TO STUDY PROTECTIVE EFFECTS
OF BIOFLAVONOIDS ON RETINAL
NEUROVASCULAR DEGENERATION
IN DIABETIC RATS

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Synopsis

For

PhD Thesis Title

“TO STUDY PROTECTIVE EFFECTS OF BIOFLAVONOIDS ON RETINAL NEUROVASCULAR DEGENERATION IN DIABETIC RATS”

Study-1

Hesperetin ameliorates hyperglycemia induced retinal vasculopathy via anti-angiogenic effects in experimental type-1 diabetic rats

The purpose of the study was to evaluate vasculoprotective effects of Hesperetin (Hsp) in Streptozotocin induced type-1 diabetic rats at two doses (100 mg/kg Body weight (BW) and 200 mg/kg BW). The study was carried out for a period of 24 weeks and evaluated for angiogenic parameters (VEGF and PKC-β), retinal vascular leakage by fluorescein angiography and, vessel (arteriolar and venular) diameters and any morphological abnormality through fundus photographs. Apart from this, transmission electron microscopy (TEM) was done to determine capillary basement membrane (BM) thickness. The results of the present study showed a significant increase in the expression of VEGF and PKC-β in diabetic retinae as compared to normal retinae. On the other hand, Hsp-treated retinae showed marked inhibition in the expression of VEGF and PKC-β. In the present study, diabetic retinae showed increase vascular permeability and leakage as compared to normal retinae. However, Hsp-treated retinae have not shown any such vascular dysfunctions. Moreover, there was significant increase in vessel caliber recorded in diabetic retinae compared to normal retinae, on the contrary Hsp-treated retinae showed lesser dilated vessels. Further, TEM study showed thickened BM in diabetic group as compared to normal group. However, Hsp-treated retinae showed marked prevention in BM thickness. In conclusion, it can be sated that Hsp has potential vasoprotective effects and can be useful in preventing diabetes induced vasculopathy.

Study-2

Hesperetin rescues retinal oxidative stress, neuroinflammation and apoptosis in type-1 diabetic rats
The purpose of the study was to evaluate the effects of hesperetin (Hsp) on diabetes-induced retinal oxidative stress, neuroinflammation and apoptosis in rats. The Hsp treatment (100 and 200 mg/kg BW) was carried for twenty four weeks in STZ-induced diabetic rats and evaluated for antioxidant (Superoxide dismutase; SOD, Catalase; CAT and glutathione; GSH) enzymes, inflammatory cytokines (TNF-α, IL-1β), caspase-3, glial fibrillary acidic protein (GFAP) and aquaporin-4(AQP4) expression. Histological changes were evaluated by light and transmission electron microscopic (LMand TEM) studies. Retinal GSH levels and anti-oxidant enzymes (SOD and CAT) activity were significantly decreased in diabetic group as compared to normal group. However, in Hsp-treated rats, retinal GSH levels were restored close to normal levels and positive modulation of anti-oxidant enzyme activity was observed. Diabetic retinae showed significantly increased expression of Pro-inflammatory cytokines (TNF-α and IL-1β) as compared to normal retinae. While Hsp-treated retinae showed significantly lower levels of cytokines as compared to diabetic retinae. Diabetic retinae showed increased caspase-3, GFAP and AQP4 expression. However, Hsp-treated retinae showed inhibitory effect on caspase-3, GFAP and AQP4 expression. LM images showed edematous Müller cell endfeet, and also degenerated photoreceptor layer; however, protective effect of Hsp was seen on Müller cell processes and photoreceptors. TEM study showed increased basement membrane (BM) thickness in diabetic retina, while relatively thin BM was recorded in Hsp-treated retina. It can be postulated that dietary flavanoids, like Hsp, can be effective for the prevention of diabetes induced neurovascular complications such as diabetic retinopathy.

Study-3

Quercetin protects retinal vascular degeneration through antiangiogenic mechanisms in STZ-induced type-1 diabetic rats

The objective of the study is to evaluate the protective effects of Quercetin (Qctn, Oral Dose-25 and 50 mg/kg BW), a plant based flavonol, on hyperglycemia induced retinal vasculopathy in diabetic rats. The study was carried out for a period of 24 weeks in STZ-induced diabetic rats. The study groups (normal, diabetic & Qctn-treated diabetic rats) were evaluated for retinal angiogenic parameters (VEGF and PKC-β) by ELISA and retinal vascular leakage by fluorescein angiography. Retinal vessel diameters [retinal arteriolar (AD) and venular (VD) diameter] were estimated in fundus images. Retinal vessel abnormalities were observed through periodic monitoring of rat fundus. Further,
transmission electron microscopy (TEM) was done to determine capillary basement membrane (BM) thickness and Endothelium/Pericyte (E/P) ratio. A significant rise in the expression of VEGF and PKC-β in diabetic retinae was recorded as compared to normal retinae. On the other hand, Qctn-treated retinae showed significant inhibition of VEGF and PKC-β expression. Fundus Fluorescein angiograms from diabetic retinae showed increased retinal vascular permeability and leakage, but was absent in Qctn-treated retinae. Moreover, there was significant increase in vessel caliber in diabetic retinae compared to normal retinae. On the contrary, Qctn-treated retinae showed lesser dilated retinal vessels. TEM showed degenerated capillary pericytes and endothelium, and swollen Muller cell processes. However, Qctn-treated retinae showed normal endothelium and pericyte structures. Further, TEM study showed thickened BM and increased E/P ratio in diabetic retinae as compared to normal retinae. On the contrary, Qctn-treated retinae showed comparatively thin BM and decreased E/P ratio. Based on the results, it can be concluded that Qctn may be effective for the prevention/treatment of diabetes induced retinal microvascular complications.

