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# To Study the Role of Rutin as Antioxidant and its Neuroprotective Effect in Type II Diabetic Retinopathy

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Abstract: Type II Diabetic Retinopathy is most common cause of visual loss in the world and it is referred as a blinding complication of diabetes that damages retina of the eye. In hyperglycemic condition it is been reported there is reduction in synthesis of various neuroprotective factor in diabetic patients retina as comparison of non diabetic. Neuroprotective Factors includes pigment epithelial-derived factor, brain derived neurotrophic factor, nerve growth factor, somatostatin, and interstitial retinol-binding protein.

Prior studies have discovered that T2DR is associated with neurodegeneration and apoptosis that take place in the retina and the hyperglycemia-induced overproduction of reactive oxygen species. Thus, oxidative stress has been regarded as key factor for causing ocular disease and induces local inflammation, mitochondrial dysfunction, and microvascular dysfunction, disruption of blood- retinal barrier and cell apoptosis of retinal capillary cells.

Keywords: Epigenetics, T2DR, BDNF, oxidative stress, antioxidants

# I. INTRODUCTION

Diabetes is a disease of abnormal glucose metabolism resulting in the hyperglycemia (high blood glucose) due to either a deficiency of insulin resistance or insulin secretion due to pathogenic changes that involve small or large blood vessels, skin, nerves, and lens of the eye. Thus hyperglycemia is considered as the major factor for triggering the development of diabetic complications (Eshaq et al., 2017). Biochemical alteration such as activation of protein kinase C, formation of advanced glycation end products and oxidative stress have been detected as a response of the retina to hyperglycemia (Ahsan, 2015). With recent progress in the management of diabetes, fortunately, the macro vascular mortality is declining, but, diabetic patients are living longer and the incidence of retinopathy and loss of vision associated with this is increasing. BDNF is physiologically involved in maturation of neurons from the neural progenitor and stem cells as well as providing them protection against various noxious stimuli, thereby enhancing their survival rates.

The quick research in the epigenetic modifications and mechanisms controlling BDNF gene expression indicate that growth in BDNF epigenetics will have vast application in prognosis, diagnosis and biomarkers for psychiatric disorders (Pharm and Kotwani, 2017).

Diabetic Retinopathy (DR) is a polygenic disease, characterized by hyperglycemia, micro inflammation, microvascular damage, increased vascular permeability, leukostasis, vascular occlusion, general neurodegeneration and local ischemia. Due to continual hyperglycemia condition there induces imbalance of cellular metabolism including excessive oxidation of glucose, production of Reactive Oxygen Species (ROS). There is activation of local inflammation because of oxidative stress and endothelial cell death occurs (Aiello et al., 1982).

Hyperglycemic condition causes endothelial cell apoptosis, necrosis, and mitosis which direct to microvascular dysfunction and local inflammation in the retina, which promote blindness. So, formation of inflammation, Reactive Oxygen Species and cell death form a roguish (vicious) cycle, promoting the development of Diabetic Retinopathy (Wu et al., 2018).

Due to the hypermetabolic state the retina is susceptible to oxidative stress (Kagan et al., 1973) and the markers levels for oxidative stress are associated with the severity of Diabetic Retinopathy (Hartnett et al., 2000). Due to the pathogenesis of diabetic complications many of different mechanisms including increased superoxide production/decreased scavenging, auto oxidation of glucose, polyol pathway activation and protein kinase C pathway activation, and increased formation of advanced glycation end products leads to increase in oxidative stress (Hamada et al., 2009). Oxidative stress not only can damage cell membrane integrity

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but also, inducing apoptosis, barrier damage and microvascular damage, and finally leading to development of Type II Diabetic Retinopathy (Bonnefont-rousselot, 2002).

According to previous study it has been reported that decrease level of BDNF in diabetic retina damages of retinal neurons that leads to Type II Diabetic Retinopathy.

Therefore, to preserve NTF and NGF, by use of flavonoids to improve retinal damage by inhibiting oxidative stress and decreasing blood glucose level thereby up-regulate the decreased level of *BDNF*. Rutin as a flavonoid exhibit various significant benefits that include anti-oxidant, anti-diabetic and anticancer, anti-inflammatory, neuroprotective effects and cardiovascular activities. Thus rutin help in reserving the functional and structural integrity of cells by decreasing oxidative stress (Pangeni et al., 2014).

The problem is despite of a number of beneficial effects of rutin; to our knowledge no studies reported the influence rutin supplementation on antioxidant, antiapoptotic status, and neurotrophic support in the diabetic retina. Therefore, the present study was designed to explore the ameliorative effect of rutin in combating neurodegeneration in case of Type II diabetic Retinopathy in human eye thus maintaining BDNF level which can prevent Type II Diabetic Retinopathy compared to that of non diabetic healthy controls.

# II. MATERIALS AND METHODS

Human retinal epithelial layer (donated) were collected from city prestigious institute Department of Ophthalmology, Gandhi Medical College (Bhopal). Proper concern and handle must be taken for the entire provision for the collection of retinal endothelial cell and its transportation from this source station to our labs of our institute, School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal.

# A. Cell Culture and Treatment

Human retinal epithelial cells were isolated by gentle brushing retinal pigment epithelium with a sterile spatula from fresh sclera segments.

