Embelin prevents amyloid-beta accumulation via modulation of SOD1 in a Streptozotocin-induced AD-like condition: An evidence from in vitro investigation

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ABSTRACT

Embelin is a neuroprotective compound with therapeutic benefit against experimental Alzheimer’s disease (AD)-like condition. In the quest of untangling the underlying mechanism behind the neuroprotective effect of Embelin in AD, an in-vitro study of Embelin against neuronal damage induced by Streptozotocin (STZ) in rat hippocampal neuronal culture was performed. Current findings demonstrated that Embelin (2.5–10 μM) has efficiently protected hippocampal neurons against STZ (8 mM)-induced neurotoxicity. An increase in amyloid precursor protein (APP), microtubule-associated protein tau (MAPT), glycogen synthase kinase 3 alpha (GSK-3α) and glycogen synthase kinase 3 beta (GSK-3β) expression levels was observed when STZ (8 mM) stimulation was done for 24 h in the hippocampal neurons. A significant downregulation in the mRNA expression levels of APP, MAPT, GSK-3α, and GSK-3β upon pre-treatment with different doses of Embelin (2.5 μM, 5 μM and 10 μM) reflects that Embelin attenuated STZ-induced dysfunction of insulin signaling (IR). Embelin significantly modulated the mRNA expression of scavenger enzyme Superoxide dismutase (SOD1). Furthermore, STZ had significantly upregulates an expression of Aβ. On the contrary, pre-treatment with three doses of Embelin reversed an Aβ-induced neuronal death. Our findings suggest that, Embelin prevents Aβ accumulation via SOD1 pathway to protect against AD-like condition.

1. Introduction

Alzheimer’s Disease (AD) is a devastating neurodegenerative disorder where the neuropathological hallmarks is characterized by an accumulation of amyloid beta (Aβ) into the amyloid plaques and intraneuronal neurofibrillary tangles (NFTs), consisting of accumulated Tau protein which might be due to hyper- and/or abnormal phosphorylation (Laurent et al., 2018; Paudel et al., 2020). AD is acknowledged as a leading cause of dementia which is characterized by gradual decline in memory and cognitive impairment (Association, 2019).

AD exists in two major types such as sporadic AD and familial AD. Sporadic AD is referred as the late-onset AD (LOAD) and is the most prevalent AD types (90% of AD cases), afflicting people of any age (but mainly above the age of 65 years) (Saido, 1998). Early-onset familial AD (EOFAD) is detected in families that have more than one member with AD in which the age of onset is steadily before age 60–65 years and frequently before age 55 years (Sherrington et al., 1995) caused by missense mutation or inheritance (Salkovits-Petsi, 2008). Nonetheless, these two AD conditions have been reported to have a relationship with common pathological hallmarks with extracellular Aβ plaques and intracellular neurofibrillary tau tangles that disrupt synaptic connections, leading to neuronal death (Butterfield et al., 2006).

Pathogenetic link between sporadic AD and type 2 diabetes mellitus (T2DM) has been well reported (Plaschke and Kopitz, 2015). Both disorders share similar features associated with glucose metabolism dysfunction and insulin signalling impairment that results in neuronal damage and cognitive deficits (Candinas et al., 2012). High expression of...
insulin mRNA in the hippocampus and rise in insulin receptor (IR) at hippocampal synaptic membrane are functionally associated with improved learning and memory (Zhao et al., 1999, 2004). Nonetheless, the precise mechanisms behind the insulin influencing learning and memory remains elusive. However, growing evidence from in vitro and in vivo studies revealed that impairment in IR signalling leads to the activation of glycogen synthase kinase 3 (GSK-3) through kinase/phosphatase imbalance state (Jolivalt et al., 2008). Furthermore, studies have demonstrated that GSK-3α and GSK-3β are the two key isoforms derived from GSK-3 regulating the production of Aβ (Hooper et al., 2008; Phiel et al., 2003) and tau phosphorylation (Ishiguro et al., 1993) respectively.

