotor response; palm oil mill effluent; sorbitol dehydrogenase.

1. INTRODUCTION

Retinopathy is a disease caused by damage to the retina and it involves abnormal vascular tissue growth in the retina [1]. The progression of retinopathy shows reversible blindness with retinal blood vessel damage and light sensation to the retina [2]. In diabetic conditions, it rapidly causes retinal barrier dysfunctions triggered by the accretion of advanced glycation end products (AGE) products [3]. Clinically, diabetic retinopathy (DR) showed exudates, intra-retinal hemorrhage, micro-aneurysms, macular edema, macular ischemia, and the appearance of cotton-wool spots in the ocular (retinal) region [4,5].

Vitamin A is an essential nutrient for vision function and it reaches the retinal layer with the highest concentration (3 millimolars) [6]. Primarily retinoids and pro-vitamin carotenoids act as pro-vitamin A, retinol, and retinoic acid and they support the neurosensory functions of the retina

****Corresponding author: Arunachalam Muthuraman***
arunachalammu@gmail.com
Received: 1/9/2024 Accepted: 3/11/2024.
DOI: https://doi.org/10.35516/jjps.v18i1.3271

[7]. The 11-cis-retinal form is mostly found in the retina and it is a crucial chromophore for all photoreceptor cells. These are also bound in the form of a Schiff base with rhodopsin in rod cells and photopsins in cone cells [8]. The deficiency of vitamin A is known to enhance retinopathy progression, especially in diabetic conditions [9]. Experimentally, the administration of vitamin A is reported to decrease the possibility of DR progression via control of multiple biochemical and molecular pathways [10].

The carotenoids *i.e.,* BC are a vitamin A precursor that acts on retinal chromophores and initiates photo-transduction, & maintains the retinal epithelial barrier functions [11]. A large quantity of beta carotene is present in carrots, tomatoes, spinach, sweet potatoes, broccoli, and winter squash including palm oil and their waste (palm oil mill effluent; POME) [12,13]. It produces therapeutic actions via free radical scavenging, anti-inflammatory, immune cell function, and regulation of vascular barrier functions [14]. Streptozotocin (STZ) is a natural alkylating agent (glucosamine-nitrosourea compound) and it induces diabetes mellitus and its complications via carbonium ions associated deoxyribonucleic acid (DNA) damages of islet β cells and other localized cells [15]. In experimental animals, DR is commonly produced by the administration of STZ [16]. The pathogenesis of DR is mostly caused by the development of retinal oxidative stress, inflammation, vascular dysfunction, accumulation of AGE products, and alteration of polyol pathways which leads to visual and cognitive dysfunctions [17–19]. The zebrafish is the finest model for assessment of diabetes mellitus and retinal & visual functions [20–22]. Zebrafish possess various significant advantages over mammalian animal models like rats and mice due to their rapid growth, easy handling, and clear visual functions [23]. Furthermore, the phenotypes of zebrafish closely resemble animal models and also mimic human diabetic microvascular complications like retinopathy [24]. Besides, the DR-associated behavioural assessment is easier in zebrafish than in rodents [25,26]. Moreover, beta carotene potentially attenuates diabetes-associated neuropathic pain in female zebrafish via inhibition of matrix metalloprotease-13 action [27]. The manifestation of diabetic complications like retinopathy is varied in male rats than female rats. The rate of diabetic complications development in female rats is only 30% [28]. However, the gender basis effects in the progression of DR in zebrafish models are not clear [29]. Generally, the female reproductive hormone i.e., estrogen possesses the potential neurovascular and retinal tissue-protective actions whereas testosterone induces the risk of chorioretinopathy [30]. Therefore, the current research work was planned to perform the vitamin A precursor *i.e.,* PBC action in STZ-induced DR in male adult zebrafish and the regulation of oxidative, inflammatory, and polyol pathways.

2. MATERIALS AND METHODS

2.1 Animals

The 8-month-old wild kind of male-grown zebrafish (**Danio rerio**) was utilized. Zebrafish were kept in 10 L of consumable water (in a fish tank). The fish tank contains a stone bubble aerator for the enrichment of dissolved oxygen in water; a glass thermometer for maintaining water temperature (26 ± 1 °C); and a light-emitting diode (LED) with a timer switch for providing the 14 hours light & 10 hours dark cycles environment. This study was supported by the Institutional Animal Ethics Committee (AUAEC/FOM/2022/01) and animal care was followed as per the rules of the Institutional Animal Ethics Committee. Animals were quarantined for 14 days before the initiation of the experiment. The neurobehavioural monitoring **i.e.,** optokinetic motor response (OMR) and startle response (SR) were evaluated as shown in the experiment protocol.

2.2 Drugs and chemicals

1,1,3,3-Tetra methoxy propane, 5,5-dithibis(2-nitrobenzoic acid), reduced glutathione, thiobarbituric acid, and streptozotocin (STZ) were purchased from Merck - Life Science, Darmstadt, Germany. PBC was

isolated as described in our previous article [13]. The positive control drug *i.e.,* dexamethasone sodium phosphate vial was purchased from Mylan Pharmaceutical Industry Company, Pennsylvania, United States.

2.3 Induction of diabetic retinopathy (DR)

The diabetes mellitus was initiated by the application of streptozotocin (STZ; 350 mg/kg) as portrayed by Olivares *et al.* [31] with minor changes of Wang *et al.* [32]. Briefly, zebrafish were anesthetized with an ice-cold water solution and 0.05 mL of STZ was administered intraperitoneally (**i.p.**) using an insulin syringe with a 27 gauge needle. The STZ stock solution was prepared by mixing 7 mg of STZ in 1000 µL of sodium citrate buffer (pH-5.5) solution. Thereafter, fish were kept in 1 % W/V glucose solution for 30 minutes to avoid hypoglycemic impact on fish health. After 24 hours, the zebrafish blood drops were collected by gentle application of a 1 mm outer-diameter glass capillary with a needle puller in the caudal vein location as described by Zang *et al.* [33]. The rise in blood glucose level was measured by utilizing a commercial glucometer. The level of blood glucose raised above 15 mM of glucose was considered a diabetic condition. The development of DR progression was raised by intravitreal (**i.vit.**) injection of STZ (20 µL of 7 % w/v of STZ stock solution) on the 7th day in diabetic animals under stereomicroscopic observation.

2.4 Experimental protocol

Five groups of healthy male zebrafish (*Danio rerio*; n = 20) were used in this research.

Group 1: Naive control group. Animals were not involved in the administration of any drugs and chemicals.

Group 2: DR control group: Animals were injected STZ (350 mg/kg; *i.p.* and 20 µL of 7 % w/v of STZ *i.vit.*) as described in the previous section.

Group 3: PBC (50) treatment group. Animals were exposed to PBC (50 mg/L) for 21 continuous days in DR-induced conditions from the 8th day of the study protocol. Dimethyl sulfoxide was used as a solvent for PBC solution preparation by sonication method.

Group 4: PBC (100) treatment group. Animals were exposed to PBC (100 mg/L) for 21 continuous days in DR-induced conditions from day 8 of the study protocol.

Group 5: Dexamethasone (10) treatment group. Animals were exposed to dexamethasone (DEX, 10 mg/L) for 21 continuous days in DR-induced conditions from day 8 of the study protocol. Ethanol was used as a solvent for DEX solution preparation. The summarized procedure of the investigation procedure is shown in Figure 1.

Thereafter, the neurobehavioural monitoring responses *i.e.,* optokinetic motor response (OMR) and startle response (SR) were evaluated on 0, 7, 14, and 21st days as described below (from the 7th day of the first STZ (*i.p.*) injection). On the 22nd day, every animals were used in the collection of blood, retina, and lens tissue for the evaluation of biomarker alterations like plasma homocysteine (HCY); retinal tissue thiobarbituric reactive substances (TBARS) [34]; reduced glutathione (GSH) [35]; tumor necrosis factor-alpha (TNF-α), superoxide dismutase (SOD) and total protein [36] levels. The lens was used for the assessment of polyol pathway markers *i.e.,* sorbitol dehydrogenase (SDH), aldose reductase (AR) activity, and total protein [36] levels as described in the following sections. The details of the study protocol and timelines of assessments are illustrated in the following Figure 2.

2.5 Assessment of zebrafish visual functions

The DR-induced alteration of visual functions *i.e.,* OMR and SR were assessed at variable time interval points *i.e.,* 0, 7, 14, and 21st days. OMR test is working based on stereotyped eye movement in response to the movement of objects in the surrounding environment. SR test response is working based on the generation of reaction to unexpected intense stimuli. The visual startle response is designed to elicit the eye blink reflex and sudden visual-motor response reflex. The information on the evaluation methods is described in the following segment.

