



# Ameliorative Effect of Grape Seed Oil on Aluminium Chloride Induced Neurotoxicity on Rats

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## Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** Neurodegenerative disorder is characterized by progressive loss of structure and function of neurons. Exposure to aluminum causes neurodegenerative disorders like dementia, and Alzheimer's disease (AD). The present study was designed to examine the ameliorative effect of grape seed oil extraction aluminium chloride induced neurotoxicity in Wistar rats.

**Methodology:** Wistar rats were administered with aluminium chloride (175 mg/kg. p.o.) for 28 days to generate neurotoxicity model. Attenuation effect of grape seed oil against aluminium chloride toxicity by oral administration adjunctly from day 18. Behavioral and locomotor activity was determined using passive avoidance test, open field test, actophotometer and rota rod test. Biochemical parameter such as acetylcholinesterase (AChE) activity and superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) activity were assessed in brain samples and histology of brain tissue were assessed on the final day of the experiment.

**Results:** Aluminium chloride treatment significantly decreases cognitive function and open field test with a significance decrease in the acetylcholinesterase and antioxidant (SOD, CAT, GR) activity

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level. Also, the histopathological examination shows significance decrease in the neuronal cell density in hippocampal region. Grape seed oil treated with aluminum chloride neurotoxic groups alleviates all the toxicity induced by the aluminum chloride. Histopathological studies in the hippocampus and cortex of the rat brain also supported that the grape seed oil markedly reduced the toxicity of aluminium chloride.

**Conclusion:** Supplementation of grape seed oil exhibited with beneficial and neuroprotective role on aluminium chloride induced neurotoxicity in Wistar rat model by improving the cognitive memory and antioxidant enzyme level.

**Keywords:** Aluminium chloride; neurotoxicity; antioxidant; acetylcholinesterase; grape seed oil.

## 1. INTRODUCTION

Neurodegenerative disorders are characterized by progressive loss of structure and function of neurons and have created a great burden to the individual and society. The global number of AD patients was 44 million in 2015; however, this number is expected to triple, reaching 115 million individuals by 2050 [1]. Each year around 4.6 million new cases were arising globally [2]. In healthcare, the actual cause of various neurodegenerative diseases remains a mystery. Protein degradation, oxidative stress, inflammation, environmental factor, mitochondrial defects, familial history, and abnormal protein accumulation are some of the environmental factors that cause neurodegenerative diseases. Aluminium is a potential causative agent for causing AD reported by numerous studies [3]. Aluminium (Al) affects the human population around the world, which is abundantly present. Besides its presence in nature, Aluminium is presented in antacids, foils, cosmetics, deodorants, and vaccines, also added in tap water as a flocculating agent during the water purification process. It is important to study the factors that might increase the absorption of aluminum across the gastrointestinal barrier. Hence, aluminum in the environment makes humans at high risk of exposure via air, water, and food [4]. Normal Al concentration in the human body is in the range of 0.1-0.4 µg Al/ gm tissue in dry wt. [5]. Multiple pathological conditions including Alzheimer's disease, Autism, multiple sclerosis [6], Parkinson's disease, and Dementia [7] are caused when the presence of aluminum is beyond this limit. It promotes the aggregation of the  $\beta$ -amyloid protein. Al can penetrate the blood-brain barrier (BBB) [8]; accumulate in different brain regions, including the cortex, cingulate bundles, corpus callosum, and hippocampus [9]; and enter different parts of the cell, including the mitochondria, lysosomes, and nucleus. To treat psychological and neurodegenerative diseases, therapeutic plants are utilized. Different Western medications being