Streptozotocin (STZ) is chemically known as (2-deoxy-2-[3-methyl-3-nitrosoure]a) 1-D-glucopyranose), which is widely used agent to induce diabetes in an experimental animals (Goud et al., 2015). In AD related studies, STZ administration induced IR signalling impairment along with neuroinflammation and oxidative stress in vivo and in vitro model, which mimic the human AD pathology (Agrawal et al., 2011; Javed et al., 2012; Kamat, 2015).

Embelin, an active ingredient isolated from the fruits of Embelia ribes Burm has been traditionally used as brain tonic for the treatment of neurological disorders (Kundap et al., 2017). Recently, neuroprotective effect of Embelin has been reported in an animal model of AD (Arora and Deshmuk, 2017; Bhuvanendran et al., 2018a). However, no earlier in vitro or in vivo studies has reported the changes of STZ induced Aβ plaques and how pre-treatment with Embelin could inhibit Aβ expression. Herein, the primary culture of hippocampal neurons was utilised as a model to study the neuroprotective effect of embelin in STZ-induced neurotoxicity. Utilizing the in vitro model, we aimed to precisely understand the underlying mechanism that can reverse through IR signalling pathways mimicking the sporadic AD pathology by Embelin derived benzoquinone.

2. Materials and methods

2.1. Embelin and its physiochemical properties

Embelin in the purest form was purchased from YUCCA Enterprises, Mumbai, India. The solubility of Embelin is found on hot organic solvents, alkali hydroxide solutions whereas Embelin is slightly soluble in petroleum ether and insoluble in water. The Embelin was in orange coloured powder with a molecular weight of 294.39 and moisture content of 1.9%. The purity of Embelin was found to be 97.90% on dry weight basis as evident by HPLC analysis.

2.2. Primary neuronal culture

New-born P4 Sprague Dawley (SD) rat pups were acquired from the Animal Facility of Jeffery Cheah School of Medicine and Health Sciences, Monash University Malaysia. All animal experimentation was approved and performed in agreement with Monash Animal Research Platform (MARP) Animal Ethics Committee with reference number MARP/2017/032. Primary cultures of hippocampal neurons were prepared as described by Kaech and Banker (2006) and Qu et al. (2012) with a minor modification. After treatment with 5 mL of trypsin for 15 min at 37°C, the hippocampal neurons were washed in Krebs buffer for 5 min to stop the trypsin reaction. Then, the hippocampal neurons were suspended in fresh 2.5 mL Krebs buffer with 5 μL DNase and slowly triturated. Neurons were plated in poly-D-lysine-coated well, followed by 2 h incubation at 37°C in a humidified atmosphere comprising 95% air and 5% CO2. Once the neurons adhered to the coated well, the medium was substituted with serum-free Neurobasal A added with 2% B27, 1.34 mM glutamine, 28 mM Glucose and 1.34 mL antibiotic-antimycotic solution (100X) followed by 8–10 days incubation period with half of the medium being changed each 2 days to guarantee neuronal growth (Chen et al., 2008, 2009).

2.3. In vitro neuroprotection test

To evaluate the protective role of Embelin against STZ instigated neurotoxicity on primary hippocampal neuronal culture, three independent experiments were directed to examine (i) the effect of various concentrations of Embelin on neuronal cultures, (ii) the impact of various concentrations of STZ on neuronal cultures viability and lastly (iii) the defensive role of various Embelin concentrations against the toxicity caused by STZ on the primary hippocampal neuronal cultures. Poly-D-lysine-coated 96 well flat-bottomed plates were used for seeding the neurons at 3x10⁴ neurons per well. STZ and Embelin were dissolved in DMEM at the chosen concentrations before adding them to neuronal culture.

