UNIVERSITI TEKNOLOGI MARA

THE NEUROPROTECTIVE EFFECT OF TRANS-RESVERATROL AGAINST NMDA-INDUCED RETINAL EXCITOTOXICITY IN SPRAGUE DAWLEY RATS VIA NMDA-MEDIATED CALCIUM SIGNALLING AND ADENOSINE A1 RECEPTOR

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Thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy
(Medicine)

Faculty of Medicine

September 2024

ABSTRACT

Glaucoma is the second leading cause of irreversible blindness worldwide. Elevation of intraocular pressure (IOP) is the common risk factor in glaucoma. However, a considerable number of individuals still suffer glaucoma with normal IOP. Glutamate-mediated excitotoxicity plays a key role in development of glaucoma including in those patients with normal IOP, which is termed as normal tension glaucoma (NTG). Modulation of adenosine A1 receptor (AA1R) has been shown to counteract glutamate-mediated excitotoxicity. Therefore, compounds with AA1R agonistic property have potential to be used as antiglaucoma agents. Trans resveratrol (TR), a polyphenolic compound known for its antioxidants properties has been reported to stimulate AA1R. Meanwhile, another synthetic compound of interest named RU615, has also shown to have high affinity towards AA1R via computational analysis. Hence, the aim of this study was to investigate the effect of TR towards NMDA-induced retinal excitotoxicity rat model through AA1R modulation. In the first study, TR and RU615 were used to investigate their effect towards retinal morphology and apoptotic changes in two different doses. Whereas, for second study, in-depth mechanism on retinal oxidative stress, apoptosis, and visual-behavioural test through modulation of AA1R by TR were elucidated. In the first study, *Sprague-Dawley* rats were divided into six groups; group 1 received intravitreal vehicle, group 2 received intravitreal NMDA, group 3 and 4 received intravitreal NMDA and pre-treatment of TR at two different doses and group 5 and 6 received intravitreal NMDA and pre-treatment of RU615 at two different doses. Rats were euthanised seven days later and retinal morphology, TUNEL staining for retinal cell apoptosis and optic nerve degeneration were evaluated. In the second study, rats were divided into four groups; group 1 and 2 were similar as in first study, group 3 received intravitreal NMDA and pre-treatment of TR, and group 4 received intravitreal NMDA and DPCPX (an AA1R antagonist) and pre-treatment of TR. Rats were subjected to visual-behavioural test 24 hours prior to euthanisation. As in first study, rats were euthanised seven days later. Retinal tissues were collected for oxidative stress markers (catalase, superoxide dismutase, glutathione, inducible nitric oxide synthase, 3-nitrotyrosine and lipid peroxidation levels) and retinal apoptotic markers (caspase-3, BAX and BCL-2 levels) including calcium dependent markers (calcineurin, calpain-1 and cabin-1).TR and RU615 at both doses preserved retinal and optic nerve morphology against NMDA-induced retinal excitotoxicity. This is evidenced by preservation of retinal nuclei, layers and optic nerve structure. In second study, TR group preserved retinal antioxidant levels, reduced retinal lipid peroxidation, nitrosative markers and calcium mediated apoptotic markers accompanied by improved visual behavioural function. Whereas reversal of TR effects by DPCPX were observed on those parameters which suggested the role of AA1R in its protective mechanism towards NMDA-induced retinal excitotoxicity. In conclusion, intravitreal TR administration protected NMDA-induced retinal excitotoxicity changes by improving the retinal redox status, reducing retinal apoptosis, that preserved retinal morphology and leads to improvement of visual-behavioural function through AA1R modulation.

ACKNOWLEDGEMENT

Alhamdulillah, completing this PhD has been a miracle for me. With Allah's blessing and guidance, it has been a wonderful 5 years of my life. I am ever thankful for both of my parents and Selamat for their constant guidance, support both mentally and fiscally. Undoubtfully my strongest pillar of inspirations. My dear brothers, sister and nephew whom always there to cheer me up I couldn't possibly complete this thesis without my spectacular and ever supporting supervisor Associate Profesor Dr Nurul Alimah whom constantly pushed me towards my end goal. Dr Norhafiza Razali whom always guide me during my rough starting years let till the end. Both of my terrific Profs, Prof Renu and Prof Igor who has been a great motivator and source of knowledge, also the ones whom accepted me into this great team hence helped me to convert my master study into PhD. I would also like to express my gratitude to Faculty of Medicine, Universiti Teknologi MARA and their supporting technical and administrative staffs. I would like to thank my dearest beloved best friend whom always help me let it be my labwork and thesis writing. I am also ever thankful for my Pharmaco teammates and my other peers who has been staying by my side here in UiTM. Special thanks to fellow staffs of Institute of Medical Molecular Biotechnology, Centre for Neuroscience Research and Laboratory Animal Care Unit for the endless knowledge and supports. I hope this thesis serves as an academic kudos in fulfilling the knowledge gap as well becomes an inspiration for future generation that have yet to come including my dear grandchildren.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Glaucoma is a cluster of eye diseases characterized by the death of retinal ganglion cells (RGC) and optic nerve damage (Weinreb et al., 2014). Globally, approximately 3.5% of the world population aged 40 to 80 years are suffering from glaucoma with around 60 million people in total affected (Allison et al., 2020). According to the World Health Organization, glaucoma is the second leading cause of blindness and is the leading cause of irreversible blindness (li et al., 2020). Clinically, glaucoma is characterized by the damage of optic disc, loss of visual fields and the gradual loss of vision (Kuehn et al., 2005).

Glaucoma is further divided into two subclasses which are primary and secondary glaucoma (Chang et al., 2021)To date, the exact mechanisms underlying RGC death in glaucoma remain widely researched on and several associating risk factors have been identified. One common prominent risk factor is the increase in intraocular pressure (IOP), which ranges between 12 – 21 mmHg (Savinova et al., 2001) However, glaucomatous changes are also reported in patients with normal IOP, which is referred as normal tension glaucoma (NTG).

Research reported glutamate excitotoxicity contributed to the progression of RGC death (Christensen et al., 2019). Glutamate is the major excitatory neurotransmitter in retina, which acts via ionotropic and metabotropic receptors. The ionotropic receptors include N-methyl-D-aspartate (NMDA), A-amino-3-hydroxy-5-methyl-4-isoxazalepropionic acid (AMPA) and kainate receptors. Glutamate neurotransmission is essential for normal physiological retinal functions, however, excessive glutamate availability results in excessive excitatory neurotransmission leading to tissue damage. NMDA subtypes are among the common glutamate receptors widely implicated to play significant role in excitotoxicity. The activation of NMDA receptor leads to intracellular influx of calcium ions (Ca^{2+}) which in turn activates Ca^{2+}-dependent pathways involving calpain 1, cabin 1 and calcineurin. Activation of these Ca^{2+}-regulated proteins promotes both caspase-dependent and caspase-independent apoptotic pathways.