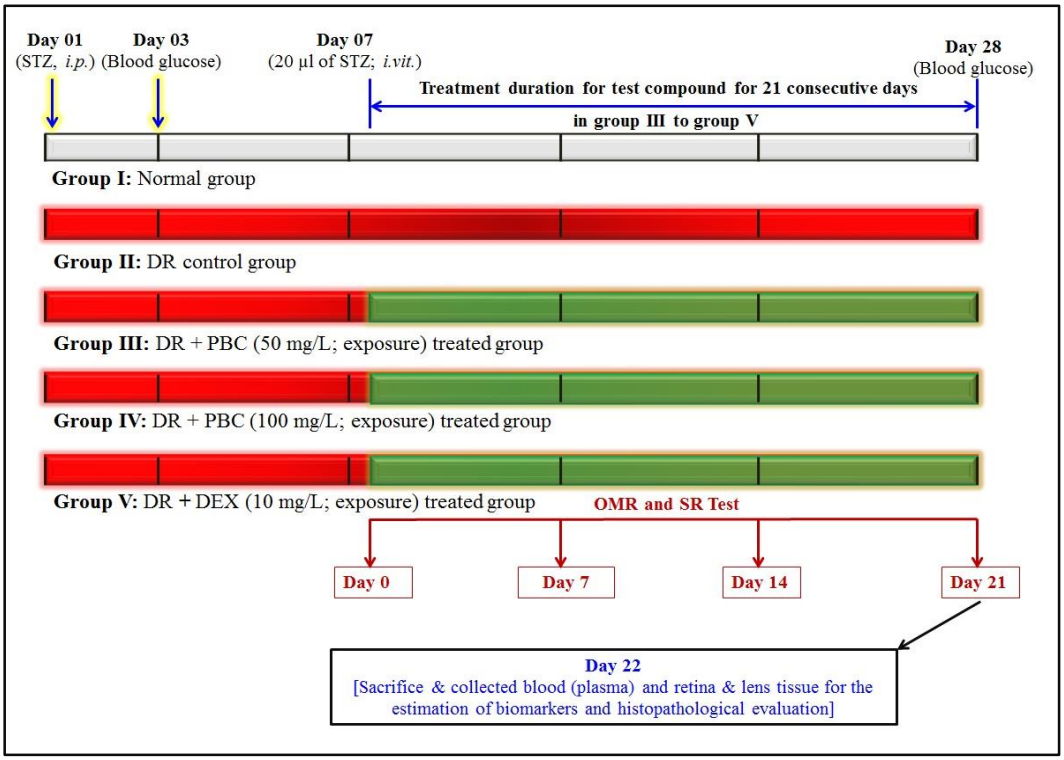

Figure 2: The details of the study protocol and timelines of assessments.

Abbreviations: DEX, dexamethasone; DR, diabetic retinopathy; *i.p.*, intraperitoneal; *i.vit.*, intra-vitreal; OMR, optomotor response; PBC, palm oil mill effluent derived beta carotene; SR, startle response; STZ, streptozotocin.

2.5.1 Evaluation of zebrafish OMR

The OMR test is adopted for the assessment of zebrafish visual acuity functions. It indicates the swimming capabilities against the moving black and white stripes as described by Fleisch and Neuhauss [37] with a slight modification of Fu *et al.* [38]. Briefly, the OMR apparatus has both concentric circular acrylic drums. The inner drum was made up of transparent acrylic sheets whereas the outer drum was made of non-transparent acrylic drum. The inner drum chamber space consisted of 120 mm inner diameter and 100 mm of height. At the center of the inner chamber, a 30 mm diameter and 70 mm height pole was fixed so that fish were able to swim in the space between the center pole and the inner drum wall. The inner drum was with filled water for 30 mm of height. The outer drum consists of 200 mm of width and 100 mm of height and it has 10 mm of width and 80 mm of height vertical slits alternatively. The outer drum was covered with a dark chamber and the inside was fixed with white LED lights. The outer drum was connected to 10 revolutions per minute (RPM) speed (CW, Clockwise and CCW, Counter Clockwise) of a 220V direct current (DC) synchronous motor. It acts as black-and-white strips of movement object stimuli to explore the zebrafish's visual behaviour response in the OMR test as described by Ninkovic and Bally-Cuif [39]. During the OMR assessment, the room light intensity was maintained at 300 lux at the water surface level.

Basel training was given to all the zebrafish one day before the investigation. The zebrafish were put into the

middle chamber with all basic environmental conditions for 3 minutes without rotation of the outer drum. Thereafter, the clockwise revolution of the external chamber was begun to evaluate the fish swimming developments against the visual strips stimuli movement. The Basel preparing preliminary was stopped when the fish could complete five circles. The OMR visual acuity test was performed on 0, 7, 14, and 21st days; by measuring the fish swimming movements against visual stimuli by clockwise and counterclockwise rotation of the outer chamber. The rising number of cycles finished by 4 4-minute periods (2 minutes of clockwise and 2 minutes of counterclockwise rotations of the outer chamber) was noted as spatial frequency threshold (SFT) in the OMR test. The decline in the SFT indicates the loss of visual acuity functions with a correlation of rods and cone cell dysfunctions. Unerringly, zebrafish are more delicate to visual boosts at sunset than at sunrise[26]. Hence, the OMR test was carried out between 3.00 PM to 4.00 PM. The optokinetic motor apparatus for the evaluation of zebrafish visual acuity functions is shown in Figure 3.

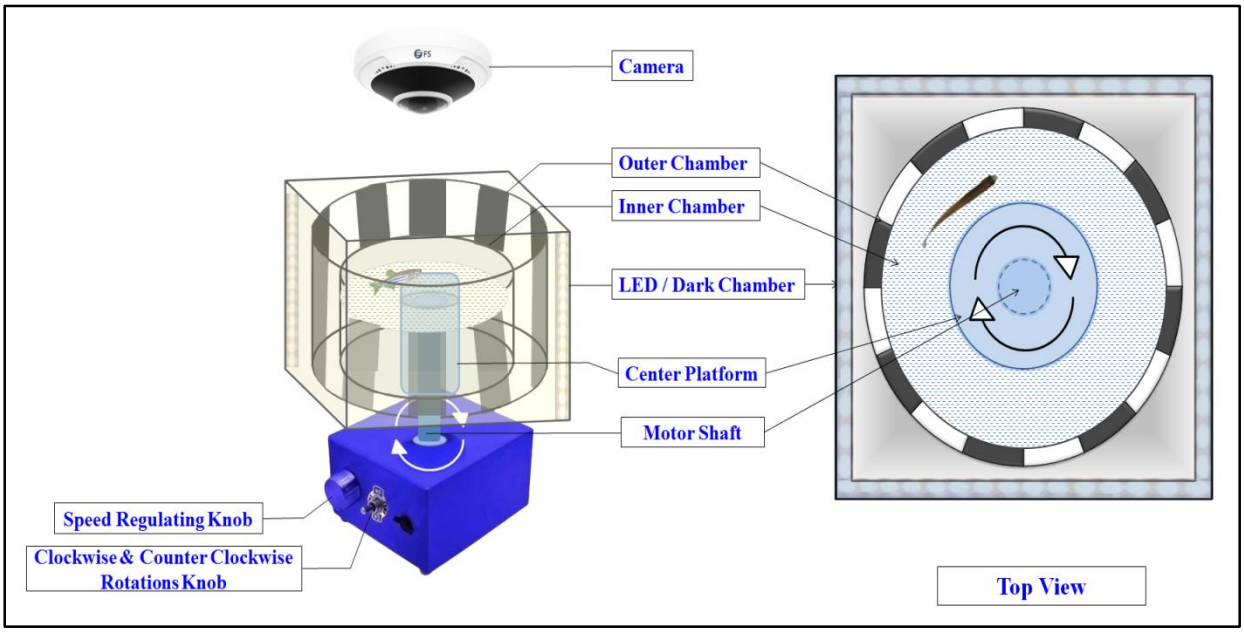

Figure 3: Illustration of OMR response test device for the assessment of zebrafish visual acuity functions.

The above figure helps to understand the design of the optokinetic motor response test apparatus. The response of fish movements was recorded by using a CCTV camera at a top angle and recorded video was used for further analysis of OMR responses.

2.5.2. Assessments of zebrafish startle response (SR)

Zebrafish readily react to unexpected stimuli as startle response (SR). In this condition, animals are showing erratic movements as narrated by Burton *et al.* [40] with a slight change to Wang *et al.* [32]. Shortly, the SR test chamber has a transparent square (25 x 25 cm) chamber with a 10 cm height. The water was topped up to 5 cm. The back side of the chamber (outer surface) was surrounded with waterproof graph paper. The recorder implement was placed at the front side of the SR test apparatus for the noting of animal movements. The animals were allowed to adapt for a 3 3-minute period in

this chamber. Basel swimming behavior was recorded by the count of line passing in 2 2-minute periods. The application of startle stimuli *i.e.*, continuous flashing light (every 10 seconds) by LED strobe light device (300 lux) for 2 minutes was applied. The unusual changes in body position and increase in erratic swimming patterns were noted. The SR recorded the count of line crossings in between 4-minute duration. The SR test was performed in the interval of 4.00 PM to 5.00 PM. The percentage startle response was calculated by using the following formula and the SR test device is illustrated in Figure 4.

$$\%SR = \frac{Number\ of\ line\ crossing\ after\ light\ exposure}{Number\ of\ line\ crossing\ before\ light\ exposure}\ X\ 100$$

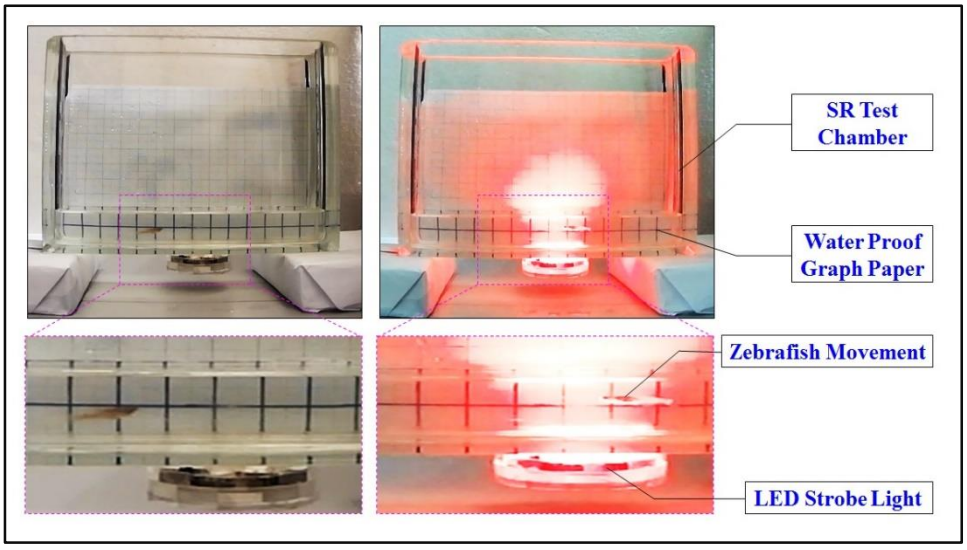

Figure 4: Illustration of SR test device for the assessment of %SR against unexpected flashlight stimuli. *Abbreviations:* SR, startle response; LED, light-emitting diode.