Study-4

Retinal neuroprotective effects of quercetin in STZ-induced type-1 diabetic rats

The aim of the study was to evaluate the effects of Quercetin (Qctn), a plant based flavanol, on retinal oxidative stress, neuro-inflammation and apoptosis in STZ-induced diabetic rats. The Qctn treatment (25 and 50 mg/kg BW) was given orally for six months in diabetic rats. Retinal antioxidant (SOD, CAT & glutathione) enzymes were estimated using commercially available assays and inflammatory cytokines (TNF-α, IL-1β) were estimated by ELISA method. Immunofluorescence studies were performed for caspase-3, glial fibrillary acidic protein (GFAP) and aquaporin-4(AQP4) expressions. Structural and Ultra-structural changes were evaluated by light and transmission electron microscopic (TEM) studies, respectively. Retinal GSH levels and anti-oxidant enzymes (SOD & CAT) activity were significantly decreased in diabetic group as compared to normal group. However, in Qctn-treated rats, retinal GSH levels were restored close to normal levels and positive modulation of anti-oxidant enzyme activity was observed. Diabetic retinae showed significantly increased expression of Pro-inflammatory cytokines (TNF-α & IL-1β) as compared to normal retinae. While Qctn-treated retinae showed significantly lower levels of cytokines as compared to diabetic retinae. Diabetic retinae showed increased caspase-3, GFAP and AQP4 expression. However, Qctn-treated retinae showed decreased caspase-3, GFAP and AQP4 expression. LM study showed significantly increased ganglion cell death in diabetic retinae compared to
normal retinae; however, protective effect of Qctn was seen on ganglion cell death. Further, TEM study showed increased basement membrane (BM) thickness, edematous and degenerated Müller cell processes in diabetic retina, while relatively thin BM and normal muller cell processes was recorded in Qctn-treated retina. It can be concluded that bioflavanoids, like Qctn, can be effective for diabetes induced retinal neurodegeneration and oxidative stress.

**Study-5**

Protective effects of bioflavanoids (hesperetin and quercetin) on gene expression of tight junction proteins and protein expression of extracellular matrix in diabetic rat retina

In the this study we evaluated effects of bioflavanoids (Quercetin-25 mg/kg BW and Hesperetin – 100 mg/kg BW) on gene expression of tight junction proteins (Occludin, Claudin-5 and ZO-1) and protein expression of extracellular matrix (Collagen-IV and Fibronectin) in diabetic rat retina. We found that bioflavanoids prevents inhibition of gene expression of tight junction proteins and inhibits increase expression of collagen-IV and fibronectin in diabetic retinae as evident from real time-PCR, western blot and immunofluorescence studies.

**Study-6**

Effects of herbal drugs and polyherbal combination on experimental diabetic retinopathy via antioxidant, anti-inflammatory, and anti-angiogenic mechanisms in streptozotocin-induced type-1 diabetic rats

The present study was aimed to evaluate the retinoprotective effects of herbal drugs [Moringa oleifera (100 and 200 mg/kg), Fenugreek (100 and 200 mg/kg) and Triphala (175 and 350 mg/kg BW)] in Streptozotocin-induced type-1 diabetic rats. The study was continued for 24 weeks and evaluated for inflammatory (TNF-α and IL-1β), angiogenic (VEGF and PKC-β) and antioxidant (GSH, SOD, and CAT) parameters. Retinal leakage was checked by Fluorescein angiography (FA) and fundus photographs were evaluated for retinal vessel caliber (arteriolar and venular). Transmission electron microscopy was done to determine basement membrane (BM) thickness. The results of the present study showed potential hypoglycemic and retinal antioxidant effects of herbal drugs. In the present study, a
significant rise in the expression of retinal inflammatory (TNF-a and IL-1b) and angiogenic (VEGF and PKC-b) parameters was observed in diabetic retinae as compared to normal retinae. However, herbal drugs-treated retinae showed marked inhibition in the expression of inflammatory and angiogenic parameters. Further, in the present study, diabetic retinae showed dilated retinal vessels as compared to normal. However, herbal drugs-treated retinae showed marked prevention in the dilatation of retinal vessels. Fluorescein angiograms obtained from diabetic retinae showed leaky and diffused retinal vasculature. On the other hand, herbal drugs-treated retinae showed intact retinal vasculature. Further, results of the transmission electron microscopy study showed thickened capillary BM in the diabetic retina as compared to normal retinae. However, treatment with herbal drugs prevented thickening of capillary BM. Our result suggests that herbal drugs may be useful in preventing diabetes induced retinal dysfunction.

Therefore, based on the encouraging results of the individual drugs, a polyherbal combination (Moringa oleifera at a dose 100 mg/kg BW, Fenugreek at a dose 100 mg/kg BW and Triphala at a dose 175 mg/kg BW) was evaluated for its efficacy against diabetic retinopathy in rats. The novel polyherbal combination has shown overall better efficacy for diabetic retinopathy as compared to individual test herbal drugs in diabetic rats. The polyherbal combination showed potential hypoglycemic, anti-oxidant, anti-inflammatory, anti-angiogenic. Therefore, we came to the conclusion that the present novel polyherbal combination produces synergistic effects in comparison to individual herbal drugs and can be used as therapeutic intervention in diabetic patients.