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Neuroprotective effect of derris indica seed extract attenuates amyloid beta plaques induce Alzheimer's in mice model

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***Abstract*---**The progression of Alzheimer's disease type dementia is carried on by the neurotoxic effects of amyloid beta ($A\beta_{25-35}$) peptide accumulation. The hypothalamic-pituitary-adrenal(HPA) axis malfunctions and leads to higher levels of amyloid beta(Aβ) accumulation. In this work, we looked at how Derris indica extract affected mice brains that had undergone neurodegeneration caused by the Aβ. Derris indica seed extract(DISE) were administered to mice over the course of 21 days in two dosages (50 mg/kg and 100 mg/kg). On the 21st day, tests for spatial learning, habituation memory, short- and long-term memory, and stepdown inhibitory responses were carried out. Pro-and anti-inflammatory cytokines and antioxidants were assessed in brain tissues. A significant ($p<0.01$) improvement in habituation memory and the step-down inhibitory avoidance test was seen after receiving the DISE therapy, reduced escape delay considerably ($p<0.01$) enhanced cognitive progress in spatial learning. DISE may have an impact on neuroprotection as seen by the significant ($p<0.01$) decrease in proinflammatory and enhancement in IL-10 along with improved antioxidant indicators. According to investigations, the DISE enhance cognition by controlling neuro-inflammation and neuroimmune function, which are both controlled by the HPA axis, which mitigates stress. DISE might be promising therapeutics for neurodegeneration disorders such as AD.

***Keywords*---**derris indica, Alzheimer's disease, amyloid beta, neuroprotective.

Introduction

Alois Alzheimer, a psychiatrist, defined AD in the early 1900s. AD causes dementia. Alzheimer's, a progressive neurological condition, primarily affects older adults(1). The Alzheimer's Association of India estimates there are 45 million dementia sufferers worldwide and 4 million in India(2). AD is the fifth-leading cause of death worldwide, affecting 45 million people. 5.8 million Americans have AD dementia, and 13.8 million are expected by 2050(3). In 2050, Europe could have 18.9 million dementia sufferers and East Asia 36.5 million(2, 4). AD is caused by plaque deposition, oxidative stress, neurofibrillary tangle formation, cholinergic shortfall, cholinergic dysfunction, and neuroinflammation(5). FDA-approved medications for AD cognitive symptoms include cholinesterase inhibitors and NMDA antagonists(6). These medications only treat symptoms, have a short half-life, and can cause stomach pain, liver problems, nausea, diarrhoea, and severe vomiting(7). Despite the effectiveness of many curative therapies, many people may react negatively or relapse. Therefore, a unique, safe, and effective AD medication with few side effects is needed.

Derris indica (Lamk.) Bennet is used to treat bronchitis and whooping cough in traditional medicine. Numerous studies show that this plant has rheumatoid arthritis, anticancer, anti-microbial, anti-diabetic, anticonvulsant, gastroprotective, and anti-oxidant properties(8-11). Derris indica inhibits pro-inflammatory cytokines, previous experiments. Derris indica efficacy in treating AD by inhibiting AchE in in vitro models, but not preclinical model, is unknown(9). The most common in vivo model to study AD progression is mice with beta amyloid plaques. This study examined the effectiveness and primary molecular mechanisms of Derris indica seed extract on Aβ-induced AD(9). Derris indica seed extract may help develop natural chemicals that affect Alzheimer's therapy.

Material and Methods

Collection of plant material

The Derris Indica seeds were harvested in Warangal, Telangana state, India, in February 2020. Dr. Mohd Mustafa, a taxonomist from Kakatiya University in Warangal, Telangana, India, verified the plant's authenticity. The Department of Botany at Kakatiya University in Warangal, India, maintains a specimen accession number for the species KUDM-1084 in its herbarium.

Plant material and extraction

The seeds were fully dried in the shade, coarsely ground, macerated in methanol for seven days while being stirred at regular intervals, and then filtered by using the Soxhlet apparatus. Derris indica seeds that had been finely ground (300g) were refluxed for 3h in 1L of methanol. A 3h later, greasy residue was collected. After being washed twice with petroleum ether, the oily residue was transformed into a thick yellow residue that was then dissolved in methanol and preserved for 48h. After 48h, a white precipitate was produced, which was redissolved in a small amount of methanol before being filtered, dried, and recrystallized with

methanol(12). The semisolid mass (18.5%) is then concentrated under decreased pressure using a rotavapor evaporator (Buchi Rotavapour, Switzerland), and identified as aqueous methanolic Derris Indica Seed Extract (DISE).

