Effects of Curcumin on Restoration and Improvement of Microvasculature Characteristic in Diabetic Rat's Choroid of Eye

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Objective: *To investigate the effect of curcumin on microvasculature changes in STZ-induced diabetic rat's choroid of eye.*
Material and Method: *Male rats were divided into three groups: control (C) Diabetic rats were induced by streptozotocin (STZ) (60 mg/kg BW) (DM) diabetic rats treated with curcumin (DMC) (200 mg/kg BW). After 8 weeks of experiments, microvasculature changes of rat's choroid were studied under vascular corrosion cast technique with scanning electron microscope (SEM).*
Results: *There were pathology and destruction of choroid microvasculature of DM group that revealed reduced and shrunken sizes of large and small blood vessels, compared with control group; long posterior ciliary arteries (LPCAs) (C = 113.70±1.38, DM = 83.53±2.70, DMC = 109.64±3.41 μm), choroid arteries (C = 94.97±2.79, DM = 59.36±2.61, DMC = 80.31±3.73 μm), vortex veins (C = 74.11±3.24, DM = 46.71±2.56, DMC = 64.66±3.60 μm), and Choriocapillaris (choroidal capillaries) (C = 13.61±0.62, DM = 4.46±0.24, DMC = 9.96±0.70 μm), respectively. In DM group, LPCAs and Choroid arteries were tortuous and showed shrinkage. Vortex veins became narrow. Choriocapillaris showed the pathological characteristics of vascular lesions including of shrinkage, constriction, microaneurysm and blind ending. Fascinatingly, Choroid microvasculature of the eye in curcumin treated group developed into regenerate and repaired conditions with healthy and normal characteristics.*
Conclusion: *Efficiency of curcumin treatment beneficially repaired and regenerated the redevelopment of choroid's microvascular complications of eye in 8-week experiments. Potential treatment with curcumin in diabetes has demonstrated in a meaningful way the therapeutic consequences in the improvement and recovery of choroidal blood vessels in eye pathology of diabetic rats.*

Keywords: *Diabetes mellitus, Streptozotocin, Curcumin, Microvasculature, Vascular corrosion cast, Scanning electron microscope*

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The complications of diabetes which can eventually lead to blindness, causing the important disease of diabetic retinopathy. Concerning microvascular retinal changes in hyperglycemia condition, induced intramuscular pericyte death and thickening of the basement membrane lead to incompetence of the vascular wall. Small blood vessels in the eye are especially vulnerable to poor blood glucose control, then the accumulation of blood glucose can damages the tiny blood vessels in the eye. Generally, diabetic retinopathy is a major clinical problem, therefore human and animal studies have been focused on retinal, rather than choroid vasculature. Normally, the retinal vasculature supplies the inner retina, while the choroid lies outside the retinal pigment epithelium (RPE), nourishing the outer retina. The choroidal vasculature provides oxygen and nutrients to the outer retina. Therefore, destruction and nonperfusion of choroidal capillaries, the chorio-capillaris, could result in functional visual loss[1].

Although diabetic retinopathy is well characterized and used diagnostically to monitor disease progression, the routine clinical ophthalmic assessment of the choroid is really rare[2]. Clinically, preproliferative diabetic retinopathy is characterized by tortuousity of vessels, microaneurysm, and vascular

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nonperfusion[3]. The recent evidences suggest that similar events may occur in the diabetic choroid[4,5]. Diabetic retinopathy have also been described in streptozotocin (STZ) and alloxan models of diabetes[6], while choroidal vascular leakage and capillary dropout occur in spontaneously diabetic rats and monkey[7,8]. Hidayat and Fine[5] was the first group that suggested the term of diabetic choroidopathy demonstrated capillary dropout, basement membrane thickening, and choroidal neovascularization. The choroidalvasculopathy in diabetes may play a more prominent role in the pathogenesis of further diabetic retinopathy. In addition, the unexplained loss of visual function that may occur in diabetic subjects without retinopathy may be result of diabetic choroidopathy[9].

