

p-Coumaric acid protects against D-galactose induced neurotoxicity by attenuating neuroinflammation and apoptosis in mice brain

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Abstract

D-galactose (D-gal) induced senescence in rodents is a widely used model for assessment of molecules affecting brain ageing. Chronic administration of D-gal causes neuroinflammation leading to cognitive deficit and memory impairment which represent Alzheimer's dementia. In present study, we investigated the neuroprotective effects of the natural phenol, *p*-Coumaric acid (PCA) and its underlying mechanism in the chronic D-gal treated mice. Subcutaneous administration of D-gal (150 mg/kg) to Swiss albino mice for 42 consecutive days resulted in cognitive impairment as observed in Morris water maze (MWM) and Y maze test, which was ameliorated by concurrent treatment with PCA (80 mg/kg, and 100 mg/kg, *p.o.*). Importantly, PCA treatment attenuated the D-gal induced oxidative stress and significantly inhibited acetylcholinesterase (AChE) activity in mice brain. Furthermore, PCA treatment significantly lowered levels of inflammatory marker nuclear factor kappa B (NF κ B) and reduced levels of proapoptotic enzyme caspase 3. We also observed that PCA treatment exhibited β -secretase enzyme (BACE1) inhibitory effect. However, our results revealed that PCA treatment failed to decrease the level of advanced glycation end products (AGE) both *in vitro* and *in vivo*. Taken together, current study demonstrated the significant neuroprotective effect of PCA against D-galactose induced neuroinflammation, cognitive impairment and apoptosis.

Introduction

Brain aging is proved to be a prominent and critical factor contributing to pathophysiology of Alzheimer's disease (AD). Population suffering from dementia accounts to almost 9.9 million per year, globally (Shwe et al., 2018). The deterioration of memory in dementia and AD is accompanied with structural changes in brain such as deposition of A β plaques, neurofibrillary tangles and damaged cellular proteins, nucleic acids or lipids and functional alterations such as reduced synaptic plasticity, neurogenesis and stimulation of various oxidative and inflammatory pathways (Wu et al., 2008).

In AD, as the age progresses some of the factors contributing predominantly to oxidative stress and neurodegeneration include mitochondrial abnormalities, A β plaques, hyperphosphorylated tau deposition, hyperactivation and abnormal function of astrocytes, microglia and excessive generation of AGEs (Su et al., 2008). Imbalance between production and inactivation of reactive oxygen species in brain culminates into neuronal damage leading to neurodegeneration and impairment of vital brain functions including learning and memory (Xian et al., 2014).

Considerable research has highlighted the crucial role of excessive level of free radicals and reactive carbonyl compounds mainly methylglyoxal, glyoxal, triosephosphates in causing protein crosslinking. These carbonyl compounds react nonenzymatically with the proteins, more specifically, N-terminal amino group and side chains of lysine and arginine resulting in formation of amadori products which subsequently undergo oxidation and dehydration resulting in formation of AGE and advanced lipoxidation end products (ALEs) (Srikanth et al., 2011). Elevated levels of AGE contribute to increasing the risk of development of AD (S. Y. Ko et al., 2010).

D-gal is a reducing sugar, which readily gets metabolized to glucose. However, at higher concentrations, it triggers the formation of ROS and leads to the formation of AGE, that do not get metabolised but expedites the aging process in brain. AGEs cause oxidative stress and *vice versa* (Sato et al., 2006). Interaction of AGE with its receptors (RAGE), has many crucial consequences such as activation of NFκB pathway which culminates into enhanced synthesis of ROS and inflammatory mediators like TNF-α, IL-1, IL-6 via activated microglia and astrocytes (Lu, Wu, Zheng, et al., 2010; Srikanth et al., 2011). Further, activation of NFκB pathway leads to neuronal apoptosis (Mastrocola et al., 2005). Increased ROS enhances amyloid precursor protein (APP) expression (S.-Y. Ko et al., 2015). APP thus formed is normally subjected to successive cleavage by enzymes β-secretase (BACE-1) and γ secretase resulting in Aβ generation. BACE-1 is the first enzyme which acts on rate limiting step in Aβ production. This supports the finding that AGEs could accelerate Aβ aggregation and accumulation which led to generation of Aβ plaques (Vitek et al., 1994). Taken together, chronic D-galactose induced senescence and neuronal dysfunction serves as a promising model for neurodegenerative disorders (Shwe et al., 2018).

In spite of an alarming increase in population suffering from Alzheimer's disease worldwide, there is lack of effective therapeutic agents. The clinically available drugs offer only symptomatic relief. Thus, emphasizing an urgent need for search of effective disease modifying or therapeutic intervention.

A plethora of studies performed previously has revealed neuroprotective potential of PCA in embolic cerebral ischemia (Güven et al., 2015). PCA was found to attenuate oxidative stress induced by cisplatin in rat brain (Ekinci-Akdemir et al., 2017). PCA enhanced long term potentiation and facilitated recovery of scopolamine induced memory impairment (Kim et al., 2017). Further, PCA was also found to protect against type 2 diabetes induced neurodegeneration in rats (Abdel-Moneim et al., 2017).

Additionally, some of the important pharmacological activities of PCA include antioxidant (Abdel-Wahab, 2003), anti-inflammatory (Pragasam et al., 2013), antimutagenic (Shailasree et al., 2014), antitumor (Hudson et al., 2000) and antiangiogenic (Kong et al., 2013). *In vitro* studies on PC-12 cells demonstrated that p-CA suppressed ERK1/2 and JNK phosphorylation, thus attenuating Aβ induced toxicity through regulation of NFκB pathway (Yoon et al., 2014).