utilized in cognitive decline are taken from plants. Plant-inferred alkaloids, for example, anticholinesterase have been utilized to treat AD. One of the most widely grown fruit crops in the world is black grapes. Wine, Jams, and raisins are also important commodities in the market of the whole world. Grape seed is a by-product of the winemaking process [10], and its oil content is traditionally extracted using either an organic solvent or mechanical techniques [11], which is used as a skincare product. Numerous studies are carried out on grapes and their by-products for health-promoting and antioxidant effects. As black grapes contain high phenolic contents, the interest in health benefits has increased. Most phenolics in Black grapes are located in the seeds [12]. The main phenolics found in Black grapes seeds are gallic acid, catechin, and epicatechin, while ellagic acid and myricetin are the major ones in the skins. Black grapes have shown promise as novel antimicrobial agents [13], anti-cancer properties [14], anti-inflammatory activity [15], antimicrobial activity against *Escherichia coli* O157:H7 [16], nootropic activity, anti ulcerative, antiarthritic, anti-viral prevent skin aging, inhibit UV-radiation induced peroxidation activity [17] and scavenge free radicals [18]. In view of these facts, the present study was designed to test the hypothesis of whether a nutritional strategy like the administration of grape seed oil could prevent aluminum chloride-induced neurotoxicity in rats.

## 2. MATERIALS AND METHODS

Grape seed collection and cold-pressed extract preparation:

The seeds obtained from fresh whole black grapes (*Vitis vinifera*) were purchased from Madhavaram, Chennai district of Tamil Nadu in the month of January 2022 and the voucher was deposited in the corresponding author's laboratory, Department of Pharmacology, C.L.BaidMetha College of Pharmacy, Affiliated to

The Tamil Nadu Dr. M.G.R Medical University, Chennai, Tamil Nadu, India.

The seeds of the grapes were carefully removed using a strainer/ hands, washed, and dried for approximately 24 hours. Cold pressing is adopted to collect the extract from the dried seeds using mortar and pestle (~100 g of dried seeds) until ground as a fine powder with a dropwise addition of methanol. Pressing the extracts until the oil shows a visible separation from the grape seed powder. Approximately after 16-18 hours, the cold-pressed oil was separated and stored in closed glass bottles (~50 ml).

## 2.1 Experimental Animals

Male Wistar rats weighing 180-200g of around 2-3 months old were used for the study. All experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethical Committee (IAEC).

### IAEC Approval no:

01/321/PO/Re/S/01/CPSEA/dated 17/11/2021 valid up to 18/11/2021.

## 2.2 Experiment Design

On the first day of experiment, the animals had been divided randomly into 5 groups of 4 animals each. Neurotoxicity is induced using Aluminium chloride (175mg/kg, p.o.) for the II, III, IV, V groups have been carried out for 30 days (29/01/2022 – 27/02/2022) and GSO (3.7g/kg) treatment was carried for 10 days (17/02/2022 – 27/02/2022). Control animals were given (0.9% NaCl 5ml/kg, p.o.) by means of using oral gavage. The final dose was given 60 min prior to behavioural checking and on 30<sup>th</sup> day, scarification of animal has been done for exvivo studies.

## 2.3 Assessments Passive Avoidance Test

This test is used to assess short term memory. Pole climbing apparatus chamber is used for passive avoidance response where pole is replaced by a wooden platform fixed on electrified grid floor. When rats stepped off the platform, they receive a continuous foot shock from grid floor. The normal reaction of rat was to jump back to the wooden platform. After about 4–5 trials, the animals acquired the passive avoidance response and they refrained from

stepping down. The criterion was reached when the animal remained on the platform for at least 60 seconds [19].

**Table 1. Treatment use for different animal groups**

Animals	Treatment
Group-I	Normal saline (0.9% NaCl 5mg/kg) p.o.
Group-II	Aluminium chloride (175mg/kg) p.o.
Group-III	Aluminium chloride (175mg/kg) p.o. + Donepezil HCl (1mg/kg) p.o.
Group-IV	Aluminium chloride (175mg/kg) p.o. + 2ml Grape seed oil (3.7g/kg) p.o.
Group-V	Aluminium chloride (175mg/kg) p.o. + 4ml Grape seed oil (3.7g/kg) p.o.

## 2.4 Open Field Test

Exploratory behavior was evaluated in an open discipline paradigm. The open arena was made of plywood and accommodated 40 x 50 x 60 cm dimensions. The entire apparatus become painted black and divided into 16 squares with white strains on the floor. Each animal was located on the nook of the equipment and for the next 5 mins they have been discovered for ambulation which includes line crossings and head dipping [20].

## 2.5 Measurement of Locomotor Activity

The spontaneous locomotor activity of each rat was recorded individually for 10 min using an actophotometer. The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in a circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded [21].