Determination of total phenolic, DPPH and ABTS in DISE

The Folin-Ciocalteu method was used to compute the total phenolic compounds in DISE, which were then expressed as mg of gallic acid per 100g of DISE (13). The DPPH scavenging capacity of DISE was evaluated(14). The ABTS scavenging capacity of DISE was evaluated (15).

Acute oral toxicity study

Using male Swiss albino mice, an acute toxicity investigation for the proportion of DISE was conducted in accordance with OECD 423 recommendations. For 72h, animals were monitored for indicators of toxicity and mortality.

Animals

The study protocol was approved with the number IAEC/15/UCPSc/KU/2019. Male Swiss albino mice weighing 20–22g were obtained from Mahaveer Agencies, Uppal, Hyderabad, India, with prior approval from our institutional animal ethics committee. In a lab setting with 12h of darkness and 12h of light, animals will be housed. Every experiment was conducted in a light-filled room at a constant temperature of 20±2°C, a relative humidity of 45±15%, with a regular pellet diet, and unlimited access to water throughout the study.

Drugs and dose selection

The DISE-50 mg/kg body weight, per oral route (p.o) and DISE-100 mg/kg body weight, p.o doses used in this study were based on previous research(10, 11, 16-18). Amyloid beta peptide ($A\beta_{25-35}$) injection was used for inducing AD at a single dose of 3 mg/kg body weight on the 15^{th} day, intracerebralventricular(i.c.v) route at the bregma point by using stereotaxic apparatus. The intraperitoneal route (i.p) of 0.1ml/20gm of mouse Ketamine and Xylazine (87.5mg/kg and 12.5mg/kg) were used for anaesthesia procedures. Donepezil 5mg/kg was used as the standard in this study(19).

Experimental design and treatment regimen

The animals were sorted into five groups. Animals in Group 01 (vehicle control; oral administration of a 2% acacia suspension from day 1 to day 21). On the 15^{th} of the experiment, animals in Group 02 (disease control or Aβ control) received a single injection of $A\beta_{25-35}$ (3 mg/kg BW) i.c.v. Animals in Group 03 (Standard control) received an i.c.v injection of $A\beta_{25-35}$ on day 15, a single dose of Donepezil (5 mg/kg BW) orally from day 15 to day 21, and a 2% acacia suspension. Animals in Group 04 (DISE-Low dosage) received an i.c.v injection of $A\beta_{25-35}$ on the fifteenth day, a single dose of 3 mg/kg body weight, and DISE 50mg/kg BW orally every day from the first to the twenty-first day with 2% acacia suspension. Animals in Group 05 (DISE-high dose) received an i.c.v injection of $A\beta_{25-35}$ on the

15th day, a single dose of 3 mg/kg BW, and DISE 100 mg/kg BW orally from the 1st to the 21st day with 2% acacia suspension. Pre-treatment for the fourth and fifth groups began on the first day and lasted until the 21st day. On day 15, all groups, with the exception of the vehicle control, received a single i.c.v dose of $A\beta_{25-35}$ 3 mg/kg. The Donepezil treated groups receive treatment beginning on the 15th day of the experiment and continuing until the 21st day. Behavioural data were collected after the induction phase and on the termination day. The obtained brain tissues were fixed in buffered formalin for further examination.