However, the effect of PCA against D-gal induced oxidative stress and neurocognitive impairments remains to be explored. The current study aims to investigate the potential protective effect of PCA in D-gal induced neurochemical and memory alterations and understand the underlying mechanisms.

Materials And Methods

Animals

Male Swiss albino mice (10 weeks old weighing 30–32 g) were procured from National Institute of Biosciences (NIBS), Pune. 6 mice per cage were housed in opaque polypropylene cages and kept under standard laboratory condition i.e. 22 ± 2 °C temperature, 60–70% humidity, maintenance of 12 hours

natural day and night cycle with food and water *ad libitum*. Prior to the testing, the mice were allowed to get habituated to the testing rooms for 1 week.

Ethics approval

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Chemical Technology, Mumbai, India with protocol number: ICT/IAEC/2018/P21. All experimental procedures were performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Drugs, reagents and treatment schedule

PCA (≥ 98 % HPLC), Rivastigmine (RVS), D-gal and methylglyoxal were purchased from Sigma Aldrich Co. (St. Louis, MO). L-Reduced glutathione (GSH), Tris-buffer Thiobarbituric acid (TBA) and 5, 5'- dithiobis (2-nitrobenzoic acid) (DTNB) were procured from Himedia Laboratories, Mumbai, India. Aminoguanidine HCl was purchased from TCI Ltd., bovine serum albumin (BSA) was purchased from S.D. Fine chemicals, nitroblue tetrazolium and sodium azide were purchased from Loba chemie pvt ltd. Mouse ELISA kits for NF κ B, BACE-1, Caspase-3 and AGE were purchased from KinesisDX, USA. All other chemicals used were of analytical grade. PCA was suspended in Tween 80 for oral administration. All other drugs were dissolved in 0.9% saline. At the end of study, the animals were sacrificed by carbon dioxide asphyxiation.

The dose of PCA was determined based on earlier studies (Sakamula and Thong-asa 2018). The total 40 mice were divided into five groups, each group having 8 mice:

1. Group 1: Vehicle control (0.2 ml of saline) for 42 days
2. Group 2: Negative control (Saline + D-gal (150 mg/kg, s.c.) for 42 days
3. Group 3: Positive control (RVS (0.25 mg/kg i.p.) + D-gal (150 mg/kg, s.c.) for 42 days
4. Group 4: PCA (80 mg/kg p.o.) + D-gal (150 mg/kg, s.c.) for 42 days
5. Group 5: PCA (100 mg/kg p.o.) + D-gal (150 mg/kg, s.c.) for 42 days

The work plan of the study is illustrated in Fig. 1.

In vitro bovine serum albumin (BSA) glycation study

In vitro protein glycation was carried out as described previously (Peng et al. 2008). For AGE formation 200 μ l of BSA (35 mg/ml) containing sodium azide (0.1 g/ml) was incubated with 400 μ l of glucose (175 mg/ml) at 37°C for 21 days, in absence or presence of PCA solution at different concentrations (10–200

µg/ml). Aminoguanidine (AG) (4 mg/ml) was used as a standard. AGEs, pentosidine and fructosamine compounds were assessed.

In vitro glycation assay with BSA-MGO

BSA-MGO assay was performed as described previously (Lunceford & Gugliucci, 2005). 200 µl of BSA was incubated with 400 µl of MGO (0.4 mg/ml) at 37 °C for 14 days in absence or presence of different concentrations of PCA (10-200 µl/ml). Blanks containing BSA-glucose but no test sample were kept at -80°C, until the measurement. Aminoguanidine (AG) (4 mg/ml) was used as a standard.

Determination of fructosamine (amadori) content

The fructosamine content was measured by nitroblue tetrazolium (NBT) reduction assay (Islam et al., 2014) . The reduction of NBT was recorded by increase in absorbance at 530 nm from 5 to 15 min at 37 °C and expressed as % inhibition.

$$\% \text{ Inhibition} = \left[\frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}} \right] \times 100$$

Behavioural assessment

Morris water maze (MWM) test

The spatial memory and learning in mice were assessed using MWM test. A plastic pool having 122 cm diameter and 50 cm height and divided into 4 quadrants was filled with water (22 ± 2 °C) up to 30 cm and rendered opaque by dissolving powdered milk. In one quadrant, a white platform was placed 1 cm below water level. 3 bright-coloured visual clues were fixed around the pool. Position of escape platform and visual cues were kept unchanged during entire experiment. The MWM test was divided into two phases as follows:

Maze acquisition phase

On day 20, mice underwent 4 consecutive training sessions, with 15 min intertrial interval. Each time, mice were carefully put in one of the four quadrants and allowed to explore the platform for 90 s. In case the mice could not locate the platform during this time, it was gently guided to the platform. The mouse

was allowed to remain on the platform for 60 s. The latency period was recorded as initial acquisition latency (IAL).

Maze retention phase

After 24 hours of maze acquisition phase, the escape latency was again evaluated and first retention latency (FRL) was recorded. Similarly, second retention latency was calculated on day 40 of treatment and recorded as second retention latency (SRL).

Probe test

The memory consolidation in mice was assessed by probe test performed 24 hours after recording of SRL. In probe test the platform was removed and mice were allowed to swim freely for 60 seconds. Time spent by individual mice in the target quadrant was recorded.