2.3.1. Effect of different concentrations of embelin on primary hippocampal neuronal culture

Hippocampal neuronal cultures were treated with Embelin at concentrations of 2.5, 5, 10, 20, 40, 80, and 160 μM for 24 h. The untreated neuronal cultures served as control. Each sample were screened in triplicates and each test was repeated thrice to confirm the accuracy of the results.

2.3.2. MTT viability assay

The hippocampal neuronal culture viability was analysed using the standard MTT assay (Chen et al., 2009). After embelin and STZ treatment, 20 μL MTT (5 mg/mL) was added to the culture and incubated for 4 h. Then, 100 μL DMSO was replaced with the culture medium and absorbance was read at 570 nm using a Microplate reader. The data was presented as a percent of control value. The percentage of hippocampal neuronal culture viability was ascertained using the formula as follows.

\[
\text{Percentage of neurons viability} = \left(\frac{\text{absorbance of treated neurons}}{\text{absorbance of untreated neurons}}\right) \times 100
\]

2.3.3. Effects of different concentrations of STZ on primary hippocampal neuronal culture

Hippocampal neuronal cultures were treated with STZ for 24 h at varied concentrations of 0.39, 0.78, 1.56, 3.125, 6.25 and 12.5 mM. The control neurons did not receive any treatment. Each sample test was run in triplicate. The half maximal inhibitory concentration (IC₅₀) of STZ was determined using following formula as described by Qu et al. (2012).

\[
\text{Lg (IC₅₀)} = \text{Lg (Xₘ)} - \text{Lg (I)} \times \left[\frac{Pₘ - (3 - Pₘ - Pₛ)}{4}\right]
\]

in which, \(Xₘ\) is the maximal concentration; \(I\) is the dilution factor; \(Ps\) is the sum of inhibition ratio; \(Pₘ\) is the maximal inhibition ratio; \(Pn\) is the minimal inhibition ratio and Lg is the common logarithm.
2.3.4. Protective effects of different embelin concentrations against STZ induced toxicity

Hippocampal neuronal cultures were pre-incubated with Embelin for 2 h at concentrations of 0.04, 0.08, 0.16, 0.63, 1.25, 2.5, 5, 10 and 20 μM before exposure to STZ for 24 h at the IC50 concentration of 8 mM. Untreated neuronal cultures served as control.

2.4. Grouping and drug treatment for gene expression and immunofluorescence studies

The hippocampal neurons were cultured on 24 well flat-bottomed plates coated with poly-D-lysine at 5x10^5 neurons per well. The treatments were divided into 5 groups as below with triplicate well for each group as shown in Table 1.

The treatment began once the neurons reached confluence. Further, the hippocampal neuronal cultures were pre-treated with Embelin or basic media for 2 h and then were induced with STZ 8 mM for 24 h. At the end of the experiment, the hippocampal neurons were extracted for gene expression and immunohistochemistry studies.

2.5. Total RNA extraction and Real-Time PCR

Total RNA was extracted from hippocampal neurons using Trizol reagent and phenol-chloroform extraction as described by Bhuvanendran et al. (2018a) with a minor adjustments. The hippocampal neurons were homogenised in 200 μL Trizol solution. Then by adding 40 μL of chloroform, the homogenate was centrifuged at 13,500 rpm for 15 min at 4 °C. After that, the supernatant was deliberately removed and then precipitated with same volume of isopropanol in another Eppendorf tube. This was then pursued by centrifugation at 13,500 rpm for 10 min at 4 °C. Then, the supernatant was deliberately removed, and the pellet was washed twice with 70% ethanol. Lastly, the pellet was suspended in 20 μL of RNase free water. Nanodrop Spectrophotometer was utilised to obtain the total RNA concentration and purity. Later, 300 ng of total RNA from each sample was reverse transcribed to complementary DNA (cDNA) by using QuantiTect® Reverse Transcription Kit as described by the manufacturer’s instruction. Next, the mRNA gene expression for encoding amyloid precursor protein (APP), microtubule-associated protein tau (MAPT), GSK3β, Superoxide dismutase 1 (SOD1) and IMPDH2 in the treated neuronal cultures were estimated by using the below primer sets procured from Qiagen.