## 2.6 Rota-Rod Test

The effect of aluminum as well as grape seed oil treatment on muscle performance was evaluated using Rota-rod (Techno) test. All the rats were given two initial training trials of 300 s, approximately 10 min apart, to maintain posture on the Rota-rod (3 cm in diameter and rotating at a constant 20 rev/min). After the initial training trials, a baseline trial of 120 s was conducted. The time each animal remained on the Rota-rod was recorded. The animals that did not fall off the Rota-rod were given a maximum score of 120 seconds [21].

## 2.7 Assessment of Antioxidants and Neurotransmitter

The animals were anesthetized using chloroform and sacrificed. The whole brain was carefully removed from the skull. For the preparation of the homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer, and a 10% (w/v) tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.4, stored at  $-2$  to  $-8^{\circ}\text{C}$ ). The homogenate was centrifuged at 3000 rpm for 10 min, and the resultant cloudy supernatant liquid was used for antioxidant and neurotransmitter assessments.

## 2.8 Histopathological Studies

The animals were anesthetized using chloroform and sacrificed. The whole brain was carefully removed from the skull. The brain sample from each group was selected and stored in 10% buffered formalin solutions and further embedded in paraffin with wax. The blocks were processed for sectioning; the sections were then stained with hematoxylin and eosin as nuclear and cytoplasmic stains, respectively to assess the ameliorative effect of grape seed oil. Pathological changes, if any, were viewed under a light microscope and recorded.

## 2.9 Statistical Analysis

The statistical analysis was carried out with the aid of one-way ANOVA followed through Dunnett's-t test. P values  $<0.05$  (95% confidence restrict) were considered to be significant, by the use of software graph pad 9.

## 3. RESULTS

### 3.1 Passive Avoidance Test

The Step-Down Latency (SDL) of Group II animals shows a significant decrease ( $p<0.0001$ ) when compared with Group I animals. On treatment with Grape seed oil (2ml), the Step-Down Latency (SDL) of Group IV animals shows significant decrease ( $p<0.001$ ) when compared with Group III animals. On treatment with Grape seed oil (4ml), the Step-Down Latency (SDL) of Group V animals shows significant increase ( $p<0.05$ ) when compared with Group III animals. Results are plotted in Fig. 1.

### 3.2 Open Field Test

The Group II animals show a significant decrease in head dipping and line crossing when

compared with Group I animals ( $p<0.0001$ ). The Group IV animals show a significant decrease in head dipping and line crossing behavior statistically when compared with Group III animals ( $p<0.001$  and  $p<0.01$ ). The Group V animals show a significant increase in head dipping and a significant decrease in line crossing when compared with Group III animals ( $p<0.05$  and  $p<0.05$ ). Results are plotted in Figs. 2a and 2b.