2.6 Estimations of plasma HCY biomarker changes

The blood tests were gathered on the 21st day from zebrafish according to the explanation of Pedroso *et al.* [41] with minor changes from Wang *et al.* [32]. Briefly, the animal was placed in an ice-cold solution (as an anesthetic solution). The diagonal cut was made between anal and caudal fin. Thereafter, the animal was placed in a 2 mL Eppendorf tube which contains 0.1 mL of 11% w/v of sodium citrate solution (as an anti-coagulant). The tubes were vortexed at 112 g centrifugation force for 10 minutes at 4°C. The gathered plasma samples were diluted with sodium citrate solution (11 % w/v; 1:4 ratios) for the estimations of plasma HCY levels.

The plasma HCY levels were estimated by using the commercial HCY ELISA kit (Abbexa Ltd., Cambridgeshire, United Kingdom). Briefly, the 0.1 mL of plasma samples were applied in a microplate reader (ClaIR™, Photon Etc., Montreal, QC, Canada) and mixed with 100 µL of detection reagent A and incubated for 1 hour at 37 °C. Then, the plate was rinsed and discorded with washing buffer. The washing process was done three times. Thereafter, 0.1 mL of detection reagent B was added to the plate and incubated for 1 hour at 37 °C. Then, the washing process was applied five times. Crucially, 90 µl of 3,3′,5,5′-Tetramethylbenzidine (TMB) chromogenic substrate was added into each well and incubated at 37 °C. The plate was placed dark chamber to avoid exposure to the light. The reaction was stopped with a mixing of 0.05 mL of stop solution after 20 minutes of incubation period. The standard plot of HCY was prepared with 0, 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 ng/mL of HCY standard. The changes of absorbance were measured immediately at 450 nm wavelength. The HCY content of plasma was calculated by using the following formula.

$$Relative\ Absorbance\ (OD) = OD_{Each\ Well} - OD_{Zero\ Well}$$

The standard plot was made with relative OD value in the X axis as the reference standard and respective HCY concentration of each standard solution in the Y axis. Test sample concentrations were calculated by using the HCY standard plot and multiplied by the dilution factor. The collection of zebrafish plasma sample procedure is illustrated in Figure 5.

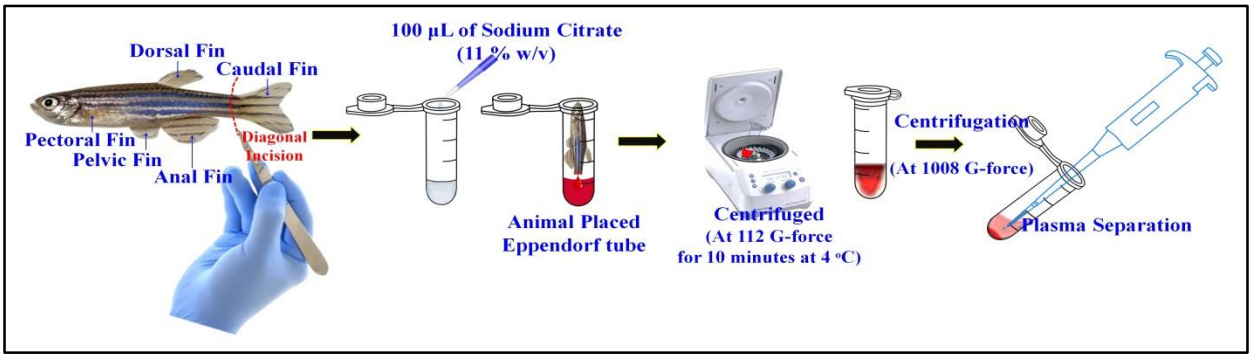

Figure 5: The procedure for the collection of zebrafish plasma samples.

The above illustration reveals the steps involved in the collection of plasma samples for the assessment of biomarker changes.

2.7 Estimations of zebrafish retinal and lens tissue biomarker changes

Zebrafish retina and lens samples were collected and homogenized with a phosphate buffer (pH 7.4) solution. The homogenized samples were vortexed to 1372 g force for 15 minutes at 4 ºC. The retinal tissue samples were used for the quantification of TBARS [34]; GSH, [35]; SOD (Cayman chemical superoxide dismutase assay kit); and TNF-α (ELISA kit method) levels. The aliquots of lens tissue were used for the assessment of SDH (Abcam SDH activity assay kit) and AR (Abcam AR activity assay kit) activity and total protein [36] levels as described in the following sections. The collection of zebrafish retina and lens samples is illustrated in Figure 6.

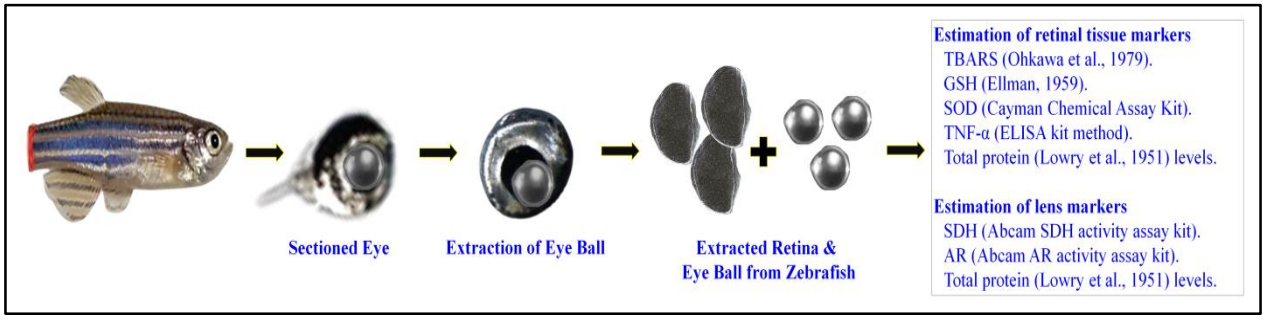

Figure 6: The collection of zebrafish retina and lens samples for the assessment of tissue biomarker changes.

The retina and lens samples help to determine the tissue oxidative stress markers (TBARS, GSH, and SOD); inflammatory biomarker (TNF-α); and polyol pathway markers (SDH and AR) levels. *Abbreviations:* TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha; SDH, sorbitol dehydrogenase; and AR, aldose reductase.

2.7.1 Estimation of retinal TBARS content

TBARS is one of the major markers of oxidative stress. The free radicals make the peroxidation of lipids (cell membrane) leading to the malondialdehyde (MDA) products. The level of tissue TBARS content was quantified as narrated by Ohkawa *et al.* [34]. Briefly, 0.2 mL of retinal tissue aliquot was disparate with 0.2 mL of sodium hydroxide (8.1%) solution; 0.2 mL of sodium dodecyl sulfate (8.1%) solution; 1.5 mL of acetic acid (30%) solution; and 1.5 mL of 2-Thiobarbituric acid (TBA: 0.8%) solution. The final volume was made with 4 mL of tribble distilled water. Thereafter, test tubes were kept at a 90 °C conditioned environment for 1 hour. Thereafter, 100 µL of distilled water was mixed and vortexed at 1792 G-force for 10 minutes. The pink-coloured chromogen absorbance variations were recorded with a spectrophotometer (DU 640B Spectro-photometer, Beckman Coulter Inc., Brea, CA, USA) instruments at 532 nm wavelength. The standard plot was made with 0 - 1000 nano-moles of 1,1,3,3-Tetramethoxypropane per mL solutions. The level of lipid peroxidation was indicated as nmol of MDA molecules per mg of protein.

2.7.2 Estimation of retinal GSH content

GSH is a non-enzymatic endogenous antioxidant molecule and it scavenges the free radicals. Hence, it is also known as a non-enzymatic oxidant marker. The tissue GSH content levels were quantified as narrated by Ellman [35]. Briefly, the aliquot was prepared by homogenate of tissue with 10% w/v of tri-chloroacetic acid (1:1 ratio) solution. The mixtures were vortexed at 4 °C at 112 G-force for 10 minutes. About 500 µL of aliquot was disparated with 2 mL of disodium hydrogen phosphate (0.3 M) solution and 0.25 mL of 0.001 M of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB or Ellman's reagent) solutions. Here, DTNB solution was prepared by using sodium citrate (1% w/v) solution. The changes in yellow chromogen absorbance were measured using a spectrophotometer (DU 640B Spectrophotometer, Beckman Coulter Inc., Brea, CA, USA) instruments at 412 nm wavelength. The standard plot was made with 0 - 100 micromoles of GSH per mL. The level of retinal tissue GSH was expressed as µmol of GSH per mg of protein.

