2015

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AMLA ENHANCES AUTOPHAGY AND MODULATES BETA AMYLOID METABOLISM IN AN *IN VITRO* MODEL OF ALZHEIMER'S DISEASE

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INTRODUCTION

Beta-amyloid (Aβ) deposition in the brain as extracellular plaques is a neuropathological hallmark of Alzheimer disease (AD)(2). The accumulation and aggregation of beta amyloid (Aβ), resulting from its over-production or impaired clearance from the CNS plays a key role in the Alzheimer disease pathogenesis (1).

Reducing production, preventing Aβ aggregation or improving its clearance are currently some areas of active research in the development of therapeutic agents to ameliorate neurodegeneration in AD. The Indian plant amla (*Emblica officinalis*), commonly known as Indian gooseberry, has widely been utilized in traditional Ayurvedic medicine preparations in the treatment of a variety of disease conditions including cardiovascular disease, diabetes. Accumulating evidence also suggests that amla may be beneficial in AD (3). Recent evidence reports increased autophagy following amla treatment. (4). Amla has shown enhanced the expression of the autophagic proteins LC3B-II and beclin1 in an *in vitro* significantly (4). Autophagy is a vital mechanism involved in the clearance of aggregated proteins and damaged organelles in cells. Enhancing autophagy has shown to reduce amyloid deposition and neurodegeneration in AD models and is a potential therapeutic strategy for AD and several neurodegenerative disorders (5). The main aim of this study is to investigate the effects of amla on modulation of autophagy and beta amyloid metabolism underlying.

METHODS

Human embryonic kidney 293 APP cells are transfected with human APP bearing the Swedish mutation (HEK293 APPsw). HEK293 APPswe cell line was kindly provided from Prof Paul Fraser laboratory.

The purified, standardized dried extract of amla which is known as Amlamax is used for treating the cells.

3mg of amla powder was dissolved in 10 liters 37 ° C DMEM medium and vortex several times.

HEK293 cells expressing APPswe line were treated with amla (50-300ug/ml) for 24 hours.

Expression level of Full length APP, APP C-terminal fragment and marker of autophagy LC3 in HEK293 APPswe cells were measured in cell lysates following amla treatment.

The primary antibodies used in this project are summarised in Table1.

	Antibodies	WB	Host
Primary	Anti-Amyloidβ Antibody (WO2) (M)	1:3000	Mouse
Primary	Anti-APP CTF (C99) (C1/1.6) IgG (M)	1:1000	Mouse
Primary	Anti-LC3 (p.m03.6)	1:2500	Rabbit
Primary	Anti-Glyceraldehyde-3-phosphatedehydrogenase (GAPDH) (M)	5:000	Rabbit

