ABSTRACT

Oxidative stress-induced neurodegenerative diseases have become more prevalent lately due to the stressful environment and lifestyle. Growing empirical scientific evidences which support the use of plant-derived antioxidants in the control of neurodegenerative disorders has been validated in the present investigation. *Loranthus parasiticus* (L.) Merr, a chinese traditional folk medicine which has been used in treating brain diseases was selected for the present study. Therefore, *L. parasiticus* was hypothesized to exhibit antioxidative and neuroprotective properties in NG108-15 neuroprotection model. *Loranthus parasiticus* aqueous fraction (LPAF) which showed the highest antioxidative and neuroprotective activities against H_2O_2 among the tested extract and fractions was subjected to a bioassay-guided fractionation and isolation approach to identify the most potent neuroprotective compound. (+)-Catechin was found to be the most potent neuroprotective compound and its underlying mechanisms were evaluated subsequently. (+)-Catechin significantly reduced reactive oxygen species production, phosphatidylserine externalization, mitochondrial membrane potential depolarization, sub-G_1 apoptotic fraction induction, and increased the percentage of cell viability following H_2O_2-induced oxidative stress insult. Moreover, (+)-catechin increased the H_2O_2-induced reduction of SOD and GPx activities. (+)-Catechin also upregulated Bcl-2 and downregulated Bax, resulting in a decreased ratio of Bax/Bcl-2. Interestingly, oxidative stress-induced overexpression of chemokine CCL21 was significantly attenuated by (+)-catechin, indicating a novel role of (+)-catechin in neuroprotection context via the regulation of neuronal chemokine CCL21. Collectively, the present findings have proven our hypothesis and support the use of *L. parasiticus* in managing oxidative stress related neurodegenerative diseases.

ABSTRAK

Penyakit neurodegeneratif akibat daripada tekanan oksidatif persekitaran dan gaya hidup semakin mendapat perhatian pada masa kini. Semakin banyak bukti saintifik empirikal yang menyokong penggunaan faktor antioksida tumbuh-tumbuhan dalam pengawalan neurodegeneratif telah dibabitkan dalam pengesahan ini. *Loranthus parasiticus* (L.) Merr, perubatan tradisional Cina yang telah lama digunakan dalam rawatan penyakit-penyakit otak dipilih untuk kajian ini. Oleh itu, *L. parasiticus* dihipotesiskan mempamerkan sifat-sifat antioksida dan neuroprotektif dalam model perlindungan saraf yang menggunakan NG108-15. Fraksi akueus *L. parasiticus* (LPAF) yang menunjukkan aktiviti antioksida dan neuroprotektif tertinggi di antara ekstrak dan pecahan lain terhadap kesan H_2O_2 dipilih untuk fraksinasi dan isolasi berpandukan bioassai untuk mengenal pasti sebatian yang paling neuroprotektif. (+)-Catechin ditemui sebagai sebatian yang paling neuroprotektif dan mekanisme dasarnya telah dinilai kemudian. (+)-Catechin didapati mengurangkan pembentukan spesies oksigen reaktif, pengeluaran phosphatidylserine, potensi mendepolarisasi membran mitokondrion, menginduksi pecahan apoptotic sub-G_1, dan meningkatkan peratusan daya maju sel selepas induksi tekanan oksidatif oleh H_2O_2. Tambahan pula, (+)-catechin meningkatkan aktiviti SOD dan GPx selepas induksi H_2O_2. (+)-Catechin juga meningkatan Bcl-2 dan menurunkan Bax, dan mengakibatkan penurunan nisbah Bax/Bcl-2. Keputusan yang menarik telah dijumpai di mana tekanan oksidatif yang meningkatkan pengawalaturan CCL21 chemokine telah dilemahkan oleh (+)-catechin dan ini telah menunjukkan peranan novel (+)-catechin dalam konteks perlindungan saraf melalui pengawalaturan CCL21 chemokine. Secara kolektif, pengesahan saintifik ini telah membuktikan hipotesis kami dan menyokong penggunaan *L. parasiticus* dalam pengurusan penyakit neurodegenaratif yang berkaitan dengan tekanan oksidatif.

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LIST OF ABBREVIATIONS

ATCC	American type culture collection
AIF	Apoptosis-inducing factor
APAF	Apoptosis protease activating factor
BH	Bcl-2 homology
BHT	Butylated hydroxytoluene
BD	Becton Dickinson
CD	Cluster of differentiation
CDK	Cyclin-dependent kinase
DAPI	4',6-diamidino-2-phenylindole
DCH-DA	2,7 dichlorofluorescein diacetate
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
DTNB	5',5'-dithio-bis(2-nitrobenzoic acid)
EDTA	Ethylenediaminetetraacetic acid
EGCG	(−)-epigallocatechin-3-gallate
FACS	Fluorescent activated cell sorting
FADD	Fas-associated protein with death domain
$FeCl_3$	Ferric chloride
$FeSO_4$	Ferrous sulfate
FITC	Fluorescein isothiocyanate
FBS	Fetal bovine serum
GAE/g_{DW}	Gallic acid equivalent per gram of dry weight
GPCR	G-protein coupled receptor

GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Oxidized glutathione
GST	Glutathione-S-transferase
HAT	Hypoxanthine-aminopterin-thymidine
HMBS	Hydroxymethylbilane synthase
HNE	4-hydroxy-2-nonenal
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
IC_{50}	50% inhibitory concentration
IgG	Immunoglobulin G
JC-1	5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide
LC-MS	Liquid chromatography mass spectroscopy
LPAF	*Loranthus parasiticus* aqueous fraction
LPEAF	*Loranthus parasiticus* ethyl acetate fraction
LPEE	*Loranthus parasiticus* ethanol extract
MTT	3-(4,5-domethylthiazol-2-yl)-2,5-diphenyltetrazolim
NADPH	Nicotinamide adenine dinucleotide phosphate
Na_2HPO_4	Disodium hydrogen phosphate
NaH_2PO_4	Sodium dihydrogen phosphate
Na_2CO_3	Sodium carbonate
NG108-15	Mouse neuroblastoma x rat glioma hybrid cell line
NMDA	N-methyl-D-aspartate

NMR	Nuclear magnetic resonance
NO	Nitric oxide
O_2^-	Superoxide anions
$OH^{\bullet}$	Hydroxyl radicals
$ONOO^-$	Peroxynitrite
PBS	Phosphate buffer saline
PE	Phycoerythrin
PI	Propidium iodide
Q-PCR	Real time-polymerase chain reaction
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TLC	Thin layer chromatography
TNF	Tumor necrosis factor
WST-1	2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt
XO	Xanthine oxidase