2.7.3 Estimation of retinal SOD activity

SOD is an enzymatic endogenous antioxidant molecule and it makes the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide. Hence, it is also known as an enzymatic oxidative stress marker. The retinal tissue SOD content levels were estimated as described in superoxide dismutase assay kit (Cayman Chemical, Michigan, United States). Briefly, about 10 µL of aliquot was disparate with 0.2 mL of radical detector and 20 µL of xanthine oxidase. The microplate was maintained at 37 °C for 30 minutes with a shaker. The changes in absorbance were measured using a microplate reader (ClaIR™, Photon ETC., Montreal, QC, Canada) instruments at 450 nm. The A standard plot was prepared with 0 - 200 µL of SOD per mL.

$$SOD\ (U/ml) = \left[\left(\frac{Sample\ Linearized\ SOD\ Rate - Y_{Intercept}}{Slope}\right) x\ \frac{Well\ volume}{Sample\ volume}\right] x\ DF$$

Here, SOD is a superoxide dismutase; and DF is a dilution factor. The level of retinal tissue SOD was indicated as a unit per mg of protein.

2.7.4 Estimation of retinal TNF-α content

The retinal tissue TNF-α content level was estimated by using the commercial TNF-α ELISA kit (Assay Genie, Dublin, Ireland). Briefly, the 100 µL of tissue aliquots were placed in a microplate reader (ClaIR™, Photon Etc., Montreal, QC, Canada) and maintained at 37 °C for 2.5 hours. Then, the microplate was rinsed out with washing buffer. Then, 0.1 mL of biotin antibody was added, and maintained the plate at 37 °C for 1 hour. The second step of washing was to make the microplate wells, followed by mixing the 100 µL of Streptavidin solution & incubating it

with gentle shaking for 45 minutes at 37 °C. Crucially, the microplate washing was made with a washing buffer solution. At the end, 0.1 mL of TMB substrate solution was mixed and the plate was maintained at room temperature for 30 minutes. Thereafter, the stop solution (50 µl) of the reaction was added to each well. The standard plot of TNF-α was prepared with 0, 0.041, 0.102, 0.256, 0.64, 1.6, 4, and 10 ng/mL of TNF-α standard. The variations of absorbance were recorded at 450 nm wavelength. The plasma TNF-α content was calculated by implementing the following formula:

$$Relative\ Absorbance\ (OD) = OD_{Each\ Well} - OD_{Zero\ Well}$$

The standard plot was made with relative OD value in the Y axis as the reference standard and respective TNF-α concentration of each standard solution in the X axis. The concentration of the test sample was calculated from the TNF-α standard curve and multiplied by the dilution factor.

2.7.5 Determination of SDH activity

The lens SDH activity level was estimated as described in the SDH activity assay kit (Abcam Limited., Cambridge, United Kingdom). Briefly, the 0.05 mL of fish lens aliquot was disparated with 50 µL with SDH assay buffer and 50 µL of reaction mixtures. It consists of 50 µL of NADH (1 mM) standard solution; 250 µL of d-Fructose (1.1 M); and 117.5 µL of triethanolamine buffer (100 mM: pH 7.6). The standard plot was made with 0, 2, 4, 6, 8, & 10 nmol of NADH standard. The absorbance changes were noted immediately at 450 nm wavelength; every 20 seconds for 60 minutes at 37 °C. The SDH activity of the fish lens was calculated by using the following formula:

$$SDH\ activity = B\ /\Delta t\ x\ V\ x\ DF$$

Here, B is an NADH content from the standard plot (in nmol); Δt is the reaction time (in minutes); V is a sample volume (in mL); and DF is a dilution factor. The SDH activity level was indicated as mU per mg of protein.

2.7.6 Determination of AR activity

The AR activity level was determined as described in the AR activity assay kit (Abcam Limited., Cambridge, United Kingdom). Briefly, the 50 µL of fish lens aliquots was disparated with 30 µL of phosphate buffer (0.067 M); 60 µL of diluted nicotinamide adenine dinucleotide phosphate (NADPH); 10 µL of DL-glyceraldehyde (5×10^{-4} M). The standard plot was prepared with 0, 20, 40, 60, 80, and 100 nmol of NADPH standard. The variations of absorbance changes were recorded immediately at 340 nm wavelength every 20 seconds for 60 minutes at 37 °C using a UV/Visible spectrophotometer (DU 640B Spectrophotometer, Beckman Coulter Inc., California, United States) instrument. The AR activity was calculated by using the following formula:

$$AR\ activity = \frac{B_{Test} - B_{Sample\ Control}}{\Delta t\ x\ M}$$

Here, B is an NADPH amount from the standard curve (in nmol); Δt is a reaction time (in minutes); M is a total protein (in mg), and D is a dilution factor. The AR activity level was indicated as mU/mg protein.

2.7.7 Estimation of total protein levels

The tissue total protein was estimated as narrated by Lowry's method [36]. Briefly, about 10 µL of aliquot was disparated with 10 µL alkaline copper sulfate and conditioned at 37 °C for 10 minutes. Thereafter, 100 µL of Folin's phenol reagent was mixed and conditioned at 37 °C for 10 minutes. The standard plot was prepared with 0, 0.125, 0.25, 0.50, 0.75, 1.0, 1.5, and 2.0 mg of bovine serum albumin (BSA) per mL. The changes in absorbance were measured at 640 nm using a microplate reader (ClaIR™, Photon Etc., Montreal, QC, Canada) instrument. The concentration of tissue total protein was expressed as mg of protein per mL.

2.8 Statistical data analysis

Data were indicated as mean ± S.D. The OMR and SR behavioural test results were analyzed using a two-way analysis of variance (ANOVA) and succeeded by the

Bonferroni post hoc comparison test after the confirmation of the normality test. The biochemical marker changes were examined by one-way ANOVA and succeeded by Tukey's multiple-range tests. This data analysis was made by GraphPad Prism version 5.0 software (Dotmatics, R&D scientific software company, San Diego, United States). A statistically significant was confirmed when the probability (p) value of less than 0.05 ($p < 0.05$).

3. RESULTS

3.1 Effect of PBC in the zebrafish OMR Test

The intravitreal injection of STZ in diabetic animals showed a significant ($p < 0.01$) statistical difference for visual acuity impairment in the OMR test as a sign of reduction of SFT values when differentiated from the normal control group. It indicates that blood-retina barrier dysfunctions were achieved in DR animals by the administration of STZ. The exposure of PBC (50 and 100 mg/L) for 21 continuous days in DR-induced animals showed significant attenuation of the SFT value in a dose-dependent manner (all $p < 0.001$ *vs.* DR group). It is equivalent to the DEX (10 mg/L) treatment group. The result of the OMR test indicates that PBC possesses ameliorative effects on visual acuity functions. The result of the OMR test is depicted in Figure 7.

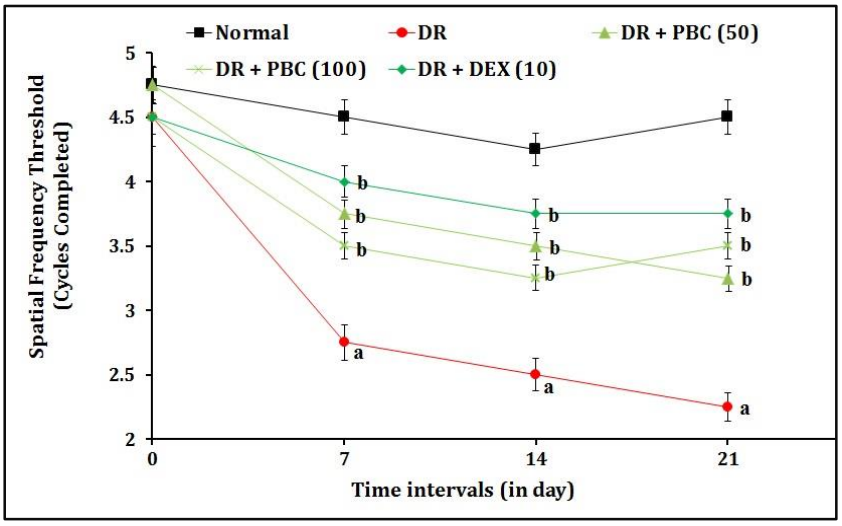

Figure 7: Effect of PBC on STZ-induced DR in OMR test.

The digits in parentheses act for a dose of mg/L. The data were expressed as standard deviation (SD; $n = 20$). $^{a}p < 0.05$ versus the control group; $^{b}p < 0.05$ versus the DR group. *Abbreviations:* DEX, dexamethasone; DR, diabetic retinopathy; PBC, palm oil mill effluent-derived beta-carotene; and SFT, spatial frequency threshold.

3.2 Effect of PBC in the zebrafish SR Test

The intravitreal injection of STZ in diabetic animals showed a significant ($p < 0.01$) statistical difference for erratic movements (as visual impairments) in the SR test as a sign of a reduction of %SR values when differentiated from the normal control group. The exposure of PBC (50 and 100 mg/L) for 21 continuous days in DR-induced animals showed significant attenuation of the %SR value in the SR test in a dose-dependent manner (all $p < 0.001$ *vs.* DR group). It is equivalent to the DEX (10 mg/L) treatment group. The result of the SR test indicates that PBC possesses ameliorative effects on visual impairments. The result of the SR test is depicted in Figure 8.

