# UNIVERSITI TEKNOLOGI MARA

# MECHANISMS UNDERLYING THE PROTECTIVE EFFECT OF TOCOTRIENOL–RICH FRACTION AGAINST RETINAL OXIDATIVE STRESS, INFLAMMATION, APOPTOSIS AND ANGIOGENESIS IN STREPTOZOTOCIN-INDUCED DIABETIC RETINOPATHY RATS

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# ABSTRACT

Diabetic retinopathy (DR) is the second commonest microvascular complication in diabetes mellitus. Its pathophysiology includes oxidative stress (OS), inflammation, apoptosis and angiogenesis. Palm oil-derived tocotrienol-rich fractions (TRF), a potent antioxidant with multiple other biological properties, may provide protection against DR development and progression. Therefore, we investigated the effect of TRF through firstly, comparing the effect of oral and topical routes of administration, and secondly, by using the best route of administration, we evaluated the mechanism of TRF on retinal oxidative stress, inflammatory, apoptotic and angiogenic status in streptozotocin-induced DR rats. Male *Sprague-Dawley* rats weighing 200-250 grams were divided into normal rats (N), which were injected intraperitoneally with citrate buffer, and diabetic rats, which were injected intraperitoneally with STZ (55 mg/kg body weight) to induce hyperglycaemia. Diabetic rats were further subdivided to diabetic-control (DV) and diabetic-treated (DT) groups. N and DV group received vehicle treatment, whereas DT received TRF daily for 12 weeks. In the first study, treatment was given through oral and topical routes. The best route of TRF administration was determined by evaluating its effect on retinal morphology, TUNEL staining for retinal cell apoptosis and angiogenic protein (VEGF) level. Using the best route of TRF administration, the effect of TRF was subsequently studied on the rats' visual-behavioural activities and retinal vessels through fundus images. The effect of TRF on retinal redox status was determined by measuring catalase, superoxide dismutase, glutathione and lipid peroxidation levels. Further analysis on retinal cell apoptosis was done through measurement of caspase-3, whereas, for retinal angiogenesis, HIF-1α, and IGF-1 were measured. Effect of TRF on inflammatory status was also determined by measuring the pro-inflammatory cytokines and NF-κB signalling pathway. In the first study, TRF supplementation through oral route increased retinal layer thickness and cells count greater than topical route. Oral TRF administration also reduced more retinal VEGF protein compared to topical. On subsequent study, rats receiving oral TRF (DT) showed improvement on visual-behaviour response with preserved retinal venous diameter compared to diabetic rats (DV). Rats in DT also had lower retinal lipid peroxidation, higher retinal antioxidants levels, lower retinal pro-inflammatory cytokines protein and gene expression, reduced NF-κB activation, lower apoptotic protein level and lower angiogenic protein and gene expression compared to DV. In conclusion, oral TRF administration protected DR progression in STZ-induced DR rats by improving retinal redox status, and reducing retinal inflammation, apoptosis and angiogenic status.

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# CHAPTER ONE
# INTRODUCTION

## 1.1 Research Background

Diabetic retinopathy (DR) is a common microvascular complication in diabetes mellitus which leads to vision impairment and blindness (Flaxman et al., 2017). Globally, prevalence of DR among diabetic patients is around 35% and among them, around 10% have vision-threatening problems (Yau et al., 2012; Wong & Sabanayagam, 2020). With increasing incidence of diabetes mellitus every year particularly in Malaysia (1.5- fold increment from year 2006 to 2015; IPH 2015), higher numbers of DR cases are expected to be seen in the future.

The pathophysiology of DR revolves around the theory of retinal oxidative stress, inflammation and angiogenesis. Excessive blood glucose level induces oxidative stress through various pathways, namely, the polyol pathway, advanced glycation end-products (AGEs), protein kinase C (PKC) and nuclear factor kappa B (NF-κB) signalling pathways (Kowluru and Chan, 2007), which leads to overproduction of free radicals. Oxidative stress has been shown to persist despite reversion of blood glucose to normal value, secondary to the epigenetic modifications or metabolic memory that happens during the hyperglycaemic period (Mishra & Kowluru). Thus, oxidative stress plays a major role in the pathogenesis of DR.

Another important factor affecting DR is chronic low-grade inflammation. Higher levels of inflammatory cytokines such as interleukin 1-beta (IL-1β), interleukin 6 (IL-6), and tumour necrotic factor- alpha (TNF-α) were seen in DR compared to normal in animal models as well as in human (Koleva-Georgieva et al., 2011). Other than that, oxidative stress also triggers cellular events that result in the activation of various inflammatory cytokines (Valacchi et al., 2018). Enhanced leucocyte-endothelial adhesion and activation of microglia, an immunocompetent cell of the retina, occur secondary to chronic inflammation and eventually result in non-perfusion of retinal tissue or hypoxia, which in combination with increased blood-retinal barrier permeability, leads to retinal damage (Wijk et al., 2017). Neurodegeneration is one of the signs of retinal cell damage seen in DR and is triggered by oxidative stress and inflammation (Barber, 2011). It is characterized by the loss of retinal neuronal function,