APP: Rn_App_1_1SG QuantiTect Primer Assay (Cat no: QT00177408)
Mapt: Rn_Mapt_1_1SG QuantiTect Primer Assay (Cat no: QT00174497)
GSK3c: Rn_RGD:620,351_1_1G QuantiTect Primer Assay (Cat no: QT00187443)
GSK3β: Rn_Gsk3b_1_1SG QuantiTect Primer Assay (Cat no: QT00182406)
Sod1: Rn_Sod1_1_1G QuantiTect Primer Assay (Cat no: QT00174888)
IMPDH2: Rn_Imph2_1_1G QuantiTect Primer Assay (Cat no: QT01576036)

The resulting cDNA and the primer set of the gene of interest were subjected to StepOne Real-Time PCR using QuantiNova™ SYBR® Green PCR kit. The thermal cycling conditions were set using a similar protocol that was used by Choo et al. (2018b). Lastly, the level of expression for six genes of interest according to fold change was measured by normalising the comparative threshold (CT) cycle of target gene against reference gene, IMPDH2 utilizing the equation: 2\(^{-\Delta\Delta CT}\) (CT IMPDH2 - CT target gene).

2.6. Ap immunofluorescent staining

The hippocampal neurons that underwent treatment were fixed with 4% paraformaldehyde (PFA) for 1 h and rinsed in TBS (50 mM Tris, 150 mM NaCl) followed by 30 min incubation in 1% BSA (Sigma) to block non-specific binding sites. Following this, incubation with the anti-beta amyloid primary antibody (1:500; Abcam; ab68896) was carried out at 4 °C and left overnight. After being washed in TBST (50 mM Tris, 150 mM NaCl, 0.05% Tween 20), the neurons were incubated with secondary goat anti-rabbit conjugated with IgG-H&L Alexa Fluor® 488 (1:2000, Abcam; ab150077) at room temperature for 1 h followed by 3 times washing in TBST. The neurons were then counterstained with mounting media ProLong Gold anti-fade Reagent with DAPI (Invitrogen). Image from the samples were photographed using fluorescence microscope (BX41, Olympus) and neurons were quantified using DigiAcquis 2.0 software. Data were expressed as the percentage ratio of amyloid beta positive neurons (green fluorescence; excitation 495 nm, emission 519 nm) to total neurons (blue fluorescence; excitation 385 nm, emission 461 nm) (Zhao et al., 2016).

2.7. Statistical analysis

All results were expressed as mean ± standard errors of the mean (SEM). One-way analysis of variance (ANOVA) with Dunnett’s post hoc test were used to calculate the significance difference between treatment groups. Meanwhile, *P < 0.05 was set as threshold of significance. Analysis were done using GraphPad Prism version 7.02 software (La Jolla, CA, USA).

3. Results

3.1. Embelin pre-treatment ameliorated STZ-induced neuronal damage in rat primary hippocampal neuronal culture

The primary hippocampal neuronal culture was treated with increasing Embelin concentrations ranging from 2.5 to 160 μM to evaluate the neurotoxic effect of Embelin to neuronal culture. We observed that cell viability was significantly decreased when primary hippocampal neurons treated with Embelin at concentrations from 20 μM and above displayed (Fig. 1A). The IC50 of Embelin for primary hippocampal neurons was found to be 37.5 μM. Thus, the concentration of Embelin from 10 μM and below was selected for the next neuroprotection assay. Further, the primary hippocampal neuronal cultures were treated with increasing concentrations of STZ for 24 h and assessed by MTT assay (Fig. 1B) to obtain an optimal dose of STZ as an inducer for neuroprotection assay. The neurotoxicity effect of STZ on the primary hippocampal neurons was dose dependent and the IC50 of STZ was found to be 8 mM. Therefore, 8 mM of STZ concentration was selected for further neuroprotection assay. In the neuroprotection assay, the primary hippocampal neurons were pre-incubated with Embelin at concentration ranging from 0.04 to 10 μM for 2 h, which was then followed by the stimulation of STZ at 8 mM for 24 h. For the neuroprotection assay, neurons viability was seen significantly enhancing neurons viability to 68.74% ± 2.96% (P < 0.0001), 60.09% ± 2.77% (***P < 0.001) and 56.98% ± 3.76% (**P < 0.01) (Fig. 2), respectively. Therefore, embelin at concentration of 2.5, 5 and 10 μM were chosen for further gene expression and immunohistochemistry studies.