Y maze test

Y-maze test is an excellent tool for assessing exploratory behaviour, spatial recognition and working memory. In Y maze test, apparatus (8 cm x 30 cm x 15 cm) with each of the arms at 120° apart was used. The test was conducted on day 22 and day 42, one hour after the dosing and included two trials. During the first trial, the mouse was allowed to explore only two arms (arm A and arm B) for 5 min keeping the third one (arm C) closed. Arm C was considered a novel arm. Post 30 min interval, the second trial was conducted, allowing each mouse to explore all 3 arms for a period of 5 min. Percentage spontaneous alteration behaviour (%SAB) and the number of entries in the novel arm were analysed for individual mouse. Consecutive entries in three different arms were counted as one alteration. Following formula was used to calculate percentage spontaneous alterations (Ghods-Sharifi et al., 2008):

$$\% \text{ SAB} = \left[\frac{\text{Number of alterations}}{(\text{Number of total entries} - 2)} \right] \times 100$$

Brain tissue collection

Animals sacrificed by CO₂ asphyxia were perfused with PBS. Isolated brains were immediately transferred in ice-cold isotonic saline and stored at -80°C.

Biochemical assessment

Brain tissue was homogenised in 0.1 M PBS (pH 7.4). The homogenate (10% w/v) was subjected to cold centrifuge (10 000 g for 15 min). The supernatant thus obtained was used for further estimations.

Evaluation of AChE

As described by Ellman et al. (Ellman et al., 1961), to 50 µl of the brain tissue homogenate, 3 ml of PBS (pH 8), 100 µl of acetyl thiocholine iodide (0.75 nM), and 100 µl of 10 mM Ellman's reagent were added. Absorbance was measured spectrophotometrically at an interval of 30 s for 5 minutes at 412 nm.

Estimation of reduced glutathione

As described by Smith et al. (Smith et al., 1988), each 3 ml reaction mixture (2.9 ml of DTNB in PBS and 0.1 ml of supernatant) was incubated at 37°C for 15 min. Absorbance was measured spectrophotometrically at 412 nm. The level of GSH/mg of protein was estimated.

Estimation of lipid peroxidation

As described by Draper et al. (Draper et al., 1993), mixture of 0.5 ml Tris-HCl buffer and 0.1 ml of the brain homogenate, was incubated for 2 h. 1 ml of 10% w/v TCA solution was then added to above mixture and it was subjected to cold centrifuge (1000 g) for 10 min. The supernatant was collected and to it, an equal volume of TBA (0.67% w/v) was added. The mixture was then subjected to heating in a boiling water bath for 15 min. The mixture was cooled in an ice bath and to it, 1 ml distilled water was added. Absorbance was measured spectrophotometrically at 532 nm.

Estimation of Superoxide dismutase

As per method by Nandi and Chatterjee (Nandi & Chatterjee, 1988), to 2.8 ml of PBS, 0.1 ml supernatant and 0.1 ml pyrogallol solution (2.6 mM in 10 mM HCl) was added. Absorbance was measured spectrophotometrically at 325 nm. The level of SOD units/mg of protein was estimated.

Immunoassays for biomarkers

NFκB, AGE, BACE-1 and Caspase-3 were estimated using ELISA kits. The manufacturer's protocol was strictly followed and reading were taken using ELISA plate reader (BIORAD).

Statistics

GraphPad Prism Version 5, San Diego, CA was used for statistical analysis. Data are represented as mean \pm SEM. Escape latency in MWM and % SAB in Y maze were analysed using repeated measure two-way ANOVA followed by post hoc Bonferroni's test. In other tests, one-way ANOVA followed by post hoc Tukey's multiple comparison test was used to calculate statistical significance.

Results

PCA does not show *in vitro* anti-glycating effect

The results of *in vitro* anti-glycating activity revealed that PCA does not inhibit formation of AGE or reduce the formation of amadori products. Also, the results of methylglyoxal assay suggested that PCA failed to neutralise the carbonyl compounds formed (results not shown).

PCA alleviates D-galactose induced memory impairment in mice

MWM test

To investigate effect of PCA on memory we evaluated escape latency by performing MWM test on day 20, 21 and 40 respectively followed by probe test on day 41.

As shown in Fig. 2a, for D-gal group, observed initial acquisition latency (IAL) ($p < 0.01$), first retention latency (FRL) ($p < 0.001$) and second retention latency (SRL) ($p < 0.001$) were significantly higher than vehicle treated group. PCA and RVS treated groups showed progressive decline in escape latency over the treatment period. Treatment with PCA (80 mg/kg) exhibited significantly decreased FRL ($p < 0.01$) and SRL ($p < 0.001$) and PCA (100 mg/kg) exhibited significantly decreased IAL ($p < 0.05$), FRL ($p < 0.001$) and SRL ($p < 0.001$) as compared to D-gal group, The results of PCA were comparable with RVS ($p > 0.05$).

To assess memory consolidation, a probe test was performed 24 h after the MWM test. During the 60 second probe trial, time spent by mouse in each quadrant was recorded. As depicted in Fig. 2 b we observed that the time spent in the target quadrant area was significantly higher in PCA (100 mg/kg) ($p < 0.05$) and RVS ($p < 0.05$) as compared to D-gal group, while D-gal group spent less time in target quadrant ($p < 0.01$) as compared to vehicle. The results of probe test for PCA (80 mg/kg) were not significant as compared to D-gal group.

