Chapter-1

Introduction

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Objectives

1. Introduction

Diabetes mellitus (DM) is a complex, progressive disease, which is accompanied by multiple complications including nephropathy, retinopathy, neuropathy, micro- and macro-vascular damage. According to the World Health Organization (WHO), 171 million people were affected by diabetes in the year 2000, and this number is likely to almost double by the year 2030. One of the major morbidity and mortality factors confronted by patients with diabetes is an increased risk of developing diabetic nephropathy (DN) that often progresses to end-stage renal disease. Approximately 30–40% of patients with type 1 and 15% with type 2 diabetes mellitus develop nephropathy. Thus, prevention or retardation of DN has become a major goal in biomedical research. Animal models of DN allow performing detailed and mechanistic testing, including direct examination of tissue to assess pathology that is difficult to perform in clinical studies and help in the development of new therapeutic strategies.

Pathogenesis of DN is multifactorial. The role of hyperglycemia in the pathogenesis of DN has been previously established by a number of studies conducted in experimental animal models and human studies. There are data indicating that hyperglycemia induces oxidative stress in the rat kidney and increased oxidative stress in the kidney may trigger apoptosis in renal cells in vitro by inducing DNA fragmentation and stimulating expression of apoptosis-regulatory genes. Hyperglycemia also leads to accumulation of advanced glycation end products (AGE's) in renal cortex. These AGE's play a role in the progression of DN through impairment of matrix proteins in vivo, leading to thickening of glomerular basement membrane and expansion of mesangial matrix. DN is also associated with dyslipidemia, which is characterized by higher plasma levels of total cholesterol, low-density lipoprotein and triglycerides, and lower levels of high-density lipoprotein. Reportedly, lipids may induce both glomerular and tubulointerstitial injury through mediators such as cytokines, reactive oxygen species, chemokines, and through hemodynamic changes. A growing body of evidences also suggests that transforming growth factor-β (TGF-β), a fibrogenic cytokine plays a key role in the development of DN. TGF-β is reported to be the final common mediator of the principal lesions of renal

disease in DM such as renal glomerular hypertrophy and extracellular matrix expansion and function as a proinflammatory cytokine in various epithelial types including the proximal tubular cells of the kidney. Although many mechanisms have been postulated, but the precise mechanism remains unknown and it appears that all these mechanisms are additive in producing the kidney injury.

Vitamin E is one of the most important phytonutrients in edible oils. It consists of eight naturally occurring isomers, a family of four tocopherols (alpha, beta, gamma and delta) and four tocotrienols (alpha, beta, gamma and delta) homologues. Tocotrienol rich fraction (TRF) is the non saponifiable vitamin E fraction extracted from oils in which tocotrienols constitute equal to or more than 50% of the total constituents. Oils from palm (*Elaei guineensis*) fruit mesocarp and Rice (*Oryza sativa*) bran represent two major natural sources having high concentration of tocotrienols in their TRF's. Palm oil TRF consists of approximately 70-80% tocotrienols and 20-30% tocopherols whereas rice bran oil TRF consists of approximately 40-50% tocotrienols and 50-60% tocopherols. TRF from palm oil (PO) and rice bran oil (RBO) have been shown to lower the blood glucose level in patients and preclinical animal models. The hypolipidemic and antioxidant activities of TRF from PO and RBO have been also established in several studies.

2. Significance of the present study

- Studies reporting the effectiveness of TRF supplementation in preventing or reducing diabetic nephropathy are lacking and require extensive research. This study was thus undertaken to investigate if the treatment with TRF from PO and RBO, could improve the renal function in diabetic rats and also to find out the optimum dose of these TRF's that would prove to be beneficial for health.
- The present study is the first to compare the hypoglycemic and nephroprotective effects of TRF obtained from PO and RBO in diabetes in order to settle down to some extent the long and yet unconcluded controversy on the establishment of superiority of one oil over the other and similarly over the superiority of the TRF from PO and RBO.

- The study also aimed to find out the probable mechanism behind the nephroprotective action of PO-TRF and RBO-TRF in diabetes.

3. Aims and Objectives

Aim

To investigate the hypoglycemic and nephroprotective effects of tocotrienol rich fraction (TRF) from palm oil and rice bran oil in experimental animal models of type-1 and type-2 diabetes.

Objectives

- To establish a non genetic rat model of type-1 and type-2 diabetes.
- Dose-dependent study of hypoglycemic effect of palm oil TRF and rice bran oil TRF in type-1 diabetic rat model to find out the optimum dose.
- Comparative study of hypoglycemic activity of palm oil TRF and rice bran oil TRF.
- Investigation of nephroprotective action of palm oil TRF and rice bran oil TRF in diabetic rats.
- Investigation of oxidative damage in diabetic rats and the protective effect exerted by the palm oil TRF and rice bran oil TRF.
- Western blot analysis of transforming growth factor (TGF β), fibronectin and type IV collagen for mechanistic studies on diabetic nephropathy.