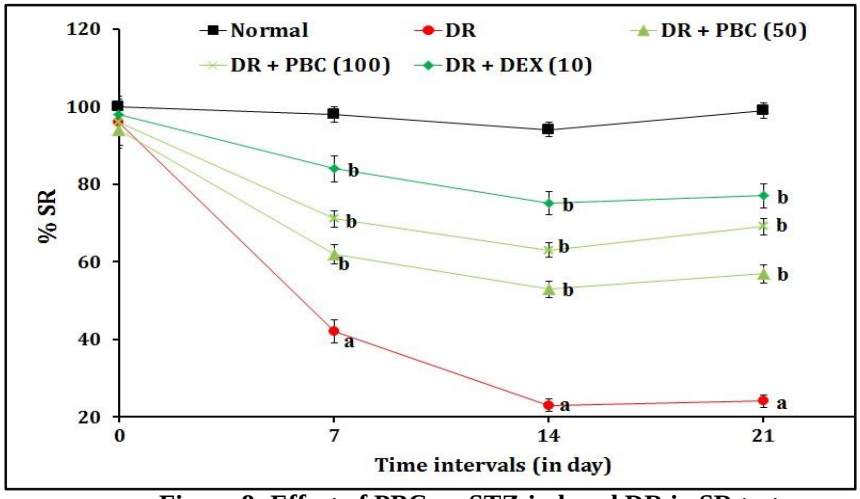

Figure 8: Effect of PBC on STZ-induced DR in SR test.

The digits in parentheses act for a dose of mg/L. The data were expressed as standard deviation (SD; $n = 20$). $^{a}p < 0.05$ versus the control group; $^{b}p < 0.05$ versus the DR group. *Abbreviations:* DEX, dexamethasone; DR, diabetic retinopathy; PBC, palm oil mill effluent-derived beta-carotene; and SR, response.

3.3 Effect of PBC on blood glucose level changes

The intravitreal injection of STZ in diabetic animals showed a significant ($p < 0.01$) statistical difference for the rising of blood glucose levels on days 1, 3, 7, and 28 when differentiated from the normal control group. It indicates that hyperglycemia was achieved and maintained with DR progression by the administration of STZ. The exposure of PBC (50 and 100 mg/L) for 21 continuous days in DR-induced animals showed significant attenuation of the elevated blood glucose value on day 28 in a dose-dependent manner (all $p < 0.001$ *vs.* DR group). It is equivalent to the DEX (10 mg/L) treatment group. The result of blood glucose level indicates that PBC possesses ameliorative potential for diabetic conditions. The result of the blood glucose level changes is expressed in Table 1.

Table 1: Effect of PBC on STZ-induced blood glucose level changes.

Groups	Day 0m (mmol/L)	Day 3 (mmol/L)	Day 8 (mmol/L)	Day 28 (mmol/L)
Normal	5.9 ± 0.4	6.2 ± 0.8	5.8 ± 0.4	6.3 ± 0.6
DR	6.4 ± 0.6	22.5 ± 1.1[a]	24.3 ± 0.7[a]	28.3 ± 1.4[a]
DR + PBC (50)	6.1 ± 0.7	23.7 ± 1.3[a]	22.2 ± 1.1[a]	12.4 ± 1.3[b]
DR + PBC (100)	6.2 ± 0.3	21.4 ± 0.9[a]	25.4 ± 1.0[a]	10.6 ± 0.9[b]
DR + DEX (10)	5.8 ± 0.8	22.3 ± 1.2[a]	23.5 ± 1.3[a]	9.4 ± 1.2[b]

Numbers in parenthesis indicate dose mg/kg. Data were expressed as standard deviation (SD; n = 20). $^{a}p < 0.05$ Vs normal group. $^{b}p < 0.05$ Vs STZ control group. *Abbreviations:* DEX, dexamethasone; DR, diabetic retinopathy; and PBC, palm oil mill effluent-derived beta-carotene

.3.4. Effect of PBC on STZ-induced plasma HCY level changes

The intravitreal injection of STZ in diabetic animals showed a significant ($p < 0.01$) statistical difference for the rising of HCY levels when differentiated from the normal control group. It indicates that plasma HCY contributed to the progression of DR by the administration of STZ. The exposure of PBC (50 and 100 mg/L) for 21 continuous

days in DR-induced animals showed significant attenuation of the above plasma HCY level changes in a dose-dependent manner (all $p < 0.001$ *vs.* DR group). It is equivalent to the DEX (10 mg/L) treatment group. The result of plasma HCY level changes indicates the PBC possesses the ameliorative potential in the regulation of diabetes-associated metabolic vascular toxin (HCY) accumulation. The result of the plasma HCY level changes were expressed in Figure 9.

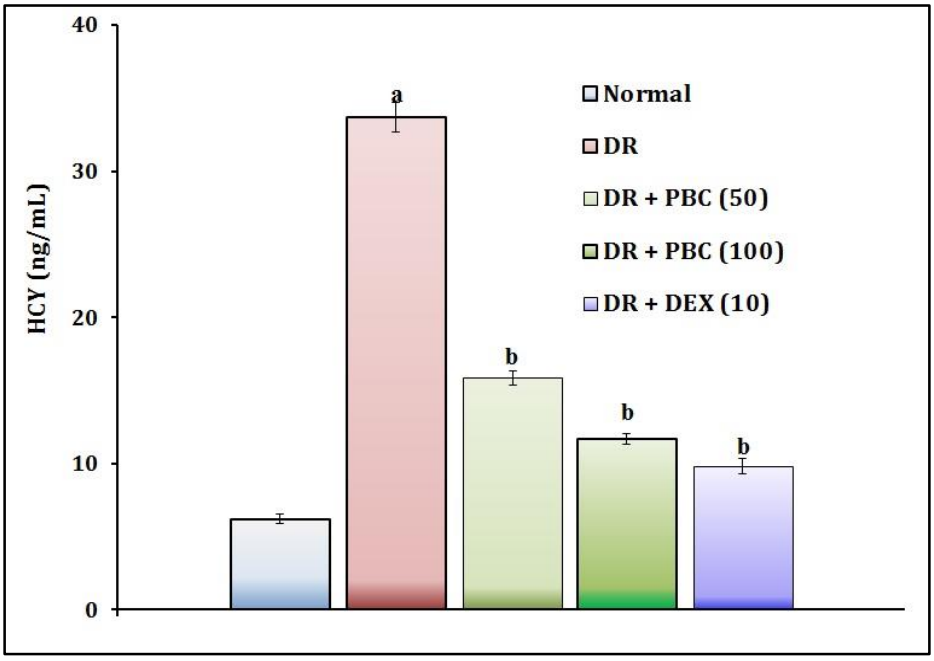

Figure 9: Effect of PBC on STZ-induced plasma HCY level changes.

The digits in parentheses represent a dose of mg/L. The data were expressed as standard deviation (SD; $n = 20$). $^{a}p < 0.05$ versus the control group; $^{b}p < 0.05$ versus the DR group. *Abbreviations:* DEX, dexamethasone; DR, diabetic retinopathy; PBC, palm oil mill effluent-derived beta-carotene; and HCY, homocysteine.

3.5. Effect of PBC on STZ-induced retinal tissue biomarker changes

The intravitreal injection of STZ in diabetic animals showed a significant ($p < 0.01$) statistical difference for the rising of TBARS, TNF-α levels, and reduced levels of GSH and SOD when differentiated from the normal control group. The exposure of PBC (50 and 100 mg/L) for 21 continuous days in DR-induced animals showed significant attenuation of the above changes of retinal tissue biomarkers in a dose-dependent manner (all $p < 0.001$ *vs.* DR group). It is equivalent to the DEX (10 mg/L) treatment group. The result of retinal tissue biomarker changes indicates the PBC possesses ameliorative potential in the regulation of diabetes-associated oxidative stress and inflammatory reactions. The results of the retinal biomarker differences are expressed in Table 2.

Table 2: Effect of PBC on STZ-induced retinal tissue biomarker changes.

Groups	TBARS (nmol/mg of Protein)	GSH (µmol/mg of Protein)	SOD (U/mg of Protein)	TNF-α (ng/mg of Protein)
Normal	1.52 ± 0.02	22.32 ± 1.1	54.21 ± 1.1	3.17 ± 0.03
DR	3.76 ± 0.06[a]	6.87 ± 1.3[a]	7.93 ± 1.6[a]	48.82 ± 0.09[a]
DR + PBC (50)	2.27 ± 0.07[b]	14.94 ± 1.5[b]	28.76 ± 0.9[b]	13.59 ± 0.08[b]
DR + PBC (100)	2.04 ± 0.03[b]	11.85 ± 0.9[b]	20.38 ± 1.2[b]	9.63 ± 0.12[b]
DR + DEX (10)	1.71 ± 0.05[b]	8.27 ± 1.2[b]	13.71 ± 1.4[b]	5.73 ± 0.08[b]

Digits in parentheses indicate a dose of mg/L. Data were expressed as standard deviation (SD; n = 20). [a]$p < 0.05$ Vs normal group. [b]$p < 0.05$ Vs DR group. *Abbreviations:* DEX, dexamethasone; DR, diabetic retinopathy; GSH, reduced glutathione; PBC, palm oil mill effluent-derived beta-carotene; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; and TNF-α stands for tumor necrosis factor-alpha.

3.6. Effect of PBC on STZ-induced lens tissue biomarker changes

The intravitreal injection of STZ in diabetic animals showed a significant ($p < 0.01$) statistical difference for the rising of SDH and AR levels when differentiated from the normal control group. It indicates that STZ alters the polyol pathway with the progression of DR. The exposure of PBC (50 and 100 mg/L) for 21 continuous days in DR-induced animals showed significant attenuation of the above variation of biomarkers in a dose-dependent manner (all $p < 0.001$ *vs.* DR group). It is equivalent to the DEX (10 mg/L) treatment group. The result of lens tissue biomarker variations indicates the PBC possesses ameliorative potential in the regulation of diabetes-associated polyol pathways alteration. The result of the lens tissue biomarker changes were expressed in Figure 10.