Streptozotocin (STZ) is a widely agent used to induce insulin-dependent diabetic mellitus in experimental models that is compatible to those of type 1 diabetes. It has the toxic effects on islet beta cells[10] and also induces potentially serious systemic microvascular alterations and complications, as observed in retina and kidney[11,12].

The vascular corrosion casting technique with scanning electron microscope (SEM) method have become a great fundamental tool for perception of organ microvasculation in order to support further conditions in terms of physiological and pathological studies. It is a standard technique to determine the three dimensional structures of the vascular bed and network[13,14]. This method clearly shows vascular architecture and provides an easily understandable beautiful and recognized three-dimensional view of an entire choroid as well as enlarged localized lesion.

In the present study, curcumin was used as daily supplement for investigation the improved and repaired structural changes of microvasculature in STZ-induced diabetic rat's choroid.

Material and Method

Induction of diabetes and experimental protocols

The procedure of the animal research was approved by Srinakarinwirot University Medical Center Animal Care Committee. Thirty male Wistar rats (200-250 g) obtained from National Laboratory Animal Center of Mahidol University were classified into three groups: control group (C) (n = 10), diabetic group (DM) (n = 10), and diabetic-treated with curcumin at a dose of 200 mg/kg BW (DMC) (n = 10). Experimental diabetic rats were induced by intravenous injection in lateral tail vein of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) (60 mg/kg BW) dissolved in 0.9% normal saline whereas control rats received injection with 0.9% normal saline alone. Rats with blood sugar level >250 mg/dl were determined as diabetic animals. At the end of 8 weeks after STZ injection and plus curcumin supplementation, the choroid microvasculature of both eyes were investigated by vascular corrosion cast technique with scanning electron microscope (SEM).

Vascular corrosion cast technique

The experimental rats were perfused with 500 ml of 0.9% NaCl solution through the left ventricle to flush out the blood from the blood vessels. Then, the Batson's No. 17 plastic mixture was infused into blood circulation of rats. The plastic injected-animals were left at room temperature for 30 minutes and immersed in hot water (80°C) for 3 hours to complete plastic polymerization. After polymerization, the eyes were disconnected and the tissues were corroded in 10% KOH solution at room temperature for 30-40 days. The eye vascular cast was rinsed in slow running tap water and cleansed in several changes of distilled water to get rid of any remaining tissues. Then, the eye casts were dried out at room temperature, mounted on a metal stub with double glue tape and carbon paint before being coated with gold on sputtering apparatus. Finally, the eye cast was examined area of choroid under SEM (JEOL JSM-5400) at accelerating voltage of 10 KV. The diameter of choroid blood vessels was measured by SemAfore computer software program.

Statistical analysis

The results were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed by using ANOVA followed by Bonferroni post test. The value of $p<0.05$ was considered to indicate statistical significance.

Results

SEM study of corrosion casts revealed clearly high magnification and detection of microvascular changes on the posterior wall of the eye. A three-dimensional illustration demonstrated the entire choroidal vasculature and view of the prominent differences choroidal vessels among three groups of rat at 8 weeks.

In normal condition of control group, numerous normal even blood vessels architectures of two long posterior ciliary arteries (LPCAs) were divided from posterior ciliary artery (PCA) (Fig. 1A) and gave branches into choroid arteries (Fig. 1B) together with recognized conspicuously vortex veins (Fig. 2A-B).

Presentation of prominent microvascular network of choriocapillaris was characterized for healthy normal sizes together with normal shape and standard pattern of choroid vasculature organization. The choriocapillaris formed with each other a large flattened sheet-like vascular sinus with little space between the capillaries. With the corresponding arterioles, the venules gathering the choriocapillaris also showed a very short course. These venules then became larger venules and drained into vortex veins (Fig. 3A).

