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Prevention and management of diabetic retinopathy in STZ diabetic rats by *Tinospora cordifolia* and its molecular mechanisms

Shyam S. Agrawal [b,*], Salma Naqvi [a], Suresh K. Gupta [a], Sushma Srivastava [a]

[a] Department of Pharmacology, Delhi Institute of Pharmaceutical Sciences and Research, New Delhi-110017, India
[b] Amity University, Sector-125, Noida – 201301, India

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ABSTRACT

We investigated the potential of *Tinospora cordifolia* (TC) in treatment of diabetic retinopathy in STZ-induced rats due to its antihyperglycemic, angiogenic, antiinflammatory and antioxidant effects. The diabetic rats, treated for 24 weeks with TC extract (250 mg/kg), were evaluated for lenticular and fundus changes. Biochemical parameters were estimated and histopathological studies performed. TC significantly reduced blood glucose and glycated hemoglobin in treated rats. It prevented cataract development in treated group. Angiogenic markers VEGF and PKC increased in diabetic retina, which reduced significantly with TC. Anti-inflammatory parameters TNF-α and IL-1β elevated in diabetic group unlike that in treated group. TC also provided defense against depletion of antioxidant enzymes- glutathione and catalase. Histopathological studies revealed thickening of basement membrane of the retinal and glomerular vasculature of diabetic rat, but no basement membrane widening was seen in treated animals. Destruction of pancreatic islet structure was observed in diabetic group, but not in treated. Thus, TC reduces blood glucose and inhibits overexpression of angiogenic and inflammatory mediators, which are distinct markers of diabetic retinopathy. It also prevents retinal oxidative stress and restores antioxidant enzyme levels. These data provide evidence for the safety and potential effect of TC in the management of experimental diabetic retinopathy.

1. Introduction

Diabetic retinopathy, the most frequent cause of new cases of blindness, progresses from mild nonproliferative abnormalities, (increased vascular permeability), to moderate and severe nonproliferative diabetic retinopathy (vascular closure), to proliferative diabetic retinopathy (growth of new blood vessels on the retina and posterior surface of the vitreous). Retinal thickening can develop at all stages of retinopathy (Donald et al., 2003). It is the result of long duration of diabetes mellitus. Diabetic retinopathy (DR) at the time of the diagnosis of diabetes is lower with type I (0.4%) than type II (7.6%) (Roy et al., 2004). Several factors influence DR viz., long duration of the disease, age, level of hyperglycemia control, hyperlipidemia, hyperviscosity, renal failure etc. Most important is the contribution of the biochemical changes associated with hyperglycemia (Abdulrahman, 2011). The exact mechanism by which hyperglycemia causes vascular disruption in retinopathy is not clear. Probably the intraocular formation of reactive oxygen species lead to pathological and biochemical changes seen in DR. These biochemical changes include protein glycation as seen in hemoglobin A1C (Brownlee et al., 1984); Protein Kinase-C activation which may lead to enhanced permeability of retinal vasculature, basement membrane thickening and cellular signaling by vascular endothelial growth factors (VEGF) (Xia et al., 1996; Miller et al., 1997). VEGF, induced by ischemic neurosensory retina, is one of the cytokines that plays a prominent role in DR. It is a marker of oxidative stress and induces hyperpermeability of macular capillaries contributing to macular edema. It also induces endothelial proliferation leading to ocular neovascularization (Abdulrahman, 2011).

Oxidative stress caused by formation of free radicals leads to retinal vasculature damage (Bursell and King, 1999; Bursell et al., 1999). Moreover, these free radicals as reactive oxygen species (ROS) are the strong stimulus for the release of proinflammatory cytokines-interleukin 1β (IL-1β) and tumor necrosis factor (TNF-α) which damage endothelial cells and play an important role in the pathogenesis of DR (Gustavsson et al., 2008).