Hippocampal neuronal cultures were pre-incubated with various concentrations of Embelin for 2 h before stimulation of 8 mM STZ.

Table 1

| S. No | Experimental groups | Description |
|------|---------------------|-------------|
| 1    | Group 1             | Normal Control, Basic Culture Medium only |
| 2    | Group 2             | Negative control, Basic Culture Medium + STZ (8 mM) |
| 3    | Group 3             | Embelin (Low dose) 2.5 μM + STZ (8 mM); |
| 4    | Group 4             | Embelin (Medium dose) 5 μM + STZ (8 mM) |
| 5    | Group 5             | Embelin (High dose) 10 μM + STZ (8 mM) |
embelin at 2.5, 5 and 10 μM exhibits significant protection against STZ-induced neurotoxicity. Data are expressed as Mean ± SEM of three independent experiments (n = 3) and statistical analysis by one-way ANOVA followed by Dunnett test *P < 0.05, **P < 0.01, and ***P < 0.001.

3.2. Changes in mRNA levels in the primary rat hippocampal neuronal culture

To observe the impact of STZ-induced neurotoxicity on the gene expression, the mRNA expression level was studied using the real time PCR analysis. The expression levels of APP and MPAT mRNA were up regulated by STZ (8 mM) exposure in primary rat hippocampal neuronal culture when contrasted with the control. This up-regulation was reduced by Embelin pre-treatment in contrast with the STZ treated group alone (Fig. 3A and B). However, elevated expression of MAPT mRNA level was seen in hippocampal neuronal culture with high dose of Embelin at 10 μM. In addition, upregulation in the expression of GSK3α and GSK3β mRNA levels were noted in STZ treated group compared to that of the control group. Moreover, 2.5 μM Embelin was seen to...
significantly decrease the expression of both mRNA levels. Nevertheless, there was a significant increment in GSK3β mRNA level by Embelin treatment at 10 μM (Fig. 3C and D).

Besides, STZ was observed to deplete SOD1 antioxidant mRNA in the hippocampal neuronal culture (Fig. 3E). Meanwhile, Embelin pre-treatment at 2.5 μM and 5 μM concentrations significantly upregulated the SOD1 expression level with a significant value of ***P < 0.001 and **P < 0.01, respectively.

3.3. Embelin pre-treatment inhibited Aβ protein expression level in the primary rat hippocampal neuronal culture

To examine whether embelin can inhibit Aβ via its neuroprotective effects, the protein expression of Aβ was studied using immunofluorescence staining method. As shown in Fig. 4A, the green Aβ-positive neurons were significantly increased in STZ-treated neuronal culture, whereas few Aβ-positive neurons were identified in the control group. Besides, the quantity of Aβ-positive neurons was seen to be reduced in a
dose dependent manner when treated with Embelin. Statistical result on the ratio of Aβ staining to total neuron number as in Fig. 4B suggested that STZ had significantly increase the expression of Aβ (25.63% ± 2.73%, ***P < 0.0001). On the contrary, Embelin pre-treatment (2.5, 5 and 10 μM) has reversed the Aβ-induced neuronal death (4.94% ± 0.58%, ***P < 0.0001; 7.64% ± 0.60%, ****P < 0.0001; 11.76% ± 1.53%, **P < 0.01).