In DM group, as the blood sugar increased progressively, the choroidal vascular changes demonstrated the LPCAs with slight tortuosity and shrinkage. The choroid arteries were often more tortuous, generalized narrowing and showed localized constrictions. The first-branch arterioles from the choroid arteries were also revealed dominant constrictions (Fig. 1C). The collecting venules converging from all directions into vortex veins were gradually decreased in numbers whereas the vortex veins became narrower. Moreover, the collecting venules converging intothe vortex veins were more decreased in numbers (Fig. 2C), irregular caliber and showed localized aneurismal dilatations (Fig. 3C). Thechoriocapillaris changes were more evident, they was elongated and decreased in numbers and also was clearly visible as sparse vessels. Constrictions, shrinkage, blind endings (Fig. 3B) and capillaries dropout of choriocapillaris were detected (Fig. 2, 3C). The intercapillaries spaces were irregular whereas the arterioles and venules showed wholesale loss (Fig. 2, 3C).

After 8 week of curcumin supplementation, the healthy normal patterns of choroidal blood vessels have recovered and got restored in DMC groups. LPCA, choroid arteries (Fig. 1D) and vortex veins (Fig. 2D) turn out to be regenerated and repaired into healthy and normal characteristics like in control group. All choroid arteriole to the choriocapillaris proceeded parallel arrays so as to interdigitate with choroid veins draining at this region. The collecting venules converging intothe vortex veins were still showed a few localized aneurismal dilatations (Fig. 3D). The choriocapillaris formed with each other as a flattened sheet-like vascular sinus with little space between the capillaries similarly to control group.

Concerning to the sizes of LPCAs, choroid arteries, vortex vein, and choriocapillaris at 8 weeks experiments were scored quantitatively in a blind fashion (Fig. 4). The average diameters about 100 μm far from the portion of main blood vessels of normal, DM, and DMC blood vessels were measured and compared as the followings; LPCAs (C = 113.70±1.38, DM = 83.53±2.70, DMC = 109.64±3.41 μm), choroid

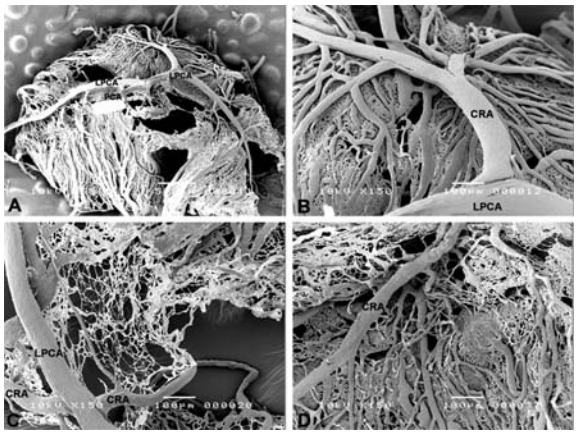

Fig. 1 SEM choroidal micrograph of posterior wall of eye vascular cast. A) Control group, showing the whole of blood vessels of choroid that come from posterior ciliary artery (PCA) and divide into 2 branches of long posterior ciliary arteries (LPCA). B-D) Illustrating choroid arteries (CRA) that divide from LPCA and give branches to supply the choroid of eye in Control, DM and DMC groups, respectively. Bar = 100 μm.

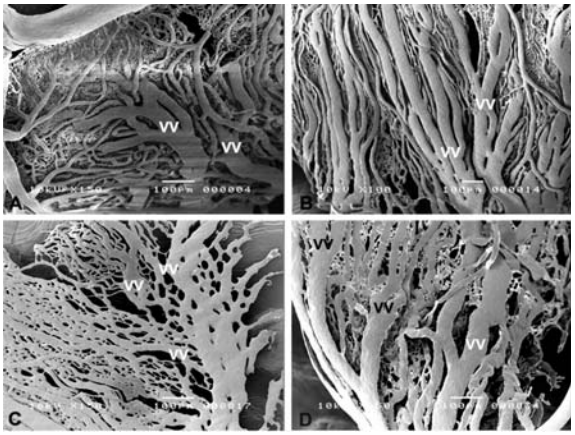

Fig. 2 SEM choroidal micrograph of vortex veins vascular cast. A, B) Control groups, showing the collecting venules converging from all directions into vortex veins of control group. C) Illustrating the choriocapillaris and the collecting venules that are gradually decreased in number. The vortex veins (VV) become narrower. D) The collecting venules converging intothe vortex veins have recovered and got restored in DMC group. Bar = 100 μm.