Y maze test

Y maze test performed on day 22 and day 42, evaluated percentage spontaneous alterations (% SAB) which signifies spatial and working memory along with learning, whereas the number of entries in the novel arm reflects locomotor activity and exploratory behaviour. % SAB was significantly lower for the D-gal group on day 22 ($p < 0.05$) and day 42 ($p < 0.001$) as compared to the vehicle control. As shown in Fig. 2c, % SAB was significantly increased in PCA treatment groups. On day 22, % SAB for PCA (100 mg/kg) but not PCA (80 mg/kg), was significantly higher ($p < 0.001$) as compared to D-gal group. On day 42, % SAB was significantly higher ($p < 0.001$) for both doses of PCA. RVS group exhibited significantly increased % SAB on day 22 and day 42 of evaluation ($p < 0.001$ on both days).

As shown in Fig. 2d, the number of entries in novel arm of Y maze were significantly reduced in D-gal treated group as observed on day 22 and day 42 ($p < 0.01$ and $p < 0.05$, respectively). However, on day 22 and day 42, PCA (100 mg/kg) ($p < 0.05$ and $p < 0.01$) and RVS ($p < 0.05$ and $p < 0.01$), treated groups exhibited significant improvement in frequency of novel arm entries as compared to D-gal group. However, PCA (80 mg/kg) did not significantly increase number of arm entries.

PCA decreases AChE activity in mice brain

As depicted in Fig. 3a AChE activity was significantly higher in D-gal group ($p < 0.001$), compared to vehicle control. In RVS group, significant inhibition of AChE was observed ($p < 0.001$). Treatment with PCA (80 mg/kg & 100 mg/kg) significantly attenuated enhanced AChE level ($p < 0.01$ & $p < 0.001$ respectively). However, AChE inhibition exhibited by PCA was lower than RVS treated group.

PCA ameliorates D-gal Induced oxidative stress

As shown in Fig. 3b, 3c and 3d we observed that D-gal treatment significantly decreased SOD and GSH levels ($p < 0.001$ and $p < 0.001$, respectively) and elevated brain MDA ($p < 0.001$) compared to vehicle control. Treatment with PCA (80 mg/kg and 100 mg/kg) significantly elevated SOD level ($p < 0.05$ and $p < 0.01$, respectively) and GSH ($p < 0.01$ and $p < 0.001$, respectively) as compared to the D-gal group. RVS significantly increased both SOD and GSH levels as compared D-gal group ($p < 0.01$ and $p < 0.001$) respectively. Further PCA (100 mg/kg) ($p < 0.01$) and RVS ($p < 0.01$) significantly attenuated D-gal induced increased MDA levels but PCA (80 mg/kg) did not significantly lower MDA level.

Effect of PCA on AGE levels in mice brain

Estimation of brain AGE level using ELISA revealed that, D-gal treatment resulted in significantly increased AGE levels in mice brain compared to vehicle control ($p < 0.001$). We observed that treatment with PCA (80 mg/kg & 100 mg/kg) did not significantly reduced the AGE levels as compared to D-gal treated group as shown in Fig. 4a. For RVS however, significant AGE inhibitory effect was observed ($p < 0.01$).

PCA reduces levels of NFκB in mice brain

The estimation of NFκB in brain homogenates was done using ELISA kits. As shown in Fig. 4b, we found that chronic treatment with D-gal markedly increased NFκB levels in brain ($P < 0.001$). PCA (100 mg/kg) significantly reduced NFκB levels in mice brain ($p < 0.05$) which was comparable with the results obtained in RVS treated group ($p < 0.001$). However, PCA (80 mg/kg) did not exhibit significant reduction in brain NFκB level.

PCA reduces levels of BACE-1 in mice brain

Estimation of BACE-1 levels in mice brain homogenates by ELISA revealed increased BACE1 levels in D-gal treated group ($P < 0.001$). As shown in Fig. 4c, PCA (80 mg/kg and 100 mg/kg) significantly reduced BACE-1 levels in mice brain ($p < 0.05$ at both dose levels). In RVS treated group also, significant reduction in BACE-1 levels was observed as compared to D-gal group ($p < 0.05$).

PCA prevents D-gal induced neuronal apoptosis

Quantitative evaluation Caspase 3 in mice brain homogenates was performed using ELISA kit. As shown in Fig. 4d, Caspase 3 levels were significantly higher after D-gal treatment which was attenuated by treatment with PCA (100 mg/kg, $p < 0.05$) and RVS ($p < 0.05$). However, we observed that there was no significant reduction in caspase-3 level in mice brain from PCA (80 mg/kg) group.

Discussion

Memory decline is a characteristic of aging and also serves as an indicator of neurodegenerative changes in brain. D-gal crosses the blood brain barrier and is taken up by brain cells via glucose transport type 1 (GLUT-1) (Shwe et al., 2018). Over accumulation of D-gal induces senescence, by causing oxidative and glycative stress, neuroinflammation and neuronal apoptosis resembling the old age related cognitive impairment and behavioural dysfunction which is suggested to recapitulate symptoms of AD (WEI et al., 2005).

