UNIVERSITI TEKNOLOGI MARA

STUDIES TO ELUCIDATE MECHANISMS UNDERLYING THE ANTICATARACT EFFECT OF ANNATTO TOCOTRIENOL IN RATS

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of the requirements for the degree of
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ABSTRACT

Cataract, the leading cause of blindness, is currently treated only by surgery. Cataract carries risk of complications and its management increases economic burden. Thus, development of pharmacological options with anticataract effects is important. Oxidative-nitrosative stress, non-enzymatic glycation and osmotic stress underlie cataractogenesis. Since tocotrienol possesses biological properties that may suppress pathophysiological mechanism of cataractogenesis, we investigated its anticataract effects in rats. Tocotrienol was formulated into two widely used colloidal drug delivery systems, microemulsion and liposome, due to its poor aqueous solubility. Firstly, the dose-dependent effects of microemulsion of tocotrienol on cataractogenesis were studied in galactose-fed rats to determine the most effective dose of tocotrienol. Different concentrations of tocotrienol were applied topically twice daily from three weeks prior to galactose diet and continued for four weeks along with galactose diet. Cataract progression was monitored and after euthanization, lenticular oxidative stress was measured. Subsequently, using the most effective dose, anticataract efficacy of microemulsion and liposomal formulation was compared in galactose-fed rats. Additionally, ocular tissue distribution of a lipophilic dye using these formulations was studied. For this distribution study, single drop of solution or formulation containing lipophilic dye was applied and animals were euthanized at different time points. Eyeglobes were dissected, cryosectioned, viewed under confocal microscope and analyzed. Lastly, mechanisms of anticataract effect of tocotrienol were studied in the rat model of streptozotocin-induced diabetes, which is a closer representation of human diabetic cataract. Using this model, the effects of tocotrienol on lenticular polyol pathway, oxidative and nitrosative stress, NFκB signaling pathway, ATP and ATPase content, calpain activity and proteins levels were studied. In dose-response study, 0.03 and 0.02% tocotrienol-treated groups showed slower cataract progression compared to vehicle-treated animals with reduction of lenticular oxidative stress. Faster cataract progression and higher oxidative stress were seen in rats treated with higher concentration of tocotrienol compared to vehicle-treated group. Both microemulsion and liposomal formulations showed similar anticataract efficacy. In ocular distribution study, better intraocular distribution was observed following single drop application of lipophilic dye in microemulsion compared to liposome and solution. In rats with streptozotocin-induced diabetes, tocotrienol delayed the progression of cataract and this anticataract effect was associated with reduction of lenticular oxidative-nitrosative stress, NFκB activation, iNOS expression, lens polyol levels, and restoration of the ATP and ATPase levels, calpain activity and lens protein levels. In conclusion, topically applied tocotrienol shows anticataract effects in rats by reducing oxidative-nitrosative stress and restoring the lens polyol levels, ATP and ATPase levels, calpain activity and lens protein levels.

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