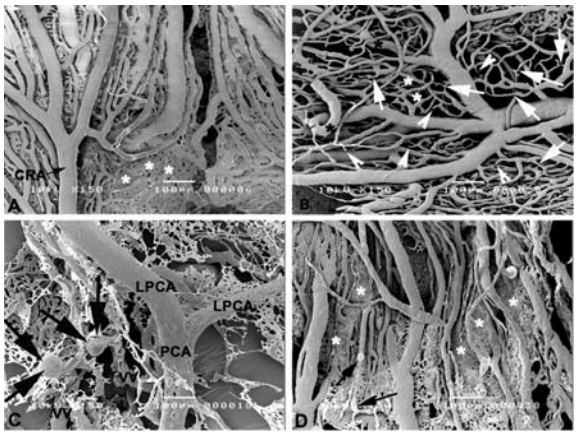

Fig. 3 SEM choroidal micrographs of posterior wall of eye vascular cast. A) Control group, showing the choriocapillarise (white asterisks) that come from branches of choroid arteries (CRA). B) Showing constrictions (white arrows), shrinkage, blind endings (white arrowhead), capillaries dropout of choriocapillaris, and tortuousity of choroid artery in DM group (black asterisks). C) Illustrating the branches of PCA, two LPCAs and thechoriocapillaris changes. The collecting venules converging intothe vortex veins (VV) are more decreased in number, irregular caliber and show localized aneurismal dilatations (black arrows). D) Showing the choriocapillaris that have recovered and got restored in DMC group. The collecting venules converging intothe vortex veins are still localized for a few aneurismal dilatations (black arrows). Bar = 100 μm.

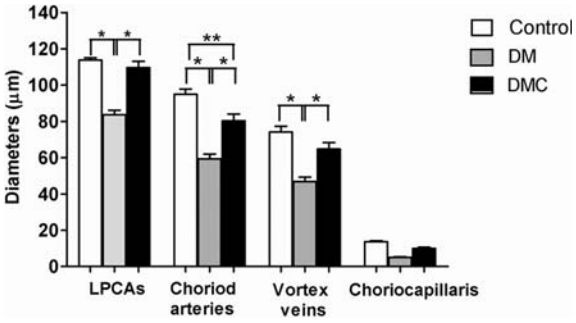

Fig. 4 The average diameters of long posterior ciliary arteries (LPCA), choroid arteries, vortex veins and choriocapillaris in control (C) diabetes (DM) and diabetes supplemented with curcumin (DMC) rats at 8 weeks. Values are mean ± SE *p<0.001, **p<0.01.

arteries (C = 94.97±2.79, DM = 59.36±2.61, DMC = 80.31±3.73 μm), vortex veins (C = 74.11±3.24, DM = 46.71±2.56, DMC = 64.66±3.60 μm) and Choriocapillaris (C = 13.61±0.62, DM = 4.46±0.24, DMC = 9.96±0.70 μm) respectively. The data were shown that the DM choroidal blood vessels had significant decrease in the diameter of LPCAs, choroid arteries, and vortex veins indices when compared to control rats (p<0.001). In contrast, the diameters of LPCAs, choroid arteries, and vortex veins were significantly increased after treatment with curcumin in DMC rats (p<0.001).

These findings confirmed previous studies[15,16] that curcumin might have efficiency to help or improve diabetic condition in term of recovery of blood vessels.