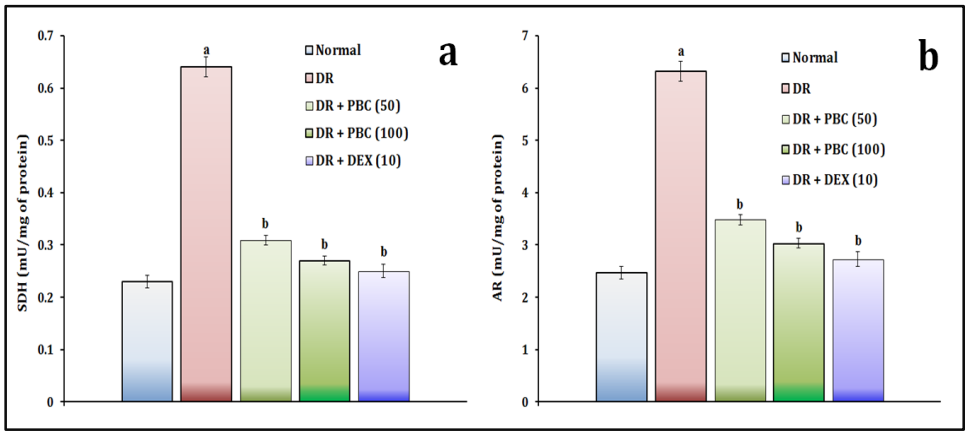

Figure 10: Effect of PBC on STZ-induced lens tissue biomarker changes.

Panel A indicates the SDH activity and panel B indicates the AR activity. The digits in parentheses represent a dose of mg/L. The data were expressed as standard deviation (SD; $n = 20$). [a]$p < 0.05$ versus the control group; [b]$p < 0.05$ versus the DR group. *Abbreviations:* AR, aldose reductase activity; DEX, dexamethasone; DR, diabetic retinopathy; PBC, palm oil mill effluent-derived beta-carotene; and SDH, sorbitol dehydrogenase activity.

4. DISCUSSION

The intravitreal injection of STZ in diabetic animals showed statistically significant ($p < 0.05$) changes in DR-associated visual-motor reflex changes via blood-retina barrier dysfunctions which are assessed by OMR and SR tests. Furthermore, it also alters the plasma (glucose and HCY) levels), and tissue biomarkers (TBARS, GSH, TNF-α, SOD, SDH, and AR activities) which indicate the amelioration of DR-associated oxidative stress, inflammatory reactions, and alteration of polyol pathways. However, the exposure of PBC (50 and 100 mg/L) and DEX (10 mg/L) for 21 continuous days showed significant betterment of STZ-induced DR and their blood-retina barrier dysfunctions and changes in biomarkers. STZ is known to induce diabetes mellitus along with the induction of local inflammatory reactions via the production of reactive methylcarbonium ions which alkylate the cellular DNA content and lead to cell damage [13,42]. Experimentally, the *i.vit.* administration of STZ in zebrafish is known to accelerate the DR progression and alter the visual acuity functions [32]. The interchangeable results were noted in this investigation *i.e.*, decreasing the SFT value in the OMR test and reducing the percentage startle response value in the SR test in 7, 14, and 21st days with the administration of PBC (50 and 100 mg/L) and DEX (10 mg/L) treatments.

In diabetic conditions, circulatory glucose readily reacts with metabolic pathways leading to accumulating the AGE products which leads to enhanced oxidative stress in retinal tissue [43]. The retinal tissue is highly sensitive to free radicals associated with oxidative damage and it plays a pivotal part in the subsequent changes of inflammatory reactions via generation of inflammatory cytokines (mainly TNF-α). The combined action of oxidative stress and inflammatory cytokines enhances the acceleration of cellular DNA damage and the development of DR [44]. A similar effect was observed in the present study, the exposure of PBC and DEX attenuated the STZ-associated TBARS, GSH, TNF-α, and SOD levels. DEX is a steroidal (glucocorticoid) medication and it is widely used for the treatment of various inflammatory disorders including DR via the reduction of hyperglycemic & oxidative stress; and inflammatory reactions [45]. It is a multi-targeted medicine, similarly, natural medicine also possesses the multi-targeted action without harmful actions to the body [46]. The abundant free radicals also interact with the cellular lipid membrane and make the lipid peroxidation which leads to cell damage [47]. Furthermore, free radical generation in diabetic animals is known to alter the enzymatic oxidative defensive system in the retina [48]. Similarly, our study also found that STZ-induced DR was shown to reduce the SOD activity levels in retinal tissue.

DR is one of the metabolic complications in diabetes mellitus which is mainly due to the alteration of polyol pathways [49]. The markers of polyol pathways are SDH and AR activities. The rising of the SDH and AR activities indicates the worsening of retinal tissue due to the activation of polyol pathways [50,51]. The changes in polyol pathways in DR are observed in lens tissue [52]. The current results revealed that STZ induces the DR complication via the rising of the SDH and AR activities. However, the multi-targeted natural medicine *i.e.*, PBC attenuates the DR via regulation of polyol pathways. PBC possesses glucose regulatory actions via the regulation of oxidative stress and inflammatory reactions [13]. Besides, our previous publication shows that BC possesses the ameliorative potential of DR via regulation of oxidative stress markers i.e., reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARSs), and catalase activity in diabetic mice [27]. Further, BC also possesses an ameliorative effect against the α-amanitin-induced nephrotoxicity via polyol pathways in rat kidneys [53]. Moreover, BC attenuates eye diseases like cataracts, age-related macular degeneration, and DR via free radical scavenging, anti-apoptosis, and regulation of mitochondrial function and inflammation [54]. The polyol pathway mechanism is reported in the progression of DR

[55]. However, there is no direct connection was reported between the BC action in the progression of DR via polyol pathway in experimental animal models (rodents & zebrafish). This is the first research report that shows evidence that BC produces the ameliorative action of DR in the zebrafish model via the polyol pathway mechanism.

Carotenoids consist of 8-isoprene units $(C5H8)_8$ skeleton (tetraterpene pigments) and it is a natural lipophilic compound present in plants (*i.e.,* (corn, carrots, oranges, and tomatoes); and microorganisms (*i.e.,* fungi, algae, and bacteria). Carotenoid compounds are known to possess potential therapeutic actions in DR [56]. The molecular mechanism of PBC is known to inhibit the TNF-α associated induced epithelial cell injury [57]. Furthermore, beta-carotene is also known to inhibit the SOD via regulation of mitogen-activated protein kinases (MAPK), nuclear factor–kappa B (NF-κB), and nuclear factor erythroid 2-related factor 2 (Nrf2) signaling [58]. However, the exact action of PBC action in the regulation of polyol pathways has not been reported yet [59][59][58][58][57][57][57][59]. Hence it is the first report that, PBC possesses the regulatory actions on polyol pathways. Moreover, PBC also converts to retinal (vitamin A) in the retina with the help of β-carotene cleavage enzyme *i.e.,* β-carotene oxygenase 1. Hence, the pro-vitamin A or vitamin A precursor *i.e.,* beta-carotene attenuates the diabetes mellitus-associated visual impairments. Hence, it can be utilized for the treatment of DR because of its multi-targeted therapeutic response. However, substantial research is expected to investigate the involvement of polyol and other pathways in the regulation of DR pathological conditions in higher vertebrate animal models before being in clinical trials. The summary of PBC molecular action in the amelioration of STZ-induced DR in zebrafish animal model has been illustrated in Figure 11.

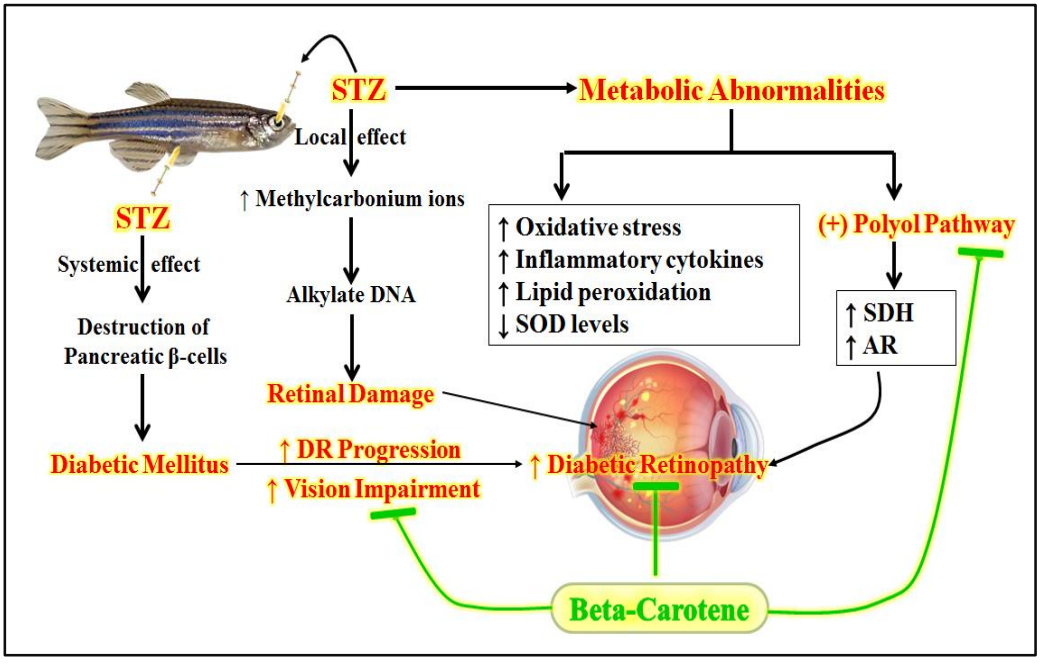

Figure 11: Molecular actions of PBC in the amelioration of STZ-induced DR in zebrafish animal model.