Consistent with the reported data (Li et al., 2015; Wang et al., 2020), we observed that the learning ability and memory function in D-gal group was significantly impaired, which was ameliorated by treatment with PCA at (80 mg/kg and 100 mg/kg) dose levels. Further, findings of probe test confirmed the memory consolidation at both dose levels of PCA. Moreover, in Y maze test increased % SAB in PCA treated groups at both dose levels indicated significantly improved short term memory and cognition compared to D-gal group. Thus, prolonged treatment with PCA protected against D-gal induced memory impairment.

Memory decline in AD is associated with cholinergic deficiency. Enhanced levels of brain acetylcholinesterase (AChE) can cause cholinergic deficiency as depicted in AD. D-gal increases brain

AChE activity (S. Z. Zhong et al., 2009) and causes impairment of brain cholinergic function (Lu, Wu, Hu, et al., 2010). Consequently, we observed increased AChE level in D-gal treated group which was significantly ameliorated by PCA at both (80 mg/kg and 100 mg/kg) dose levels. These results were comparable with RVS, indicating that protective effect of PCA against D-gal induced neurodegeneration and memory impairment could be at least partially mediated through its AChE inhibitory activity.

On overdose, D-gal is converted to aldose and hydrogen peroxide via the action of galactose oxidase, leading to the formation of a superoxide anions and oxygen-derived free radicals (Lu, Wu, Hu, et al., 2010), which ultimately results in diminished levels of SOD and GSH enzymes and elevated MDA level in D-gal group. Chronic treatment with PCA at both dose levels (80 mg/kg and 100 mg/kg), significantly attenuated the D-gal induced oxidative stress by increasing SOD and GSH level and decreasing MDA level in mice brain which suggested the neuroprotective effect of PCA through antioxidant mechanism.

These findings demonstrate protective effect of PCA in D-gal induced behavioural and neurochemical alterations. Chronic overdose of D-gal leads to formation and accumulation of advanced glycation end products (AGEs). Elevated AGE levels in body are implicated in aggravating the oxidative stress and triggering the production of proinflammatory cytokines, which culminates in impaired synaptic plasticity and neuronal death. The oxidative stress and AGE initiate a vicious cycle leading to neurodegeneration and cognitive impairment.

Investigation of in vitro antiglycation effect of PCA in glucose-BSA system and methylglyoxal scavenging assay revealed that, PCA does not exhibit significant AGE inhibitory or methylglyoxal scavenging activity *in vitro*. Our results are consistent with the reported literature (Chen et al., 2016). Further, estimation of brain AGE levels using ELISA revealed no significant decrease in AGE levels after PCA treatment, thus supporting our findings of in vitro study. This also suggests that, PCA may have shown neuroprotective effect by other than AGE inhibitory mechanisms, thus we further explored the effect of PCA treatment on inflammatory and apoptotic pathways.

Several studies have revealed that interaction between AGE and receptor for advanced glycation end products (RAGE) leads to activation of nuclear factor kappa B (NFκB) pathway, which plays a significant role in cell proliferation, apoptosis, and inflammatory response (J. Zhong et al., 2020). Consistent with these findings, we observed elevated levels of NFκB in D-gal treated mice brain. Decreased NFκB levels observed in PCA treated mice suggests that protective effect of PCA against D-gal induced neuroinflammation might result through inhibition of nuclear translocation of NFκB or its expression.

Excessive formation of AGE promotes Aβ formation by enhancing expression of APP and upregulates BACE-1 and presenilin 1 (S.-Y. Ko et al., 2015; Lubitz et al., 2016). BACE-1 is essential for cleavage of APP which is an endogenous precursor of Aβ. Our investigation revealed that chronic D-gal treatment led to upregulation of BACE-1 levels in mice brain. Significant reduction in BACE-1 level after PCA treatment suggests that PCA may protect against Aβ generation through its potential BACE-1 inhibitory effect. These findings are consistent with the study reporting BACE-1 inhibitory effect of PCA isolated from *Corni*

fructus (Yoon et al., 2014). However, further detailed investigation is required to confirm the BACE-1 inhibitory effect of PCA.

Neuronal damage due to D-gal induced oxidative stress can be reflected from the levels of proapoptotic marker caspase 3 in mice brain. In our observations, we found that PCA treatment reduced the brain caspase 3 level at 100 mg/kg dose, indicating its potential antiapoptotic effect which may be attributed to inhibition of NF κ B and BACE-1. Our findings revealed that RVS treatment also ameliorated D-gal induced apoptosis. These results demonstrate protective effect of PCA against D-gal induced neurotoxicity via attenuating D-gal induced neuroinflammation and neuronal apoptosis.

Conclusion

Based on our findings we conclude that neuroprotective effect of PCA in D-gal induced neurotoxicity and memory impairment may be at least partly mediated through: i) Protecting activity of antioxidant enzymes in brain, ii) Decreasing brain AChE activity, iii) Inhibiting BACE-1 activity in mice brain and iv) Modulating inflammatory and proapoptotic markers in mice brain. These findings provide a new insight suggesting p-Coumaric acid as a potential agent for the treatment of age-related brain disorder. However, further research is needed to elucidate the exact molecular basis for its use in the management of AD.

Declarations

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Conflicts of interest/Competing interests

The authors declare there are no conflict of interest/ no competing interests.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

Authors' contributions

Prof. Archana Juvekar: Conception of the work, supervised the research, critical revision of the article.

Pratibha Daroi: Conception and design of the work, performed experiments, data collection, analysis, Interpretation and original draft preparation.