Discussion

In the present study of STZ induced diabetic's rats eye, vascular cast and SEM results revealed specific changes of choroidal vessels among three groups of rat. The DM rats displayed increasing choroidal damages. Generally, the architecture of the choroidal microvasculature consists of long straight LPCAs, choroid arteries, short running arterioles and venules, choriocapillaries and vortex veins. In diabetic condition, vessels tortuosity, shrinkage, and generalized narrowing were observed in the LPCAs, choroid arteries and vortex veins together with number reductions. Damage was worst in the choriocapillaris that is the smallest vessels. It was remarkably similar to a study using choroidal casts in human diabetes in which constrictions, shrinkage, blind endings micro-aneurysms and capillary dropout were observed. The intercapillaries spaces were irregular while the arterioles and venules also showed wholesale loss. There is no evidence for choroidal neovascularization as observed.

According the previous studies, the pathology of choroidal damage occurred in human diabetic retinopathy and shared many features with retinal vasculopathy including endothelial cell loss[17] tortuosity, microaneurysms, capillaries dropout[4] and reduced circulation in area of choroid[18]. In the severe phenotype in a mouse model of retinal vascular damage which appeared similar to that in diabetic retinopathy, the percentage area covered by the choriocapillaris decreased to two-thirds of that in wild-type, as indicative of capillaries dropout[19]. Capillary shrinkage may lead to the decreased blood flow as observed in human diabetic retinopathy[4]. Generally, capillary constrictions are speculated that it is a natural sequence ~~is~~ for capillary blockage to lead to discontinuity and capillary breakage. This constriction may arise via capillary damage induced by leucostasis and microaneurysms. The microaneurysms in the diabetic

Table 1. The average diameters of long posterior ciliary arteries (LPCA), choroid arteries (CRA), vortex veins and choriocapillaris in control (C) diabetes (DM) and diabetes treated with curcumin (DMC)

Groups	Diameters of blood vessels (μm), mean ± SE			
	LPCAs	Choroid arteries	Vortex veins	Choriocapillaris
Control	113.70±1.38	94.97±2.79	74.11±3.24	13.61±0.62
DM	83.53±2.70	59.36±2.61	46.71±2.56	4.96±0.48
DMC	109.64±3.41	80.31±3.73	64.66±3.60	9.96±0.70
p-value[a]	<0.001	<0.001	<0.001	<0.001

SE = standard error of mean

choroid are associated with intrachoroidalneovascular formations[20].

The current studies suggest several contributing factors that the numbers of polymorphonuclear leukocytes (PMNs) is increased in the human diabetic choroidal vasculature and are often associated with Choriocapillaries degeneration (CCD)[21]. The aggregation of PMN is generally associated with inflammatory and immune response. In diabetic choroids, it is often observed the elevated levels of P-selectin and intracellular adhesion molecule 1. These substances stimulate PMN rolling and adhesion to endothelial cells, respectively[22]. The diabetic PMN can produce a greater oxidative burst, which can contribute to the endothelial cells loss in CCD and also release proteolytic enzymes that damage endothelial cells[23].

Glucose is the main cause for microvascular complications of diabetes like retinopathy, nephropathy and neuropathy. The negative role of hyperglycemia on endothelial functions and pathological changes occur in diabetes are well established. There are four major molecular signaling mechanisms activated by hyperglycemia in endothelial cells. These include: activation of PKC, increased hexosamine pathway flux, increased advanced glycation end product formation, and increased polyol pathway flux[24].

Protein kinase C (PKC) is a family of serine-threonine kinases that plays an important role in signal transduction mechanisms[24]. The PKC pathway is further activated in diabetes as a result of hyperglycemia. In this pathway, PKC is activated by the increased amounts of diacylglycerol (DAG), which is synthesized directly from glycolytic intermediates such as dihydroxyacetone phosphate and glyceraldehyde-3-phosphate[25,26]. PKC appears to be activated in a range of diabetic tissues including the retina, kidney, heart, and aorta. An activation of PKC has been implicated in many processes relevant to diabetic complications, including regulation of vascular permeability and flow, increased production of cytokines, and increased synthesis of basement membranes[27]. In diabetes microvascular complications, for example, PKC affects the activation of a number of growth factors and changes the function of vasoactive factors. These vasoactive factors include vasodilators such as nitric oxide (NO) as well as vasoconstrictors such as angiotensin II and endothelin-1[28].

