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Aloe emodin, an anthraquinone exhibits higher cytotoxicity to hepatoma, prostate and cervical cancer cells through cell cycle arrest and apoptosis compared to normal cells. However, its underlying mechanism on ER+-breast cancer cell death remains unclear. Therefore, this study was done to investigate aloe emodin cytotoxicity and its mechanism on estrogen receptor (ER)-positive (MCF-7), ER-negative breast cancer cells (MDA-MB-231) and control breast cells (MCF-10A) in comparison with tamoxifen. Cytotoxicity was determined using WST-1 proliferation assay and Trypan blue exclusion test. Apoptosis mechanism was investigated using Annexin V-FITC/PI staining and DNA fragmentation assay. Both genes and proteins involved in the regulation of cell cycle (p53, p21, CDK1, CDK2, cyclin B1 and cyclin E1) and apoptosis (Fas, FADD, Caspase-3, Caspase-8, Caspase-9, Bax, Bcl-2, and Cytochrome c) in aloe emodin-treated MCF-7 were determined using Quantigene 2.0 Plex and protein ELISA assays respectively. Maximum Bcl-2, and Cytochrome c and Caspase-9 proteins were upregulated; while no change observed in Bcl-2 protein. Except for Caspase-9, these results are in accordance with gene expression. In intrinsic apoptosis signalling, Bax, Cytochrome c and Caspase-9 proteins were upregulated; while no change observed in Bcl-2 protein. Except for Caspase-9, these results are in accordance with gene expression. In intrinsic apoptosis, Fas and Caspase-8 were upregulated, contrary to gene expressions. These findings indicate that aloe emodin cytotoxic action on MCF-7 cells is through G2/M arrest; both extrinsic and intrinsic apoptosis pathways. Its actions on G2/M phase arrest and activation of intrinsic apoptosis pathways were p53-dependent, while extrinsic apoptosis was p53-independent. Data obtained suggests (i) aloe emodin has potential as a selective apoptotic inducer in ER+-breast cancer management and (ii) the present study could be used as a basic rationale for in vivo experiment setting.

Neuroprotective strategies are required to complement the available medical treatments in order to enhance the brain endogenous protective mechanisms and cushion the effect of stroke injury. Pharmacological preconditioning is an avenue of preventative medication anticipated to be highly effective in protecting and reducing the ischemic induced neuronal damage. Recently, in vitro preconditioning studies have shown that prior activation of group I metabotropic receptor (mGlUR) with its specific agonist (S)-3,5-dihydroxyphenylglycine ((S)-3,5-DHPG) elicits neuroprotection against excitotoxicity. Furthermore, the activation of group I mGlUR regulates the expression of DREAM. DREAM protein regulates transcription of various genes including edem1 which is a component protein of ER-associated degradation pathway (ERAD). This study elucidates the neuroprotective effect of group I mGlUR agonist preconditioning, (S)-3,5-DHPG via DREAM and ERAD in acute ischemic stroke rats. One, 10 or 100 μM (S)-3,5-DHPG was administered intracereally to 6 adult male Sprague Dawley rats 2 hours prior to the middle cerebral artery occlusion. After 24 hours, the modified neurological severity score (mNSS) and grid walking test were assessed. The rats were sacrificed and the infarct brain volumes were estimated by 2,3,5-triphenyltetrazolium chloride staining. The serum level of neuron-specific enolase (NSE) and brain tissue level of Bip/GRP78 ER stress marker were assessed by ELISA assays. The ischemic penumbra tissue surrounding the ischemic core infarct was dissected and the cytoplasmic and nuclear proteins as well as the total RNA were extracted. The protein levels of nuclear and cytoplasmic DREAM, as well as EDEM1, SEC61α and VCP were analysed by Western blot. The expression of dream and edem1 genes were analysed by qRT-PCR. Finally, the level of protein degradation activity in the ischemic penumbra tissue was determined by the 20S proteasomal assay. One or 10 μM of (S)-3,5-DHPG preconditioning in stroke rats has significantly improved the neurological functions and reduced the brain infarction as well as the NSE level. The DREAM protein has significantly increased in the nuclear compartment after 2 hours of 1 μM (S)-3,5-DHPG administration and in the cytoplasmic compartment after 24 hours of 100 μM (S)-3,5-DHPG administration. Similarly, 1 μM (S)-3,5-DHPG preconditioning has significantly reduced the levels of Bip/GRP78 ER stress marker, DREAM and ERAD proteins as well as proteasomal degradation activity after 24 hours of an ischemic stroke. The expression of dream and edem1 gene were decreased in 1 μM (S)-3,5-DHPG preconditioning compared to non-preconditioning ischemic stroke rats. In conclusion, the 1 and 10 μM of (S)-3,5-DHPG preconditioning enhanced the endogenous protective mechanism via promoting the nuclear DREAM protein to regulate the expression of EDEM1 and ERAD activities in order to alleviate subsequent ischemic injury in the brain whereas 100 μM of (S)-3,5-DHPG preconditioning exacerbated the ischemic injury.