Shrikant Dhage: Performed analysis, data collection and interpretation, critical revision of the article. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Chemical Technology, Mumbai, India with protocol number: ICT/IAEC/2018/P21. All experimental procedures were performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

References

1. Abdel-Moneim, A., Yousef, A. I., Abd El-Twab, S. M., Abdel Reheim, E. S., & Ashour, M. B. (2017). Gallic acid and p-coumaric acid attenuate type 2 diabetes-induced neurodegeneration in rats. *Metabolic Brain Disease*, *32*(4), 1279–1286. <https://doi.org/10.1007/s11011-017-0039-8>
2. Abdel-Wahab, M. (2003). Influence of p-coumaric acid on doxorubicin-induced oxidative stress in rat's heart. *Pharmacological Research*, *48*(5), 461–465. [https://doi.org/10.1016/S1043-6618\(03\)00214-7](https://doi.org/10.1016/S1043-6618(03)00214-7)
3. Chen, H., Virk, M. S., & Chen, F. (2016). Phenolic acids inhibit the formation of advanced glycation end products in food simulation systems depending on their reducing powers and structures. *International Journal of Food Sciences and Nutrition*, *67*(4), 400–411. <https://doi.org/10.3109/09637486.2016.1166187>
4. Draper, H. H., Squires, E. J., Mahmoodi, H., Wu, J., Agarwal, S., & Hadley, M. (1993). A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. *Free Radical Biology and Medicine*, *15*(4), 353–363. [https://doi.org/10.1016/0891-5849\(93\)90035-S](https://doi.org/10.1016/0891-5849(93)90035-S)
5. Ekinçi-Akdemir, F. N., Gülçin, Gürsul, C., Alwasel, S. H., & Bayir, Y. (2017). Effect of P-Coumaric acid against oxidative stress induced by cisplatin in brain tissue of rats. *Journal of Animal and Plant Sciences*, *27*(5), 1560–1564.
6. Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88–95. [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
7. Ghods-Sharifi, S., Haluk, D. M., & Floresco, S. B. (2008). Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning. *Neurobiology of Learning and Memory*, *89*(4), 567–573. <https://doi.org/10.1016/j.nlm.2007.10.007>
8. Guven, M., Bozkurt Aras, A., Akman, T., Murat Sen, H., Ozkan, A., Salis, O., Sehitoglu, I., Kalkan, Y., Silan, C., Deniz, M., & Cosar, M. (2015). Neuroprotective effect of p-coumaric acid in rat model of embolic cerebral ischemia. *Iranian Journal of Basic Medical Sciences*, *18*(4), 356–363. <https://doi.org/10.22038/ijbms.2015.4284>
9. Hudson, E. A., Dinh, P. A., Kokubun, T., Simmonds, M. S. J., & Gescher, A. (2000). Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. *Cancer Epidemiology Biomarkers and Prevention*, *9*(11), 1163–1170.

10. Islam, M. N., Ishita, I. J., Jung, H. A., & Choi, J. S. (2014). Vicenin 2 isolated from *Artemisia capillaris* exhibited potent anti-glycation properties. *Food and Chemical Toxicology*, *69*, 55–62. <https://doi.org/10.1016/j.fct.2014.03.042>
11. Kim, H.-B., Lee, S., Hwang, E.-S., Maeng, S., & Park, J.-H. (2017). p-Coumaric acid enhances long-term potentiation and recovers scopolamine-induced learning and memory impairments. *Biochemical and Biophysical Research Communications*, *492*(3), 493–499. <https://doi.org/10.1016/j.bbrc.2017.08.068>
12. Ko, S.-Y., Ko, H.-A., Chu, K.-H., Shieh, T.-M., Chi, T.-C., Chen, H.-I., Chang, W.-C., & Chang, S.-S. (2015). The Possible Mechanism of Advanced Glycation End Products (AGEs) for Alzheimer's Disease. *PLOS ONE*, *10*(11), e0143345. <https://doi.org/10.1371/journal.pone.0143345>
13. Ko, S. Y., Lin, Y. P., Lin, Y. S., & Chang, S. S. (2010). Advanced glycation end products enhance amyloid precursor protein expression by inducing reactive oxygen species. *Free Radical Biology and Medicine*, *49*(3), 474–480. <https://doi.org/10.1016/j.freeradbiomed.2010.05.005>
14. Kong, C. S., Jeong, C. H., Choi, J. S., Kim, K. J., & Jeong, J. W. (2013). Antiangiogenic effects of P-coumaric acid in human endothelial cells. *Phytotherapy Research*, *27*(3), 317–323. <https://doi.org/10.1002/ptr.4718>
15. Li, J. J., Zhu, Q., Lu, Y. P., Zhao, P., Feng, Z. B., Qian, Z. M., & Zhu, L. (2015). Ligustilide prevents cognitive impairment and attenuates neurotoxicity in d-galactose induced aging mice brain. *Brain Research*, *1595*, 19–28. <https://doi.org/10.1016/j.brainres.2014.10.012>
16. Lu, J., Wu, D. M., Zheng, Y. L., Hu, B., Zhang, Z. F., Ye, Q., Liu, C. M., Shan, Q., & Wang, Y. J. (2010). Ursolic acid attenuates D-galactose-induced inflammatory response in mouse prefrontal cortex through inhibiting AGEs/RAGE/NF-κB pathway activation. *Cerebral Cortex*, *20*(11), 2540–2548. <https://doi.org/10.1093/cercor/bhq002>
17. Lu, J., Wu, D. mei, Hu, B., Cheng, W., Zheng, Y. lin, Zhang, Z. feng, Ye, Q., Fan, S. hua, Shan, Q., & Wang, Y. jian. (2010). Chronic administration of troxerutin protects mouse brain against d-galactose-induced impairment of cholinergic system. *Neurobiology of Learning and Memory*, *93*(2), 157–164. <https://doi.org/10.1016/j.nlm.2009.09.006>
18. Lubitz, I., Ricny, J., Atrakchi-Baranes, D., Shemesh, C., Kravitz, E., Liraz-Zaltsman, S., Maksin-Matveev, A., Cooper, I., Leibowitz, A., Uribarri, J., Schmeidler, J., Cai, W., Kristofikova, Z., Ripova, D., Leroith, D., & Schnaider-Beerli, M. (2016). High dietary advanced glycation end products are associated with poorer spatial learning and accelerated Aβ deposition in an Alzheimer mouse model. *Aging Cell*, *15*(2), 309–316. <https://doi.org/10.1111/accel.12436>
19. Lunceford, N., & Gugliucci, A. (2005). Ilex paraguariensis extracts inhibit AGE formation more efficiently than green tea. *Fitoterapia*, *76*(5), 419–427. <https://doi.org/10.1016/j.fitote.2005.03.021>
20. Mastrocola, R., Restivo, F., Vercellinatto, I., Danni, O., Brignardello, E., Aragno, M., & Boccuzzi, G. (2005). Oxidative and nitrosative stress in brain mitochondria of diabetic rats. *Journal of Endocrinology*, *187*(1), 37–44. <https://doi.org/10.1677/joe.1.06269>