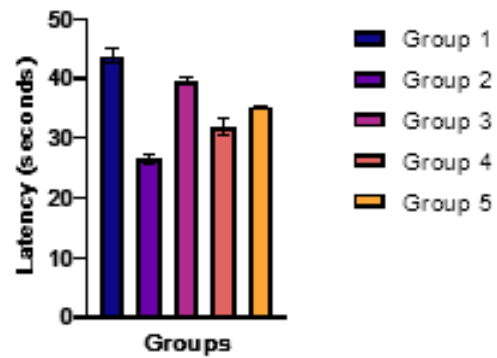


Fig. 1. Effect of grape seed oil passive avoidance test

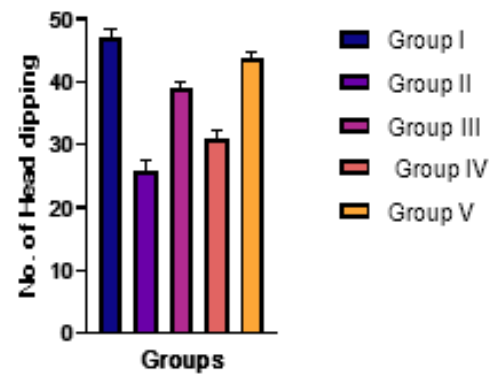


Fig. 2a. Effect of grape seed oil in head dipping

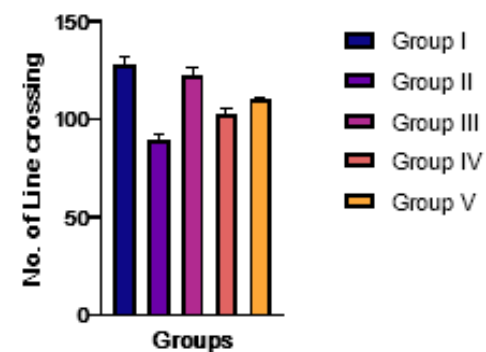


Fig. 2b. Effect of grape seed oil in line crossing

### 3.3 Rota Rod Test

The fall of time of Group II animals shows a significant decrease ( $p < 0.0001$ ) when compared with Group I animals. On treatment with Grape seed oil (2ml), the fall of time of Group IV animals shows a significant decrease ( $p < 0.001$ ) when compared with Group III animals. On treatment with Grape seed oil (4ml), the fall of time of Group V animals shows a significant increase ( $p < 0.001$ ) when compared with Group III animals. Results are plotted in Fig. 4.

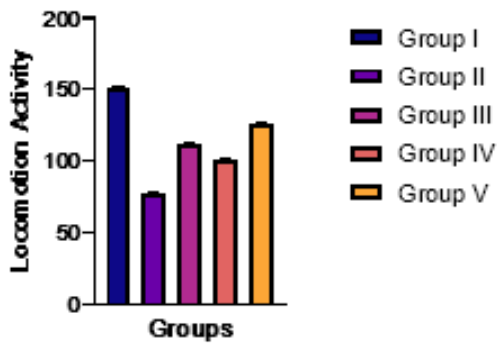


Fig. 3. Effect of grape seed oil in actophotometer

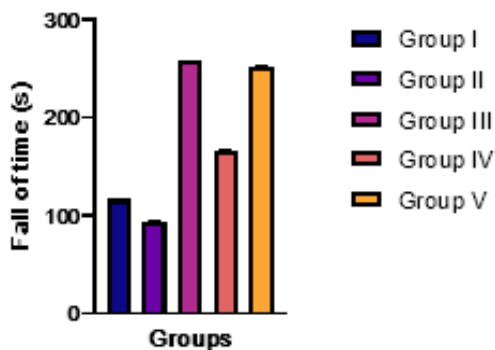


Fig. 4. Effect of grape seed oil in rota rod

### 3.4 Assessment of Antioxidant

The SOD level of Group II animals shows a significant ( $p < 0.0001$ ) decrease when compared with Group I animals. On treatment with Grape seed oil (2ml), the SOD level of Group IV animals shows a significant decrease ( $p < 0.001$ ) when compared with Group III animals. On treatment with Grape seed oil (4ml), the SOD level of Group V animals shows a significant ( $p < 0.05$ ) increase when compared with Group III animals. The results are plotted in Fig. 5.

The CAT level of Group II animals shows a significant ( $p < 0.0001$ ) decrease when compared

with Group I animals. On treatment with Grape seed oil (2ml), the CAT level of Group IV animals shows a significant decrease ( $p < 0.001$ ) when compared with Group III animals. On treatment with Grape seed oil (4ml), the CAT level of Group V animals shows a significant ( $p < 0.05$ ) increase when compared with Group III animals. The results are plotted in Fig. 6.