(M) denotes monoclonal antibody

RESULTS

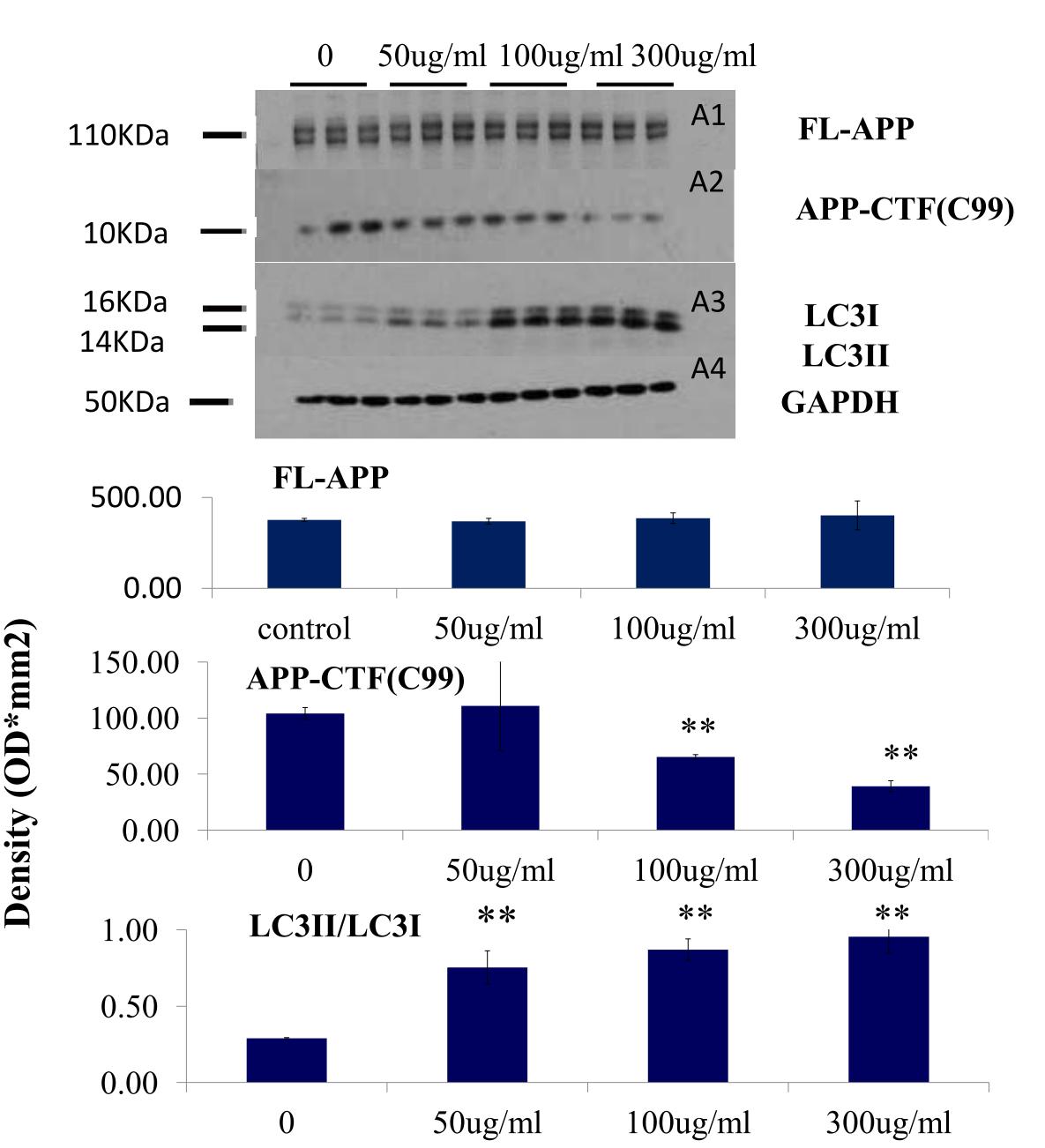**Experience of proteins of interest in treated HEK 293APPswe cell line with amla**

Figure 1 A. Expression of FL-APP (A1), APP-CTF(C99) (A2), marker of autophagy LC3 (A3) and GAPDH levels of APP in treated HEK2 93swe cells (50-300 μg/ml) are (triplicate) similar (A1) levels of increasing concentration of amla(50-300 μg/ml) significant reduced C.terminal fragment in a in HEK293swe (triplicate) (A2) and significant increased levels of LCE biomarker (A3).

Figure1B: The western blotting results aretransformed with Quantity One software The immunoblotting Quantity One software has confirmed the visualization of bands. (* p<0.05, ** p<0.01).

SUMMARY

Preliminary data shows that amla treatment enhances autophagy and modulates accumulation of proteolytic products of Amyloid precursor protein (APP) such as APP-C terminal fragments (C99).

•Amla treatment (50-300 μg/ml) induced a dose-dependent increase in autophagic flux, as measured by Western blotting utilizing an LC3 directed antibody as an autophagosome marker.

•At similar concentrations, amla treatment also reduced accumulation of APP C-terminal fragment levels by 33 to 77%.

• no significant changes were observed in APP levels, indicating that amla did not alter APP production.

•One of the valuable component of amla which has shown significant effect in inducing autophagy via increasing the expression of autophagic proteins is quercetin (4).

Future :
• To determine if amla mediated autophagy contributes to enhanced clearance/degradation of Aβ in neuronal cells.
• To determine if amla reduce Aβ aggregation and toxicity.
•Investigate the effect of amla on Aβ42 oligomerization and toxicity
Overall, our findings suggest that amla may confer beneficial effects through modulating autophagy and Aβ metabolism, and warrants further investigation as a potential therapeutic agent in AD.

ACKNOWLEDGEMENTS

The authors acknowledge Edith Cowan University and all who took part in the study for Alzheimer's disease Research.

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