Abbreviations: ANOVA, analysis of variance; CAT, catalase; DR, diabetic retinopathy; GSH, reduced glutathione; HE, hematoxylin; IL-1β, interleukin 1β; PAS, Periodic acid-Schiff (PAS); PKC, Protein Kinase-C; TC, *Tinospora cordifolia*; TNF-a, tumor necrosis factor; VEGF, vascular endothelial growth factors.

* Corresponding author. Mobile: +91 9868902055.
E-mail addresses: skgup@hotmail.com (S.K. Gupta), salmanaqvi_8@yahoo.com (S. Naqvi), shyamagrawal2006@gmail.com (S.S. Agrawal), sushma.srivastava@gmail.com (S. Srivastava).

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Because of a steep rise in the incidence and prevalence of diabetes in the last decade, diabetic retinopathy has become a matter of big concern. It is reported that nearly all persons with type 1 and more than 60% of those with type 2 diabetes develop retinopathy in 20 years (Abu and Al-Mezaine, 2011). Hence, prevention and treatment of diabetic retinopathy and other diabetic complications needs to be focused. Presently, no satisfactory pharmacological therapy for DR treatment is available. This prompted us to find a drug which may be able to inhibit the progression of diabetes mellitus to DR.

The herb *Tinospora cordifolia* (TC) commonly known as Guduchi, family: Menispermaceae, has a long history of use in Ayurvedic medicine. It possesses anti-cancer (Singh et al., 2005), immune stimulating (Rawal et al., 2004), cholesterol-lowering (Stanely Mainzen Prince et al., 2003), liver-protective (Bishayi et al., 2002) as well as anti-diabetic activities. It is reported that TC extract decreases blood glucose in diabetic rats. The possible mechanism of its hypoglycaemic action is that TC may potentiate the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the b-cells of islets of Langerhans or its release from bound insulin (Stanely Mainzen Prince et al., 2000). Apart from its antihyperglycemic actions, *T. cordifolia* has also shown some promising effects in preventing diabetic complications. It has improved healing in the diabetic foot ulcers (Purandare and Supe, 2007) and has shown prevention of experimental diabetic cataract (Rathi et al., 2002).

Based on these effects of TC, this study was carried out with an objective to explore its potential in management of diabetic retinopathy and other complications.

2. Materials and methods

2.1. Plant extract

The standardized methanol soluble extract of *T. cordifolia* stem was obtained from Sanat Products Ltd., Delhi, India, along with a certificate of analysis (Batch No1170109).

2.2. Animals, induction of diabetes and study protocol

Wistar rats (*Rattus norvegicus*) of either sex, weighing between 200 and 225 g were procured from the animal house, Delhi Institute of Pharmaceutical Sciences and Research, (Protocol No: DIPSAR/IAEC/11/2008). All experimental procedures and animal care followed the institutional guidelines and Association for Research in Vision and Ophthalmology statement for the use of animals in eye research. All animals were maintained under adequate conditions at an ambient temperature of 21 ± 2 °C, and were subjected to12 h light and dark cycle. They were fed with standard rat chow and water ad libitum.

Animals were randomly divided into normal control and streptozotocin-induced (45 mg/kg i.p) diabetic groups. The diabetic rats were further divided into diabetic control and TC (250 mg/kg, oral) treated diabetic groups. Dose was selected on the basis of literature survey. Each group consisted of 15 animals.

Treatment was initiated three days after STZ induction. Animals showing blood glucose level above 250 mg/dl were selected for the study. Blood sugar was monitored weekly till the end of the study. Photographs of anterior and posterior chamber of the eye were taken at adequate intervals to evaluate the lenticular and fundoscopic changes.

After 24 weeks of diabetes, animals were sacrificed with deep anesthesia, blood was collected in EDTA-coated vacuutainers and retinae were isolated, blotted, weighed and stored for further analysis. Kidney, pancreas, heart and liver were also isolated and fixed for light microscopy. Retinal homogenate was prepared in phosphate buffer, pH 7.4 for biochemical estimations. All estimations were done in duplicate.