Abbreviations: AR, aldose reductase; DNA, deoxyribonucleic acid; DR, diabetic retinopathy; SDH, sorbitol dehydrogenase; SOD, superoxide dismutase; and STZ, streptozotocin.

CONCLUSIONS

The current study results revealed that the exploration of PBC attenuates the DR-associated visual acuity responses in the OMR and SR tests together with changes in biomarkers *i.e.*, the elevation of GSH & SOD activity levels; decrease of plasma glucose & HCY; and tissue TBARS, TNF-α, SDH & AR levels. Hence, PBC can be used for the treatment of diabetes mellitus-associated microvascular obstructions and dysfunctions because of its anti-oxidative, and anti-inflammatory actions; and regulation of metabolic toxin & polyol pathways. Nevertheless, this study will reach out to investigate retinal–barrier functions with connection to endothelial growth factor and neutrophil recruitment pathways for the effective therapeutic management of DR progression.

Acknowledgment

This research work was funded by the Malaysian Ministry of Education through the Fundamental Research Grant Scheme, FRGS/1/2021/SKK0/AIMST/03/4. We are thankful to the Malaysian Ministry of Education, Malaysia, and the Research Management Center, AIMST University, Malaysia.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research work was supported by the Malaysian Ministry of Education through the Fundamental Research Grant Scheme, FRGS/1/2021/SKK0/AIMST/03/4.

REFERENCES

1. Gupta S. and Thool A. R. A Narrative review of retinopathy in diabetic patients. *Cureus*. 2024; 16(1).
2. Chen J., Zhuo X., Zhu Y. and Zhuo Y. Multidisciplinary approaches in the treatment of retinal degenerative diseases: A review. *Adv. Therap*. 2024; 7(2):2300162.
3. Oshitari T. Advanced glycation end-products and diabetic neuropathy of the retina. *Int. J. Mol. Sci*. 2023; 24(3):2927.
4. Srivastava N. Diabetes and the retinal changes in the eye: A threat to the sight. *Int. J. Community Med. Public Health*. 2024; 11(2):1030.
5. Al Saad M., Shehadeh A., Abu Ameerh M. A., Meqbil J., Qablawi M., AlRyalat S. A. D. and Elubous K. Evaluation of changes in the ganglionic cell inner plexiform layer and macular retinal nerve fiber layer in patients receiving hydroxychloroquine. *Jordan J. Pharm. Sci.* 2023; 16:163-170.
6. Thirunavukarasu A. J., Ross A. C. and Gilbert R. M. Vitamin A, systemic T-cells, and the eye: Focus on degenerative retinal disease. *Front. Nutr*. 2022; 9:914457.
7. Semenova Y. and Bjørklund G. Antioxidants and neurodegenerative eye disease. *Crit. Rev. Food Sci. Nutr*. 2023; 1-19.
8. Radzin S., Wiśniewska-Becker A., Markiewicz M., Bętkowski S., Furso J., Waresiak J., Grolik J., Sarna T. and Pawlak A. M. Structural impact of selected retinoids on model photoreceptor membranes. *Membranes*. 2023; 13(6):575.
9. Levine D. A., Mathew N. E., Jung E. H., Yan J., Newman N. J., Thulasi P., Yeh S., Ziegler, T. R., Wells J. and Jain N. Characteristics of vitamin A deficiency retinopathy at a tertiary referral center in the United States. *Ophthalmol. Retina*. 2024; 8(2):126-36.
10. Ichsan A. M., Bukhari A., Lallo S., Miskad U. A., Dzuhry A. A., Islam I. C. and Muhiddin H. S. Effect of retinol and α-tocopherol supplementation on photoreceptor and retinal ganglion cell apoptosis in diabetic rats model. *Int. J. Retina Vitreous.* 2022; 8(1):40.

11. Kamal S., Junaid M., Ejaz A., Bibi I. and Bigiu N. Eye sight and carotenoids. *Carotenoids: Structure and function in the human body*; Springer Cham: Switzerland. 2021; 609-647.
12. Gebregziabher B. S., Gebremeskel H., Debesa B., Ayalneh D., Mitiku T., Wendwessen T., Habtemariam E., Nur S. and Getachew T. Carotenoids: Dietary sources, health functions, biofortification, marketing trend and affecting factors - A review. *J. Agric. Food Res.* 2023; 14:100834.
13. Paramaswaran Y., Subramanian A., Paramakrishnan N., Ramesh M. and Muthuraman A. Therapeutic investigation of palm oil mill effluent-derived beta-carotene in streptozotocin-induced diabetic retinopathy via the regulation of blood-retina barrier functions. *Pharmaceuticals*. 2023; 16.
14. Zhang L., Wang K., Liang S., Cao J., Yao M., Qin L., Qu C. and Miao J. Beneficial effect of ζ-carotene-like compounds on acute UVB irradiation by alleviating inflammation and regulating intestinal flora. *Food & Funct*. 2023; 14(18):8331-8350.
15. Wan Chik M., Ramli N. A., Mohamad Nor Hazalin N. A. and Surindar Singh G. K. Streptozotocin mechanisms and its role in rodent models for Alzheimer's disease. *Toxin Rev*. 2023; 42(1):491-502.
16. Zhou J. and Chen B. Retinal cell damage in diabetic retinopathy. *Cells*. 2023; 12.
17. Biswas A., Choudhury A. D., Agrawal S., Bisen A. C., Sanap S. N., Verma S. K., Kumar M., Mishra A., Kumar S., Chauhan M. and Bhatta R.S. Recent insights into the etiopathogenesis of diabetic retinopathy and its management. *J. Ocul. Pharmacol. Ther*. 2024; 40(1):13-33.
18. Sun K. X., Chen Y. Y., Li Z., Zheng S. J., Wan W. J., Ji Y. and Hu, K. Genipin relieves diabetic retinopathy by down-regulation of advanced glycation end products via the mitochondrial metabolism related signaling pathway. *World J. Diabetes*. 2023; 14(9):1349.
19. Jagadeeshwar K., Rupaka S. R., Alavala R. R., Rao G. S. N. K. and Kulandaivelu U. Investigation of nootropic and neuroprotective activity of *Myristica malabarica* bark extracts on STZ induced cognitive impairment in experimental animals. *Jordan J. Pharm. Sci.* 2023; 16:171-183.
20. Middel C. S., Dietrich N., Hammes H. P. and Kroll J. Analysis of the morphology of retinal vascular cells in zebrafish (*Danio rerio*). *Front. Cell. Dev. Biol*. 2023; 11.
21. Middel C. S., Hammes H. P. and Kroll J. Advancing diabetic retinopathy research: Analysis of the neurovascular unit in zebrafish. *Cells*. 2021; 10(6).
22. Williams F. The use of zebrafish and in vivo pal as a novel discovery platform for psychoactive agents. *Jordan J. Pharm. Sci.* 2023; 16:559.
23. Veldman M. B. and Lin S. Zebrafish as a developmental model organism for pediatric research. *Pediatr. Res.* 2008; 64:470-476.
24. Heckler K. and Kroll J. Zebrafish as a model for the study of microvascular complications of diabetes and their mechanisms. *Int. J. Mol. Sci.* 2017; 18.
25. Lee Y. and Yang J. Development of a zebrafish screening model for diabetic retinopathy induced by hyperglycemia: Reproducibility verification in animal model. *Biomed. Pharmacother.* 2021; 135: 111201.
26. Chhetri J., Jacobson G. and Gueven N. Zebrafish on the move towards ophthalmological research. *Eye.* 2014; 28:367-380.
27. Paramakrishnan N., Chavan L., Lim K. G., Paramaswaran Y. and Muthuraman A. Reversal of neuralgia effect of beta carotene in streptozotocin-associated diabetic neuropathic pain in female zebrafish via matrix metalloprotease-13 inhibition. *Pharmaceuticals.* 2023; 16:157.
28. Shinohara M., Masuyama T., Shoda T., Takahashi T., Katsuda Y., Komeda K., Kuroki M., Kakehashi A. and Kanazawa Y. A new spontaneously diabetic non-obese torii rat strain with severe ocular complications. *Int. J. Exp. Diabetes Res.* 2000; 1:89-100.