Only a small proportion of glucose is metabolized to sorbitol during normoglycemia, while in hyperglycemia the enzyme aldose reductase (AR) is activated, leading to an accumulation of intracellular sorbitol and fructose that increase the flux through the polyol pathway[29]. Sorbitols and other polyols accumulate in intracellular vicinity, leading to osmotic damage and swelling. Moreover, aldosereductase (AR) is the first and rate-limiting enzyme of the polyol pathway, which converts monosaccharides (glucose) to their polyols or sugar alcohols (sorbitol). This enzyme is widely distributed throughout the body, including those tissues that are susceptible to chronic diabetic complications (retina, lens, cornea, glomerulus, nervous system and the blood vessels). In fact, alterations in sorbitol and fructose metabolism are implicated as factors contributing to vascular complications in diabetes mellitus[30].

After curcumin supplementation, the healthy normal patterns of choroid blood vessels have recovered and got restoration at 8 weeks. Choroid blood vessels in curcumin-treated group became regenerated and repaired in to healthy and normal characteristics. Regarding curcumin, it potentially inhibits lipid peroxidation in rat liver microsome[31], along with being an inhibitor of lipid peroxidation. It is known that curcuminhas an inhibitory on PKC activity. Activation of PKC by glucose has been implicated in

the regulation and activation of membrane-associated NAD(P)H-dependent oxidases and subsequent production of superoxide anion[32].

Various compounds may inhibit the formation of pro-inflammatory cytokines thus offering the means for modulation of the process and possibly avoiding extensive damage (inflammation) of the surrounding tissue by invading neutrophils and lymphocytes. Curcumin down-regulates the expression of various cell surface adhesion molecules that have been linked with inflammation[33], down-regulation the expression of various inflammatory cytokines, including TNF-α, IL-1, IL-6, IL-8, and chemokines[34]. Curcumin has been shown to inhibit the action of TNF-α, one of the most pro-inflammatory of the cytokines[35]. Moreover, curcumin is a potent antioxidant, which might contribute to its anti-inflammatory action[36,37].

Normal choroidal vasculature is essential for normal function and structure of the retina. The rich blood supply to the choriocapillaris with high oxygen pressure and high vascular permeability would be participated considering its role in diffusing oxygen and nutrients through the choriocapillaris to nourish the retina. Damaging of choroidal blood vessels can result in photoreceptor dysfunction and death. Vaso-occlusions and nonperfusionof choroidal blood vessels is occurred in the diabetic choroid, as well as in the retina. The patho-physiological effects of diabetic on the microcirculation of the choroid may be reflected in the choroidal vascular changes as noted in this present study. Interestingly, healthy blood vessels became recovered and redeveloped in diabetic-rats, supplemented with curcumin.

Conclusion

It is possible that curcumin could improve the destruction of the choroid blood vessels because the potential beneficial effects of curcumin have been shown to exhibit anti-inflammatory, antioxidant and anti-diabetic activities. Potential treatment with curcumin in diabetes has been demonstrated meaningfully about the therapeutic consequence in improvement and recovery choroid's microvascular complications of eye. The efficiency of curcumin might be applied to be an alternative therapeutic agent in diabetic choroidiopathy. Further studies of curcumin treated on retinal microvasculature are suggested.

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Potential conflicts of interest

None.