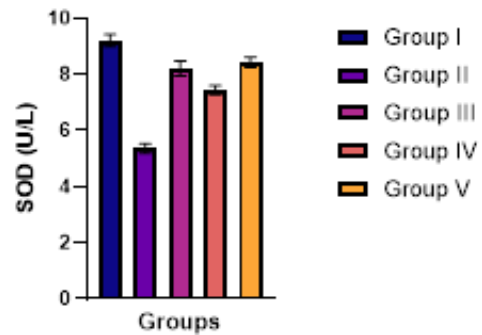


Fig. 5. Effect of grape seed oil in SOD

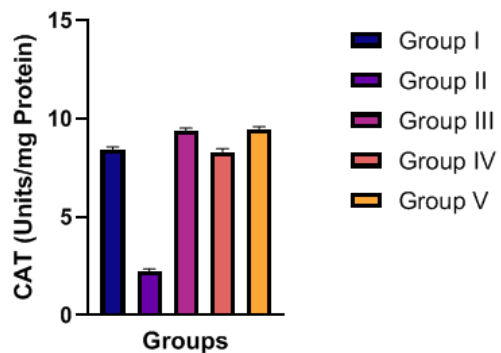


Fig. 6. Effect of grape seed oil in CAT

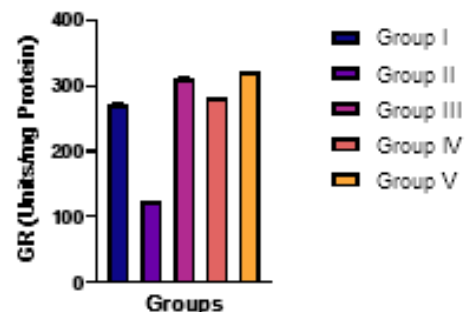


Fig. 7. Effect of grape seed oil in glutathione reductase

The GRD level of Group II animals shows a significant ( $p < 0.0001$ ) decrease when compared with Group I animals. On treatment with Grape seed oil (2ml), the GRD level of Group IV animals shows a significant decrease ( $p < 0.0001$ )

when compared with Group III animals. On treatment with Grape seed oil (4ml), the GRD level of Group V animals shows a significant ( $p < 0.001$ ) increase when compared with Group III animals. The results are plotted in Fig. 7.

### 3.5 Assessment of Neurotransmitter

The AChE activity of Group II animals shows significant ( $p < 0.0001$ ) increase when compared with Group I animals. On treatment with Grape seed oil (2ml), the AChE of group IV animals shows significant decrease ( $p < 0.001$ ) when compared with Group III animals. On treatment with Grape seed oil (4ml), the AChE of Group V animals shows significant ( $p < 0.001$ ) decrease when compared with Group III animals. The results are plotted in Fig. 8.

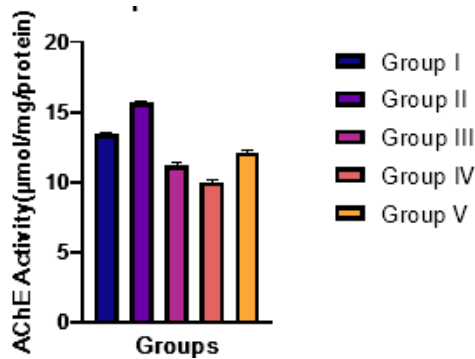


Fig. 8. Effect of grape seed oil in AChE

### 3.6 Assessment of Histopathology

On observation, it was found that there was decrease in density of neuronal cells and disruption in the normal arrangement of neuronal cells in hippocampal region of Group II animals when compared to Group I animals. Treatment groups (Group IV and V) revealed improved neuronal configuration than Group III. Group IV, V shows significant increase in the density of neuronal cells and hippocampal regions of brain when compared with neuronal loss in negative control group (Group II). Histopathological pictures are shown in Figs. 9 to 13.

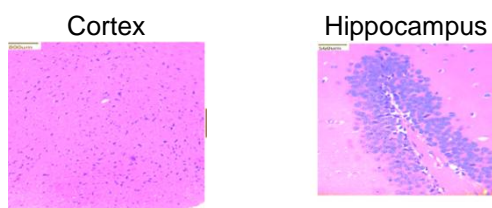


Fig. 9. Brain histopathology of control group

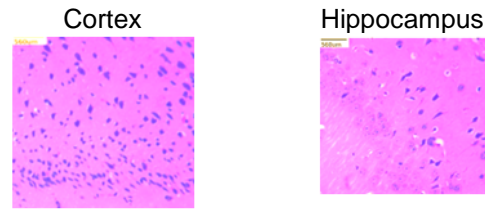


Fig. 10. Brain histopathology of negative control group

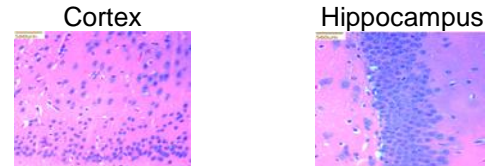


Fig. 11. Brain histopathology of standard control group

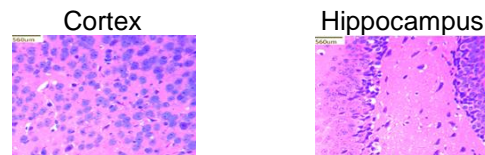


Fig. 12. Brain histopathology of grape seed oil (2ml)