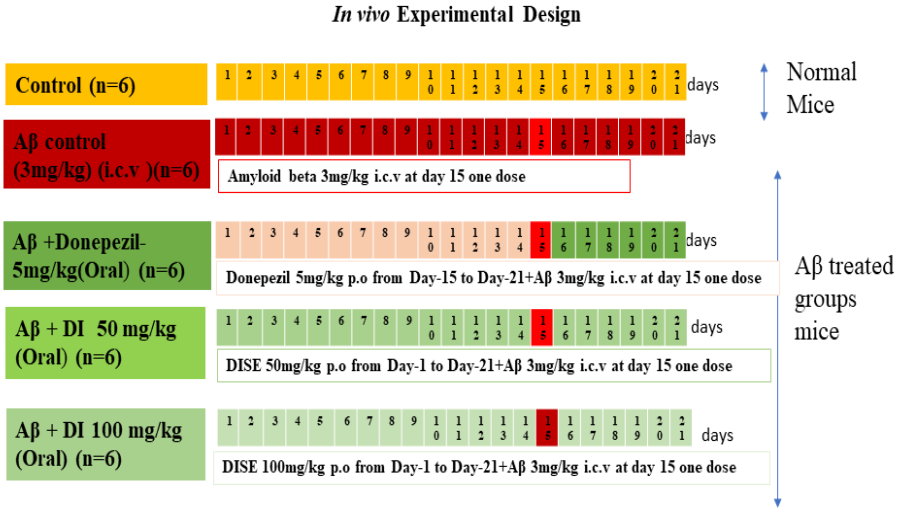

Fig. 1 Animal Experimental Design

Analysing the behavioural indicators of AD caused by beta-amyloid plaques Anti-Alzheimer's activity

With the help of the elevated plus maze concept and the Morris Water Maze (MWM) model, the anti-Alzheimer's of DISE were examined *in vivo*.

Elevated Plus Maze Test

An elevated plus maze (EPM) has four arms that are each 30 cm above the ground and 90 degrees apart from one another. Two of the arms were enclosed by tall walls, and the other two arms formed a plus sign when connected by a central region (7 cm x 7 cm) (30 cm x 7 cm x 20 cm). The floor of the maze was painted black, as were the walls of the enclosed arms. Sixty minutes before the test, animals were treated with a vehicle, DISE, Donepezil, and $A\beta_{25-35}$. The duration of time(duration) spent in the open arms and the proportion of open arm entries were then calculated for each animal. The apparatus was meticulously cleaned following each trial. From day 15 to day 21, the dose of $A\beta_{25-35}$ and the DISE 50 and DISE 100 mg/kg were maintained, with daily EPM readings. Readings were taken on the eighth day without dosing in order to assess the transfer latency using a cut off of 60 seconds(19, 20).

Morris water maze test

A water maze test was used to evaluate mice's spatial learning and memory. A 100cm-diameter pool with water was used for the Morris water maze (22^{o}C). There were visual signals in each of the pool's four equally sized quadrants. From day 1 through day 4, mice were allowed to participate in the concealed platform test. On the fifth day, a probe trial was conducted. Both the entries of crossings and the amount of time the mouse spent in the target quadrant were counted(19, 20).

Estimation of the levels of inflammatory cytokines

In the brain tissue, the concentrations of TNF- and IL-6 as well as additional anti-inflammatory cytokines (IL-10) were measured. For the quantification, R&D SYSTEMS, USA, used mouse-specific TNF-, IL-6, and IL-10 ELISA kits. The procedures were carried out precisely as instructed by the product's manufacturer.

Assessment of antioxidant levels in experimental animal brain tissue

The anti-oxidant activity and levels in the tissues of the brain were examined. Using commercially available assay kits (Cayman Chemical, USA), the total superoxide dismutase (SOD) enzyme and catalase(CAT) activities were assessed in accordance with the manufacturer's guidelines. The levels of reduced glutathione(GSH)(21) and the activities of glutathione S-transferase (GST)(22) were estimated in accordance with previous literature. The nitrite levels were analysed as a measure of nitric oxide (NO) by using Griess reagent. The quantification of nitrites is done by using a standard sodium nitrite(23).

Monitoring myeloperoxidase activity in brain tissue

Myeloperoxidase activity was measured as a measure of neutrophil infiltration(23).

Brain tissue lipid peroxidation measurement

To determine the degree of lipid peroxidation in the brain tissues, the quantities of thiobarbituric acid reactive substances(TBARS) was measured according to previous work (24). Based on the MDA extinction coefficient, the lipid peroxidation state was calculated.

Studies on the histopathology of brain tissue

The brain tissues from each group were removed on day 22nd and kept in buffered formalin. The brain tissues were processed in isopropyl alcohol after 48 hours of fixation, then embedded in paraffin, sectioned to a thickness of 5µm, and stained with H&E. 10x magnification images taken light microscopy were used to look for histological changes in the brain tissue sections (Zeiss microscope).

Analytical Statistics

The statistical analysis was carried out using GraphPad Prism 5.01. All data was represented as mean ± S.E.M(n=6). Dunnett's multiple comparison test was used post hoc to compare the Aβ control group to the other groups after a one-way ANOVA. Statistical significance was defined as a P value < 0.05. ###p< 0.001; ##p< 0.01 #p< 0.05 compared with the vehicle control group; *p<0.05, **p<0.01 and ***p<0.001compared with the Aβ alone treated group; ns, non-significant.