29. Quiroz J. and Yazdanyar A. Animal models of diabetic retinopathy. *Ann. Transl. Med.* 2021; 9:1272.
30. Nuzzi R., Scalabrin S., Becco A. and Panzica G. Gonadal hormones and retinal disorders: A review. *Front. Endocrinol.* 2018; 9:66.
31. Olivares A. M., Althoff K., Chen G. F., Wu S., Morrisson M. A., DeAngelis M. M. and Haider N. Animal models of diabetic retinopathy. *Curr. Diab. Rep.* 2017; 17:93.
32. Wang S., Du S., Wang W. and Zhang F. Therapeutic investigation of quercetin nanomedicine in a zebrafish model of diabetic retinopathy. *Biomed. Pharmacother*. 2020; 130:110573.
33. Zang L., Shimada Y., Nishimura Y., Tanaka T. and Nishimura N. Repeated blood collection for blood tests in adult zebrafish. *J. Vis. Exp*. 2015; (102):e53272.
34. Ohkawa H., Ohishi N. and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*. 1979; 95:351-358.
35. Ellman G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 1959; 82(1):70-77.
36. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. 1951; 193:265-275.
37. Fleisch V. C. and Neuhauss S. C. F. Visual behavior in zebrafish. *Zebrafish*. 2006; 3:191-201.
38. Fu R., Liu H., Zhang Y., Mao L., Zhu L., Jiang H., Zhang L. and Liu X. Imidacloprid affects the visual behavior of adult zebrafish (Danio rerio) by mediating the expression of opsin and phototransduction genes and altering the metabolism of neurotransmitters. *Sci. Total Environ.* 2024; 910:168572.
39. Ninkovic J. and Bally-Cuif L. The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse. *Methods*. 2006; 39:262-274.
40. Chhetri J., Jacobson G. and Gueven N. Zebrafish on the move towards ophthalmological research. Eye (Lond). 2014; 28:367-380.
41. Burton C. E., Zhou Y., Bai Q. and Burton E. A. Spectral properties of the zebrafish visual motor response. *Neurosci Lett*. 2017; 646:62-67.
42. Pedroso G. L., Hammes T. O., Escobar T. D., Fracasso L. B., Forgiarini L. F. and da Silveira T. R. Blood collection for biochemical analysis in adult zebrafish. *J Vis Exp*. 2012; e3865.
43. Capdevila J., Ducreux M., García Carbonero R., Grande E., Halfdanarson T., Pavel M., Tafuto S., Welin S., Valentí V. and Salazar R. Streptozotocin, 1982–2022: Forty years from the FDA's approval to treat pancreatic neuroendocrine tumors. *Neuroendocrinology*. 2022; 112(12):1155-1167.
44. Wang R., Rao S., Zhong Z., Xiao K., Chen X. and Sun X. Emerging role of ferroptosis in diabetic retinopathy: a review. *J. Drug Target.* 2024; 1–11.
45. Wal P., Wal A., Gupta D., Mantry S., Mahajan K. C., Rathore S. and Behl T. *Diabetic retinopathy: Stressing the function of angiogenesis, inflammation and oxidative stress. In: targeting angiogenesis, inflammation, and oxidative stress in chronic diseases*; Academic Press. Cambridge, United States 2024; 323-348.
46. Venugopal D., Vishwakarma S., Sharma N., Kaur I. and Samavedi S. Evaluating the protective effects of dexamethasone and electrospun mesh combination on primary human mixed retinal cells under hyperglycemic stress. *Int. J. Pharm.* 2024; 651:123768.
47. Subramanian A., Thirunavukkarasu J. and Muthuraman A. Astaxanthin ameliorates diabetic retinopathy in swiss albino mice via inhibitory processes of neuron-specific enolase activity. *Processes*. 2022; 10(7):1318.
48. Niu T., Shi X., Liu X., Wang H., Liu K. and Xu Y. Porous Se@ SiO2 nanospheres alleviate diabetic retinopathy by inhibiting excess lipid peroxidation and inflammation. *Mol. Med.* 2024; 30(1):24.

49. Pfaller A. M., Kaplan L., Carido M., Grassmann F., Díaz-Lezama N., Ghaseminejad F., Wunderlich K. A., Glänzer S., Bludau O., Pannicke T. and Weber B.H. The glucocorticoid receptor as a master regulator of the Müller cell response to diabetic conditions in mice. *J. Neuroinflammation*. 2024; 21(1):33.
50. Szałabska-Rąpała K., Zych M., Borymska W., Londzin P., Dudek S. and Kaczmarczyk-Żebrowska I. Beneficial effect of honokiol and magnolol on polyol pathway and oxidative stress parameters in the testes of diabetic rats. *Biomed. Pharmacother*. 2024; 172:116265.
51. Wang N., Singh D. and Wu Q. Astragalin attenuates diabetic cataracts via inhibiting aldose reductase activity in rats. *Int. J. Ophthalmol*. 2023; 16(8):1186.
52. Aruna V., Amruthavalli G. V. and Gayathri R. DcoD ameliorate diabetic retinopathy through aldose-sorbitol cleavage. *J. Pharm. Res. Int.* 2023; 35(34):57-61.
53. Zhang C., Gu L., Xie H., Liu Y., Huang P., Zhang J., Luo D. and Zhang J. Glucose transport, transporters and metabolism in diabetic retinopathy. *Biochim. Biophys. Acta – Mol. Basis. Dis*. 2023; 166995.
54. Gezer A., Üstündağ H., Karadağ Sarı E., Bedir G., Gür C., Mendil A. S. and Duysak L. β-Carotene protects against α-amanitin nephrotoxicity via modulation of oxidative, autophagic, nitric oxide signaling, and polyol pathways in rat kidneys. *Food Chem. Toxicol.* 2024; 193:115040.
55. Johra F. T., Bepari A. K., Bristy A. T. and Reza H. M. A mechanistic review of β-carotene, lutein, and zeaxanthin in eye health and disease. *Antioxidants.* 2020; 9:1046.
56. Lorenzi M. The polyol pathway as a mechanism for diabetic retinopathy: Attractive, elusive, and resilient. *Exp. Diabetes Res.* 2007; 2007:61038.
57. Fathalipour M., Fathalipour H., Safa O., Nowrouzi-Sohrabi P., Mirkhani H. and Hassanipour S. The therapeutic role of carotenoids in diabetic retinopathy: a systematic review. *Diabetes Metab. Syndr. Obes.* 2020; 13:2347-2358.
58. Song Y., Zhu L. and Zheng X. β-carotene inhibits MAPKs signaling pathways on rat colonic epithelial cells to attenuate TNF-α-induced intestinal inflammation and injury. *Cell. Biochem. Biophys.* 2024; 82(1):291-302.
59. Wu T., Xie Y., Wu Z., Li Y., Jiang M., Yu H., Li X., Wang J., Zhou E. and Yang Z. β-carotene protects mice against lipopolysaccharide and d-galactosamine induced acute liver injury via regulation of NF-κB, MAPK, and Nrf2 signaling. *J. Oleo Sci*. 2023; 72(11):1027-1035.
60. Zhu C. *Aldose reductase inhibitors as potential therapeutic drugs of diabetic complications*; Vol 2: Diabetes mellitus-insights and perspectives. IntechOpen, United Kingdom: 2013; 17-46.

سلائف فيتامين أ: بيتا كاروتين يخفف من اعتلال الشبكية السكري الناجم عن الستربتوزوتوسين لدى ذكور الزرد البالغين عن طريق تنظيم مسار البوليول

يامونا باراماسواران [1]، أسوينبراكاش سوبرامانيان [2]، أروناتشالام موثورامان [3]*

[1] PG باحث بحثي، كلية الصيدلة، جامعة AIMST، جالان بيدونج سيملينج، بيدونج، قدح، ماليزيا.

[2] وحدة التشريح، كلية الطب، جامعة AIMST، جالان بيدونج سيملينج، بيدونج، قدح، ماليزيا.

[3] وحدة الصيدلة وعلم السموم والعلوم الصحية الأساسية، كلية الصيدلة، جامعة AIMST، جالان بيدونج سيملينج، بيدونج، قدح، ماليزيا.

ملخص

اعتلال شبكية العين بسبب مرض السكر يعرف على أنه اضطراب وعائي عصبي تقدمي بسبب تلف الأوعية الدموية في شبكية العين. يعمل البيتا كاروتين ككروموفور في شبكية العين ويبدأ في نقل الصور وصيانة خلايا الظهارة. BC موجود في النفايات السائلة من مطحنة زيت النخيل والتي تسمى .PBCتهدف الدراسة الحالية إلى البحث عن دور PBC في الحماية من DR في نموذج حيواني وهو الزرد. تم استخدام المجموعات الخمس من دانيو ريريو الصحية في هذه الدراسة. تم تحقيق اعتلال الشبكية السكري عن طريق إعطاء الستربتوزوتوسين (STZ) داخل الصفاق متبوعًا بإعطاء STZ داخل الصفاق في اليوم السابع. تعرضت الحيوانات إلى جرعتين من 50) PBC و 100 مجم / لتر) بالإضافة إلى الديكساميثازون (DEX) لمدة 21 يومًا متواصلة. تم تقييم السلوكيات البصرية المرتبطة بـ DR، مثل الاستجابة الحركية البصرية (OMR) والاستجابة المفاجئة (SR) جنبًا إلى جنب مع التغيرات الكيميائية الحيوية، مثل الجلوكوز في البلازما والهموسيستين ((HCY وبيروكسيد الدهون، وانخفاض الجلوتاثيون (GSH)، وعامل نخر الورم ألفا ((TNF-α، ديسموتاز الفائق أكسيد (SOD)ومستويات البروتين الكلي. تم استخدام العدسات لتقييم مؤشرات مسار البوليول، مثل نشاط هيدروجيناز السوربيتول ((SDH) ونشاط إنزيم الألدوز المختزل (.(ARأظهرت النتائج أن PBC تخفف من مشاكل اعتلال شبكية العين الناجمة عن مرض السكر DR من خلال تنظيم تشوهات المسار الكيميائي الحيوي بطريقة مشابهة للمجموعة المعالجة بـ DEX. ومن ثم، يمكن استخدام PBC لإدارة وتخفيف اثار DR بسبب دوره فى الإجراءات التنظيمية لمسارات مكافحة ارتفاع السكر في الدم، ومضادات الأكسدة، والمضادة للالتهابات، والبوليول.

الكلمات الدالة: اختزال ألدوس، الجذور الحرة، الحقن داخل الجسم الزجاجي، استجابة المحرك البصري، النفايات السائلة مطحنة زيت النخيل، سوربيتول ديهيدروجينيز.

* ***المؤلف المراسل: أروناتشالام موثورامان***

arunachalammu@gmail.com

تاريخ استلام البحث 2024/9/1 وتاريخ قبوله للنشر 2024/11/3.