The retinal tissue layer were rinsed with sterile PBS and then incubated in 5mg/ml sterile filtered trypsin for 50 min at 37°C. After incubation the tissue layer was placed onto the sterile gauze and trypsin was vacuumed out and then filled with 5ml of DMEM (containing F12 media + 20% FBS) (Blenkinsop et al., 2013). Following enzyme digestion to form a single cell suspension, the human retinal epithelial cells were isolated using Matrigel<sup>TM</sup>- Membrane Matrix. The wells of culture plates containing cultured RPE cells were scrapped with the help of scraper and collected then 5 $\mu$ M, 10  $\mu$ M, 15  $\mu$ M concentration of drug was prepared for different time duration. The cells confluency were checked and were treated with prepared drug concentration and treated cells were plated to culture plate for 12hrs and 24 hrs.

# B. BDNF Quantification by Enzyme Linked Immunosorbent Assay (ELISA)

The level of brain derived neurotrophic factor was measured using ELISA kit (ab99978 BDNF Human ELISA Kit) according to manufacturer's protocol. By using BDNF Human ELISA Kit it is possible to measure specific antigen from impure samples as BDNF Human ELISA Kit provides antibody specific for Human BDNF coated on a 96 well plate. Actual concentration of BDNF in each sample was calculated using standard curve.

### C. Caspase Assay

The upregulation of enzymatic activity of the caspase-3 was measured by using Caspase-3- colorimetric assay kit (Biovision). The assay provides the spectrophotometric detection of the chromophore p- nitroaniline (pNA) after its cleavage from the labeled substrate DEVD-pNA . the results were quantified at an emission of 400nm.

### D. Estimation of Thiobarbituric Acid- Reactive Substances (TBARS) Levels

The estimation of lipid peroxidation in cell lysate was done by TBARS Assay Kit (Cayman chemical). The MDA-TBA complex formed under high temperature (90-100°C) and acidic conditions were measured colorimetrically at 540 nm.



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# III. RESULTS

# A. Effect of Rutin on the level of Brain Derived Neuroprotective Factor (BDNF)

Quantitative measurement of the BDNF level using ELISA showed decreased level in Type II Diabetic Mellitus (T2DM) and Type II Diabetic Retinopathy (T2DR) as compared to that of normal sample. Rutin treatment to T2DM and T2DR samples showed increased concentration levels of BDNF as compared to non-treated.



FIG: The above figure indicates the concentration of BDNF level on non-treated and rutin treated cells of T2DM and T2DR for 12 hours and 24 hours.

Increased BDNF level has been observed in rutin treated cells with  $15\mu$ M drug concentration in both T2DM and T2DR and no effect was observed in  $5\mu$ M and  $10\mu$ M concentration of drug on cell line.

### B. Estimation of Rutin on Oxidative Stress

The TBARS levels were measured in cell lysate of normal, T2DM, T2DR and rutin treated T2DM and T2DTR samples. The level of TBARS was found to be increased in T2DM and T2DR samples as compared to that of control whereas after rutin treatment the elevated level of TBARS was found to be decreased in T2DM and T2DR as compared to that of non-treated samples.





Decreased TBARS level has been observed in rutin treated cells with 10  $\mu$ M and 15 $\mu$ M drug concentration in both T2DM and T2DR and no effect was observed in TBARS level when cell line was treated with 5  $\mu$ M a concentration of rutin.

# C. Effect of Rutin on Caspase-3 Activity

Caspase-3 activity was measured in rutin treated and non-treated control. Caspase-3 activity in T2DM and T2DR was found to be increased as compared to normal sample. After treatment with rutin the observed the decreased level of caspase-3 concentration to that of non-treated samples.



FIG: The above figure indicates the concentration of Caspase-3 level on non-treated and rutin treated cells of T2DM and T2DR for 12 hours and 24 hours.



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Decreased caspase-3 level has been observed in rutin treated cells with 5  $\mu$ M, 10  $\mu$ M and 15 $\mu$ M drug concentration in both T2DM and T2DR as compared to that of control.

#### IV. CONCLUCIONS

DR is considered to be result of vascular changes in the retinal circulation and is one of the most common complications of diabetes that affect the blood vessels of the retina, leading to blindness. At initial stages of Diabetic Retinopathy (DR), specific retinal ganglion cells (RGCs) go through apoptosis and neurodegeneration of retina is likely to be related with a lack of *BDNF*. The neurotrophic factor (*BDNF*) was found to be down-regulated; apoptosis cytokines (caspase-3) was found to be up-regulated and lipid peroxidation products such as TBARS which is up- regulated in case of Type II Diabetic Retinopathy. The current research has shown the effect of rutin on human retinal epithelial cells of Type II Diabetic Retinopathy patients. The aim of study was to check the effect of rutin as a drug on levels of caspase-3, TBARS and BDNF on human retinal epithelial cell line (*in-vitro*).

Caspase-3 levels were measured by using Caspase-3 Colorimetric Assay Kit and the assay was measured at 405nm. The results obtained show the decreased level of Caspse-3 in both T2DM and T2DR samples as compared to non-treated samples.

BDNF levels were measured by using BDNF Human ELISA Kit in which plate is coated with Human BDNF Antibody. The ELISA results show the increased level of BDNF in both T2DM and T2DR samples as compared to non-treated.

TBARS levels were measured by using TBARS Assay Kit and assay was measured at 540 nnm.

The assay results show the decreased level of TBARS in both Type II Diabetic Mellitus and Type II Diabetic Retinopathy samples as compared to non-treated.

Present study demonstrates the inhibition of superoxide production by rutin against oxidative stress in Human Type II Diabetic Retinopathy cell lines by *in vitro*.

Based on neuroprotective and antidiabetic effect of rutin *in vitro*, further in vivo studies can be performed to investigate the pharmacological effect of rutin for control of Type II Diabetic Retinopathy.

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