2.3. Lenticular and fundoscopic changes

Pupil of the rat eye was dilated using 1% Tropicamide eye drops. Moisol 0.7% eye drops were administered to avoid cornea from drying. Changes in the anterior segment of the eye were seen and documented using slit lamp (Haag-Streit IM900 Imaging Module).

Rat fundus was seen using hand-held fundus camera (Kowa, Genesis-Df, Japan) for any changes in retinal vessels.

2.4. Glycemic parameters

Blood glucose was monitored weekly by tail prick using ACCU-CHEK ACTIVE (Roche) glucose strips. HbA1c was estimated after 24 weeks using ion exchange resin (Biosystems S.A., Barcelona) and was quantified by direct photometric reading at 415 nm.

2.5. Angiogenic parameters

Vascular Endothelial Growth Factor (VEGF) and Protein Kinase-C beta 1 (PKC-β1) levels were measured using RayBio® Rat VEGF (RayBiotech, Inc.) and PKC b1 (Uscn Life Science Inc. Wuhan) ELISA kits respectively, as per the manufacturer's instructions.

2.6. Anti-inflammatory parameters

Interleukin-1β (IL-1β) and Tumor Necrosis Factor (TNF-α) levels were estimated using RayBio® Rat IL-1β and RayBio® Rat TNF-α ELISA kits of RayBiotech, Inc. as per the manufacturer's instructions.

2.7. Antioxidant parameter

Reduced glutathione (GSH) and catalase (CAT) were measured using Glutathione and Catalase Assay kits respectively (Cayman Chemical Company, USA) as per the manufacturer's instructions.

2.8. Histopathological and immuno histochemistry (IHC) studies

Retina, pancreas, heart, liver and kidney tissues were fixed in 10% phosphate buffer formalin. Fixed tissues were cut into thin sections, slides were prepared and stained. Periodic acid-Schiff (PAS) stain was used for kidney while retina, pancreas, heart and liver were stained using hematoxylin and eosin (HE) stain. The slides were observed under high magnification. Retinal cell apoptosis was seen using BAX and BCl-2 antibodies. All histologic parameters were quantitated by an experienced observer blinded to the identity of the sample being examined.

2.9. Statistical analysis

All data are expressed as mean ± standard deviation. The groups were compared by one-way ANOVA with Tukey post hoc comparison.

3. Results

3.1. Glycemic parameters

Blood glucose level in untreated STZ diabetic rats (423.15 ± 49.28 mg/dl) was significantly higher than in the non-diabetic rats (96.07 ± 2.76; $p < 0.0001$). Oral administration of *T. cordifolia* (TC) suppressed the elevation of blood glucose level to 352.22 + 47.78 mg/dl, which was significantly lower as compared to diabetic control animals ($p < 0.001$), but was significantly more ($p < 0.0001$) than normal rats.

Similarly, %HbA1c in diabetic rats (7.04 ± 0.18) was significantly higher than normal animals (5.37 ± 0.48; $p < 0.0001$). However, %HbA1c in TC treated group was 6.30 ± 0.90, which was significantly lower than diabetic group ($p < 0.05$).

3.2. Clinical ocular examination

3.2.1. Lenticular changes

Normal group rat lenses had no signs and symptoms of cataract development. However, cataractous changes were observed in diabetic control rat over a period of 24 weeks, which were absent in treatment group (Fig. 1a–c).

3.2.2. Fundus visible changes

After 24 weeks of diabetes, a significant dilation and tortuosity of retinal vessels was seen in diabetic rats. Mean vessel diameter in normal and diabetic groups was 18.75 ± 2.36 and 35.96 ± 1.05 pixel, and that in TC treated group was 28.39 ± 0.72 pixel, which was significantly lower than diabetic rats ($p < 0.0001$) (Fig. 1d–f).