21. Nandi, A., & Chatterjee, I. B. (1988). Assay of superoxide dismutase activity in animal tissues. *Journal of Biosciences*, 13(3), 305–315. <https://doi.org/10.1007/BF02712155>
22. Pragasam, S. J., Venkatesan, V., & Rasool, M. (2013). Immunomodulatory and anti-inflammatory effect of p-coumaric acid, a common dietary polyphenol on experimental inflammation in rats. *Inflammation*, 36(1), 169–176. <https://doi.org/10.1007/s10753-012-9532-8>
23. Sato, T., Shimogaito, N., Wu, X., Kikuchi, S., Yamagishi, S. I., & Takeuchi, M. (2006). Toxic advanced glycation end products (TAGE) theory in Alzheimer's disease. *American Journal of Alzheimer's Disease and Other Dementias*, 21(3), 197–208. <https://doi.org/10.1177/1533317506289277>
24. Shailasree, S., Venkataramana, M., Niranjana, S. R., & Prakash, H. S. (2014). Cytotoxic Effect of p-Coumaric Acid on Neuroblastoma, N2a Cell via Generation of Reactive Oxygen Species Leading to Dysfunction of Mitochondria Inducing Apoptosis and Autophagy. *Molecular Neurobiology*, 51(1), 119–130. <https://doi.org/10.1007/s12035-014-8700-2>
25. Shwe, T., Pratchayasakul, W., Chattipakorn, N., & Chattipakorn, S. C. (2018). Role of D-galactose-induced brain aging and its potential used for therapeutic interventions. *Experimental Gerontology*, 101, 13–36. <https://doi.org/https://doi.org/10.1016/j.exger.2017.10.029>
26. Smith, I. K., Vierheller, T. L., & Thorne, C. A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). *Analytical Biochemistry*, 175(2), 408–413. [https://doi.org/10.1016/0003-2697\(88\)90564-7](https://doi.org/10.1016/0003-2697(88)90564-7)
27. Srikanth, V., Maczurek, A., Phan, T., Steele, M., Westcott, B., Juskiw, D., & Münch, G. (2011). Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. *Neurobiology of Aging*, 32(5), 763–777. <https://doi.org/10.1016/j.neurobiolaging.2009.04.016>
28. Su, B., Wang, X., Nunomura, A., Moreira, P., Lee, H. -go., Perry, G., Smith, M., & Zhu, X. (2008). Oxidative Stress Signaling in Alzheimers Disease. *Current Alzheimer Research*, 5(6), 525–532. <https://doi.org/10.2174/156720508786898451>
29. Vitek, M. P., Bhattacharya, K., Glendening, J. M., Stopat, E., Vlassara, H., Bucala, R., Manogue, K., & Cerami, A. (1994). Advanced glycation end products contribute to amyloidosis in Alzheimer disease (I-amyloid peptide/aggregation/nucleation-dependent kinetics/seed structure and function). *Neurobiology*, 91(May), 4766–4770.
30. Wang, C., Cai, Z., Wang, W., Wei, M., Si, X., Shang, Y., Yang, Z., Li, T., Guo, H., & Li, S. (2020). Piperine regulates glycogen synthase kinase-3 β -related signaling and attenuates cognitive decline in D-galactose-induced aging mouse model. *Journal of Nutritional Biochemistry*, 75, 108261. <https://doi.org/10.1016/j.jnutbio.2019.108261>
31. WEI, H., LI, L., SONG, Q., AI, H., CHU, J., & LI, W. (2005). Behavioural study of the -galactose induced aging model in C57BL/6J mice. *Behavioural Brain Research*, 157(2), 245–251. <https://doi.org/10.1016/j.bbr.2004.07.003>
32. Wu, D. mei, Lu, J., Zheng, Y. lin, Zhou, Z., Shan, Q., & Ma, D. fu. (2008). Purple sweet potato color repairs d-galactose-induced spatial learning and memory impairment by regulating the expression of

synaptic proteins. *Neurobiology of Learning and Memory*, 90(1), 19–27.