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ผลของ curcumin ต่อการเปลี่ยนแปลงของหลอดเลือดชั้นคอรอยด์ในตาของหนูที่เป็นเบาหวาน

วิภาพรรณ ขิมมากทอง, หัทยา เพชรพิบูลไทย, ปิยาณี ศรียา, วิภาวีอนุพันธ์พิศิษฐ์

วัตถุประสงค์: *เพื่อศึกษาโครงสร้างของหลอดเลือดชั้นคอรอยด์ในตาของสัตว์ที่ป่วยเป็นเบาหวานที่ถูกกระตุ้นให้เป็นเบาหวาน (โดยสาร streptozotocin) และเป็นเบาหวานที่ได้รับการรักษาแล้วด้วยสาร curcumin ในระดับจุลทรรศน์อิเล็กตรอนชนิดส่องกราด (SEM) ร่วมกับ vascular corrosion cast*

วัสดุและวิธีการ: *หนูเพศผู้ถูกแบ่งออกเป็น 3 กลุ่ม ได้แก่หนูกลุ่มควบคุมและหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานโดยสาร STZ (60 mg/kg BW) และหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานที่ได้รับขมิ้นชัน (200 mg/kg BW) ระยะเวลา 8 สัปดาห์ทำการฉีดพลาสติกเข้าไปสู่หลอดเลือดในดวงตาโดยวิธี microvascular casting เมื่อครบกำหนดได้ศึกษาลักษณะโครงของหลอดเลือดบริเวณชั้นคอรอยด์ในตาโดยจุลทรรศน์อิเล็กตรอนชนิดส่องกราดร่วมกับ vascular corrosion cast แล้วทำการเปรียบเทียบวิเคราะห์ลักษณะและขนาดของหลอดเลือดแต่ละระดับของหนูทั้ง 3 กลุ่ม*

ผลการศึกษา: *ผลการศึกษาพบว่าหนูกลุ่มที่ถูกกระตุ้นให้เป็นเบาหวานมีความผิดปกติของชั้นคอรอยด์ โดยมีการทำลายโครงสร้างของหลอดเลือดพบว่าหลอดเลือดในชั้นคอรอยด์กลุ่มเบาหวานมีขนาดหดเล็กลงมาก เมื่อเทียบกับกลุ่มควบคุม ทั้งในระดับของหลอดเลือดขนาดใหญ่และขนาดเล็กได้แก่ long posterior ciliary arteries (LPCA) (C = 113.70±1.38, DM = 83.53±2.70, DMC = 109.64±3.41 mm), choroid arteries (CRA) (C = 94.97±2.79, DM = 59.36±2.61, DMC = 80.31±3.73 mm), vortex veins (C = 74.11±3.24, DM = 46.71±2.56, DMC = 64.66±3.60 mm) และ Choriocapillaris (C = 13.61±0.62, DM = 4.46±0.24, DMC = 9.96±0.70 mm) ตามลำดับและพบความเสียหายของหลอดเลือดของหนูกลุ่มเบาหวานในระดับ LPCAs, choroid arteries, และ vortex veins ในลักษณะที่มีขนาดหดเล็กลง (constriction) และพบว่า choriocapillaris มีลักษณะของการเหี่ยวแฟบ (shrinkage) และหดเล็กลงเกิดการโป่งพอง (microaneurysm) ตลอดจนหลอดเลือดที่มีปลายตัด (blind ending) แต่ในสัตว์ที่ป่วยเป็นเบาหวาน และได้รับการรักษาด้วยสาร curcumin พบว่าโครงสร้างของหลอดเลือดในชั้นคอรอยด์นั้นมีการซ่อมแซมและฟื้นฟูสภาพเกือบเข้าสู่สภาวะปกติและใกล้เคียงกลุ่มควบคุมทั้งขนาดและลักษณะของหลอดเลือด*

สรุป: *สาร curcumin ซึ่งเป็นสารที่มีอยู่ในขมิ้นชันมีผลในการรักษาช่วยฟื้นฟูและซ่อมแซมสภาพหลอดเลือดตา ในชั้นคอรอยด์ที่เสื่อมสภาพจากสภาวะเบาหวานในระยะเวลา 8 สัปดาห์ มีผลที่ดีต่อการลดภาวะแทรกซ้อนของหลอดเลือดที่ตาและน่าจะมีประโยชน์ในการนำมาประยุกต์ใช้เชิงการแพทย์ทางเลือกในการรักษาโรคเบาหวาน*