UNIVERSITI TEKNOLOGI MARA

ANTI-REMODELLING EFFECTS OF *TRANS*-RESVERATROL AND RU-615 ON EXTRACELLULAR MATRIX IN STEROID-INDUCED GLAUCOMA MODELS VIA TGF-B-SMAD SIGNALLING PATHWAY

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ABSTRACT

Glaucoma is the leading cause of irreversible blindness worldwide and the only modifiable risk factor is intraocular pressure (IOP). Increased extracellular matrix (ECM) deposition in the trabecular meshwork (TM) increases aqueous humour outflow resistance leading to IOP elevation, effects modulated by the TGF-β-SMAD signalling. The similarities between steroid-induced glaucoma and primary open angle glaucoma have led to the utilisation of steroid-induced experimental models in glaucoma-related research. Resveratrol is a natural polyphenolic compound and RU-615 is a derivative of imidazo[1,2-a]benzimidazole compounds. Both compounds have been shown to counteract the steroid-induced increase in IOP. However, whether their effects were associated with modulating the ECM remodelling in TM and their related pathways remained uncertain. Therefore, this study aims to determine the effect of *trans*-resveratrol (TR) and RU-615 on steroid-induced changes in ECM remodelling and their mechanisms of action using *in vitro* and *in vivo* steroid glaucoma models. In the *in vitro* study, the effects of TR and RU-615 on steroid-induced increase ECM deposition and their cellular signalling pathway of TGF-β1-SMAD in primary human trabecular meshwork cells (HTMCs) were evaluated. Whereas *in vivo* study evaluated the effects of prolonged topical application of TR and RU-615 on the IOP, visual behaviour functions and morphology of the TM and retina. Treatment with TR and RU-615 downregulated ECM gene and protein expressions induced by dexamethasone in the HTMCs. These were associated with reduced TGF-β1 and SMAD 4, and raised SMAD 7, gene and protein expressions. Both treatments also reduced plasminogen activator inhibitor (PAI-1) and increased tissue plasminogen activator (tPA), gene and protein expressions that are involved in modulating matrix metalloproteinases (MMPs). Both topical TR and RU-615 treatments reduced TGF-β1 and increased tPA aqueous humour levels in steroid-induced ocular hypertensive (SIOH) rats. Prolonged twice-daily topical applications of TR and RU-615 significantly reduced the IOP in SIOH rats. Moreover, both treatments significantly reduced the TM cell counts and thickness, and significantly increased the retinal cell count and thickness, contributing to their effects in reducing the IOP and retinal protection in the steroid-induced glaucoma model. Despite these results, SIOH rats receiving both treatments had no significant visual improvement in the object recognition test but significantly improved visual function with the mirror chamber test. Hence, it could be concluded that both treatments using TR and RU-615 in the steroid-glaucoma model were able to reduce ECM deposition with the effects mediated through the TGF-β1-SMAD pathway associated with reduced PAI-1 and increased tPA levels. The involvement of TGF-β1-SMAD-PAI-1/tPA in the effects of TR and RU-615 in reducing the deposition of ECM components is likely the cause of changes observed in the TM cells and tissues, which improved the AH outflow leading to reduced IOP and preservation of retinal morphology in steroid-induced glaucoma model.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Glaucoma is characterised by the loss of retinal ganglion cells (RGCs) and damage to the optic nerve, causing visual field defects and subsequently leading to irreversible blindness. It is the leading cause of irreversible blindness affecting about 70 million people worldwide (Kapetanakis et al., 2016; Zhang, Wang, Li, et al., 2021). The disease is an age-related disorder with highest risk in population above 60 years (Allison et al., 2020; McMonnies, 2017). The incidence of glaucoma is expected to rise in years to come since the world is witnessing growth in size and proportion of the elderly population. With an estimate of 1 in 6 people in the world to be aged 60 and above by the year 2030, glaucomatous neuropathy is expected to bring a significant health and economic burden to the worldwide community (Delgado et al., 2019). Majority of world's glaucoma cases are from the Asian population, and this increasing trend of glaucoma prevalence has become a worrisome issue in Malaysia (Al-Naggar et al., 2020).

Glaucoma is classified into primary and secondary types. Primary glaucoma includes primary open angle glaucoma (POAG), primary angle closure glaucoma (PACG) and congenital glaucoma, among which POAG is the most common. Secondary glaucoma is a consequence of a pre-existing disease or predisposing factors such as the use of corticosteroids, topically or systemically. Steroid-induced glaucoma is widely prevalent due to the widespread use of steroids for inflammatory conditions (Feroze et al., 2023). It is clinically and pathologically akin to POAG and both are treated with similar treatment strategies, pharmacologically and surgically (Fini et al., 2017). The similarities between the two have led to the common use of steroid-induced *in vitro* and *in vivo* models for glaucoma related research (Agarwal & Agarwal, 2017).

At present, 80% of glaucoma cases are those of POAG, accounting for 68.56 million people globally (Zhang, Wang, Chen et al., 2021; Zhang, Wang, Li, et al., 2021). Although the pathophysiological mechanisms underlying glaucomatous changes are still not fully understood, however, some risk factors predisposing to the disease have been identified. Among these, the elevated IOP has been recognized as the only modifiable risk factor for the development and progression of glaucomatous neuropathy