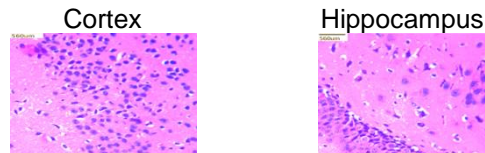


Fig. 13. Brain histopathology of grape seed oil (4ml)

## 4. DISCUSSION

- Aluminum chloride causes a class of neurodegenerative disorder. The incidence of neurodegenerative disorders increases with age. Damage of short-term memory is the characteristic feature. When the condition proceeds, extra cognitive capabilities are impaired, like ability to calculate, and use objects and equipment. Acetylcholinesterase inhibitors are the best marketers authorized by means of the Food and Drug Administration (FDA) for the treatment of AD. All different retailers prescribed for the remedy of AD are used on an off-label foundation.
- As per preceding studies, grape seed oil has superb anti-oxidant belongings,

consequently, it's far believed to have actions on CNS problems and neurodegeneration illnesses.

- The current study has revealed the ameliorative effect of *grape seed oil* on Aluminium chloride-induced neurotoxicity in rats. Aluminum chloride-induced impairment of reminiscence became assessed by using behavioral parameters like Passive avoidance task, open field test and locomotor activity using an actophotometer, rota rod. It became discovered that remedy with *grape seed oil* shields cognitive deficits in Aluminium chloride induced neurotoxicity.
- Spatial studying inside the open area habituation was used to access mastering and memory. The decline in response to regular surroundings after repeated exposures to the acquainted surroundings is cited to spatial learning. Recurrent publicity produces a lower in the exploratory tasks, that's implicative of memory referring to a selected feature of that surroundings. Exploratory activities may be reduced on subsequent touch with open subjects. In the end of the study the animals which are treated with *grape seed oil* indicated elevated spatial routine and sleep deprivation decreased spatial routine learning.
- Passive avoidance behavior is based on poor reinforcement became used to observe the level of memory. An electro-shock is given during training period for about 15 sec inside the Step-Down Latency (SDL) which is then recorded. SDL was determined by the time taken via the rat to step down from the wooden platform to grid ground with its whole paw on the grid floor. SDL is enhanced as an indication of long-term memory in which Aluminium chloride treated animals showed reduced SDL. Treatment with *grape seed oil* showed development in the longtime reminiscences index of elevation in SDL.
- Muscular and locomotion activities using Actophotometer, and Rota rod were found to be significantly decreased after Al treatment. Therefore, high levels of Al not only interfere with memory but also affect motor functions and lead to decreased motor activities and grip strength. On treatment with *grape seed oil*, animals enhanced their locomotor activity.

- Antioxidants SOD, CAT, GRD, and neurotransmitter AChE activity were evaluated where there is a significant decrease in the antioxidant level and increased AChE after Al treatment. On treatment with *Grape seed oil*, there was an increase in antioxidant level and a decrease in AChE activity.
- The histopathology studies of the brain show that on treatment with *Grape seed oil*, there is a significant increase in the density of neuronal cells and hippocampal regions of the brain while compared with neuronal loss in the Aluminium chloride treated group.

## 5. CONCLUSION

The Grape seed oil possessed significant recovery in memory and locomotor activity processes but a higher dose 4ml showed better activity than lower dose 2ml.

The current study reveals the ameliorative effect of Grape seed oil on Aluminium chloride-induced neurotoxicity in rats. From the effects, it can be concluded that grape seed oil has an extraordinary impact on behavioral & locomotor activity enhancement and oxidative stress. Further studies are required for the identification of molecular-level activity and individual phytochemical constituents which could be responsible for neuroprotective action.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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