<https://doi.org/10.1016/j.nlm.2008.01.010>

33. Xian, Y. F., Su, Z. R., Chen, J. N., Lai, X. P., Mao, Q. Q., Cheng, C. H. K., Ip, S. P., & Lin, Z. X. (2014). Isorhynchophylline improves learning and memory impairments induced by D-galactose in mice. *Neurochemistry International*, 76, 42–49. <https://doi.org/10.1016/j.neuint.2014.06.011>
34. Yoon, J. H., Youn, K., Ho, C. T., Karwe, M. V., Jeong, W. S., & Jun, M. (2014). P-coumaric acid and ursolic acid from corni fructus attenuated β -amyloid25-35-induced toxicity through regulation of the NF- κ B signaling pathway in PC12 cells. *Journal of Agricultural and Food Chemistry*, 62(21), 4911–4916. <https://doi.org/10.1021/jf501314g>
35. Zhong, J., Wang, Z., Xie, Q., Li, T., Chen, K., Zhu, T., Tang, Q., Shen, C., & Zhu, J. (2020). Shikonin ameliorates D-galactose-induced oxidative stress and cognitive impairment in mice via the MAPK and nuclear factor- κ B signaling pathway. *International Immunopharmacology*, 83(April), 106491. <https://doi.org/10.1016/j.intimp.2020.106491>
36. Zhong, S. Z., Ge, Q. H., Qu, R., Li, Q., & Ma, S. P. (2009). Paeonol attenuates neurotoxicity and ameliorates cognitive impairment induced by d-galactose in ICR mice. *Journal of the Neurological Sciences*, 277(1–2), 58–64. <https://doi.org/10.1016/j.jns.2008.10.008>

Figures

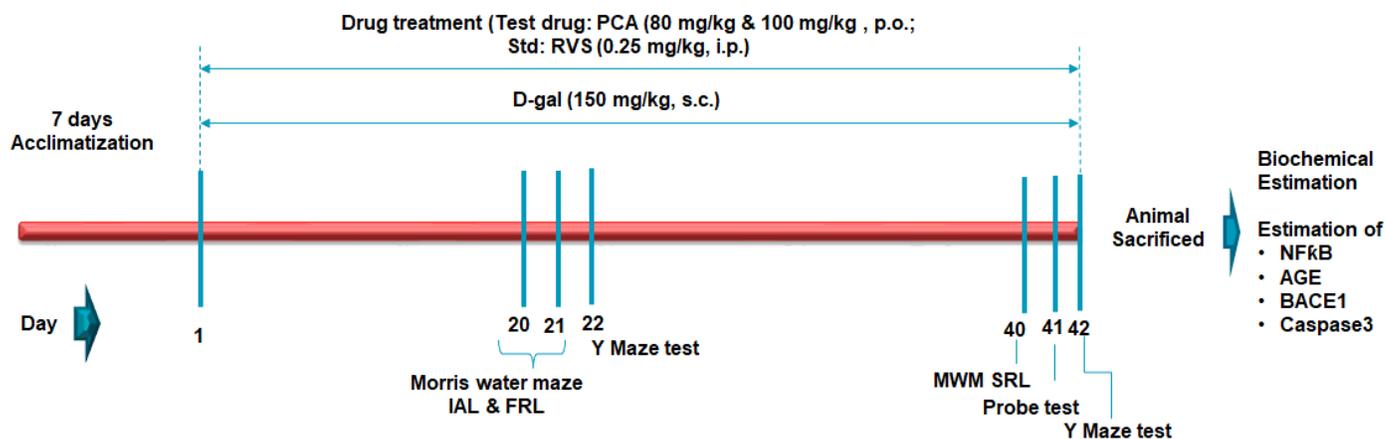


Figure 1

The work plan of the study.



Figure 2

Behavioural test results of PCA, a) Mean escape latency & (b) Time spent in the target quadrant for MWM test and c) % SAB and d) number of entries in the novel arm for Y maze test. Data expressed as mean \pm SEM (n=6). For Escape latency and % SAB, evaluation was performed by repeated measure two-way ANOVA followed by post hoc Bonferroni multiple comparison test. [#p<0.05, ###p<0.01, ###p<0.001 vs Vehicle group; *p<0.05, **p<0.01, ***p<0.001 vs D-gal group]. All other results were evaluated by one-way ANOVA followed by post hoc Tukey's test [[#p<0.05, ###p<0.01, ###p<0.001 vs. vehicle group and *p<0.05 and **p<0.01 vs. D-gal group].

Figure 3

Effect of PCA on a) AChE activity b) brain SOD level, c) brain GSH level and d) brain MDA level. Data expressed as mean \pm SEM (n = 6) and evaluated by one- way ANOVA followed by post hoc Tukey's test. [###p< 0.001 vs. vehicle group, *p<0.05, **p<0.01 and ***p< 0.001 vs. D-gal group].

Figure 4

Effect of PCA on a) brain AGE level, b) brain NFkB level c) BACE 1 level and d) brain Caspase 3 level. Data expressed as mean \pm SEM (n = 6) and evaluated by one- way ANOVA followed by post hoc Tukey's test. [##p< 0.01, ###p< 0.001 vs. vehicle group, *p<0.05, **p<0.01 and ***p< 0.001 vs. D-gal group].