4. Discussion

Despite the advances in extensive pre-clinical and clinical AD research, there lacks a disease modifying therapeutic strategy against the same reflecting the complexity in the AD pathology. In quest of exploring the therapeutic strategy that could ameliorate the AD like pathology Embelin has reported its neuroprotective effective against experimental AD like condition (Bhuvanendran et al., 2019a). Herein, in an in vitro AD model was established by inducing the rat primary hippocampal neuronal cultures with STZ, which is a glucoseamine-nitrosourea compound utilised as an experimental tool to induce AD-like condition (Saifari et al., 2020). The characteristic features of this model mimicking the clinical AD are Aβ fragments, Aβ deposition and total tau protein (Kamat, 2015). Results from the current finding demonstrated that Embelin posses neuroprotective potential against STZ induced neurotoxicity in rat primary hippocampal neurons. To the best of our knowledge, current study is the very first of its kind reporting the neuroprotective effect of Embelin on STZ induced AD-like condition in rat primary hippocampal neuronal culture. Earlier finding has reported that Embelin administration improved intracerebroventricular (ICV) STZ-induced memory deficit in rats (Arora and Deshmukh, 2017). Despite the neuroprotective effects of Embelin, the precise underlying mechanism underlying the neuroprotective effects of Embelin on STZ induced AD-like condition was not studied in detail. Therefore, this study was aimed to investigate the anti-AD effect of Embelin in rat primary hippocampal neuronal cultures exposed to STZ. The exposure of cultured neurons to STZ resulted in neuronal death. The present findings demonstrated that pre-treatment with Embelin (2.5–10 μM) has significantly thwarted STZ-induced neurotoxicity and subsequent neuronal death as shown by MTT assay. These results reflect the potential neuroprotective effects of Embelin against STZ-induced neurotoxicity without side effects. Worth mentioning here is that Embelin does not fully restore the neuronal viability. This might be attributed to that fact that more than 50% of neuronal death occurred due to exposure of 8 mM STZ during the treatment assay. Thus, pre-treatment with Embelin can only lead to 70% increase in neuronal viability and it was significant when compared to STZ treated group alone.

In ICV-STZ treated rats, alterations of brain insulin system lead to the insulin receptor (IR) and phosphatidylinositol-3 kinase (PI3K) signalling cascade dysfunction that induces insulin-resistant brain state (Salkovic-Petrisic, 2008). This further leads to the activation of GSK-3 in which isoforms alpha and beta subsequently induce Aβ accumulation and tau hyperphosphorylation (Kamat, 2015), which share many features with a sporadic AD in humans. Current investigation observed a significant upregulation in APP and GSK-3α mRNA when neurons isolated from the primary rat hippocampus were exposed to STZ for 24 h. Similar line of findings was reported earlier, stating the close relationship between the increase in GSK-3α activity and the processing of APP as well as generation of Aβ (Phipel et al., 2003). Earlier documented evidences has reported an upregulation in the APP expression level in STZ induced astrocytes for 24 h (Rajasekar et al., 2016). However, we observed that pre-treating the rat hippocampal neurons with Embelin for 2 h has significantly downregulated the APP and GSK-3α mRNA expression level. Worth reporting here is that, in the case of APP, surprisingly Embelin treated groups demonstrated significant downregulation in mRNA expression of APP below the baseline vehicle control level. Generally APP play an important role in synaptic maintenance as well as neuronal migration during early embryogenesis (van der Kant and Goldstein, 2015). Nevertheless, it is still unclear whether the reduction in APP mRNA level is due to Embelin alone or some other factors that may involve in the neuroprotection., STZ treatment significantly increased the GSK-3β mRNA level. As the GSK-3β regulates the expression level of MAPT in AD condition, it can be said that the MAPT expression level should mimic that of GSK-3β. In this study, there was an increment of MAPT mRNA expression similar to GSK-3β (Mendes et al., 2009), but not significant compared to the control group. The present results showed that only rat hippocampal neurons pre-treated with Embelin 2.5 μM concentration had significantly reduced the mRNA expression level of MAPT and GSK-3β. In the case of higher concentration, the results were unusual with embelin at 10 μM dose having a significant rise in GSK-3β mRNA level and a similar pattern that can be observed in MAPT mRNA expression even though it was not significant. One plausible reason for this is that once a drug had reached the plateau of the dose-effect curve, there is a very little benefit but a significantly greater risk for toxicity at higher doses (Lowe and Lertora, 2013). This theory is supported by the neuroprotective effect in this study showing a bell shape dose-response curve whereby the neuroprotective effect of Embelin at 5 and 10 μM dose started to reduce in comparison to 2.5 μM dose (Fig. 3).

The present study investigated whether the STZ-induced AD-like pathology is associated with alteration in the level of SOD1 in primary rat hippocampal neuronal culture. It was found that the induction of STZ decreased the expression of SOD1 mRNA expression neuronal culture. It was earlier stated that STZ can cause oxidative stress in the brain and cognitive disabilities in rats (Qu et al., 2012). Herein, Embelin with 2.5 and 5 μM dose significantly alleviated STZ-induced SOD1 down-regulation in neuronal culture. As Embelin possesses potent anti-oxidative activities (Mahendran et al., 2011; Poojari, 2014), Embelin might exert its neuroprotective effects through antioxidant activity with upregulation of SOD1 mRNA expression.

The protective effect of Embelin was further verified through immunofluorescence staining where the effect of Embelin on Aβ expression was explored. Earlier pre-clinical investigations have revealed that accumulation of Aβ found in STZ-treated rats (El Halawany et al., 2017; Wang et al., 2017). The present study showed that pre-treatment of Embelin significantly diminished the Aβ expression level induced by STZ treatment in a dose dependent manner similar to APP mRNA level. Thus, this result further supported the gene expression study as APP gene generates Aβ through enzyme secretases known as alpha, beta and gamma (Zhang et al., 2011). Earlier published finding from our research group where molecular docking study revealed that Embelin binds well with the αβpeptides actives sites (Bhuvanendran et al., 2019b) which eventually may trigger inhibition of these αβpeptides by Embelin. In addition, Embelin prevent the formation of Aβoligomers via inhibition of BACE-1 and increases Aβ clearance through P-gp induction (Nuthakkii et al., 2019).

Embelin being a compound from plant sources has demonstrated the potential therapeutic effects against several neurological disorders (Kundap et al., 2017) including AD (Bhuvanendran et al., 2018b). In regard to the recent repeated end stage failure of AD drug (Mullane and Williams, 2020), therapeutic strategy from the natural source might be an interesting alternative treatment strategy against AD. Worth mentioning here is that, drugs from natural sources are used since long time. Interestingly, around 60% of new drug candidates identified between 1980 and 2015 were from the natural sources (Pradeep et al., 2019). Hence, based on the findings from current in vitro study we can speculate that Embelin might be a therapeutic candidate of interest against AD like condition. The plausible underlying mechanism behind the neuroprotective effect of Embelin is depicted in Fig. 5. However extensive pre-clinical and clinical research are warranted for the clinical translation of Embelin against AD like condition.
5. Conclusion

In conclusion, Embelin showed a promising anti-AD like effect in vitro model. Embelin exerts its neuroprotective effect through GSK-3 pathway, reducing oxidative stress and preventing amyloidogenesis in STZ-induced neurotoxicity in rat hippocampal neuronal culture.

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CRediT author contribution statement

SB performed all the experiments and manuscript writing in its entirety. YK was involved in designing, data analysis. MFS and SB were involved in conceptualising, designing the research project, data analysis, editing and revising. IO gave critical feedback for this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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