Results

Determine the total polyphenol content on DISE extract

DISE extract was found to have 73.15 ± 0.96 mg/100g of DISE of polyphenols (n = 3).

DISE extract was evaluated for antioxidant potency by using the ABTS assay

Screening was done for DISE (5 mg/ml) and by taking Vitamin C (0.2 mg/ml) and Rutin (0.2 mg/ml) as reference standards. The IC_{50} of the DISE extract obtained was tabulated and the results obtained are as follows:

Preliminary screening

S.No.	Standards (0.2 mg/ml)	% Inhibition
S1	Vitamin-C	100
S2	Rutin	99.59
S.No.	Compound (5mg/ml)	% Inhibition
1	DISE extract	98.02

IC_{50} Values

S.No.	Standards (0.2 mg/ml)	IC_{50} (mg/ml)
S1	Vitamin-C	0.004±0.000
S2	Rutin	0.001±0.000
S.No.	Compound (5mg/ml)	IC_{50} (mg/ml)
1	DISE extract	0.22 ±0.65

Table:1 Effect of DISE extract on ABTS

DISE extract was evaluated for antioxidant potency by using the DPPH assay

Screening was done for the DISE starting at a concentration of 5 mg/ml and by taking vitamin-C and rutin (0.2 mg/ml) as reference standards. The IC_{50} of DISE was tabulated and the results obtained are as follows:

Preliminary screening

S.No.	Standards (0.2 mg/ml)	% Inhibition
S1	Vitamin-C	90.05

S2	Rutin	81.84
S.No.	Compound (1mg/ml)	% Inhibition
1	DISE extract	83.74

IC_{50} Values

S.No.	Standards (0.2mg/ml)	IC_{50} (mg/ml)
S1	Vitamin-C	0.013±0.01
S2	Rutin	0.016±0.001
S.No.	Compound (1mg/ml)	IC_{50} (mg/ml)
1	DISE extract	0.31±0.61

Table:2 Effect of DISE extract on DPPH

An acute toxicity study

The DISE was found to have no toxic signs up to a dose of 2000 mg/kg in male Swiss albino mice. The LD_{50} of the DISE was greater than 2000 mg/kg b.w. The investigations into these DISE extracts were carried out with different graded doses according to OECD guideline 423, i.e. 5, 50, 300, and 2000 mg/kg body weight. So, an extended *in vivo* study is carried out.

DISE extract effects on the body weight and brain index in Aβ-induced AD mice

We observed no significant ($p>0.05$) changes in body weights and brain index among treated and VC experimental groups compared to Aβ control (Fig. 2) on the termination day, but the Aβ-induced group had lost their body weights and brain index compared to the VC group, while the Donepezil and DISE therapy groups showed their body weights and brain index were restored compared to the Aβ treated group. The VC gained body weight and brain index compared to the Aβ control.

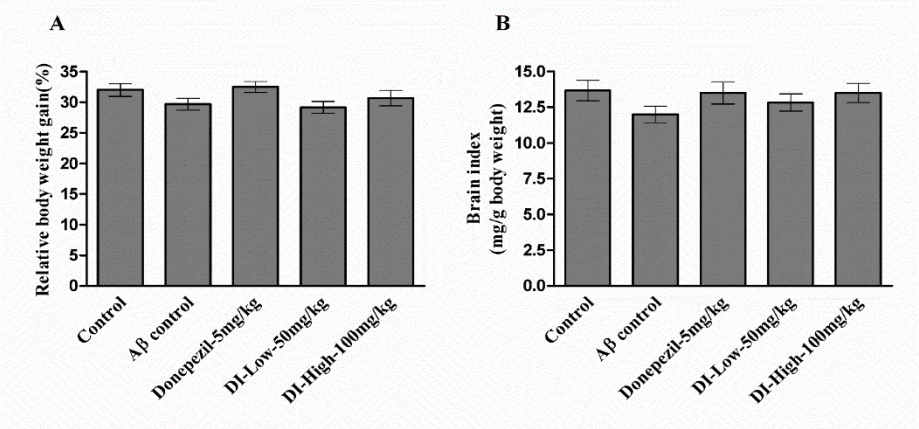

Fig. 2: DISE effect on the physical characteristics and clinical signs of mice induced with Aβ. A) Relative body weight over the course of the experiment B) Brain index.

The Y-maze test was used to evaluate the learning behaviour impact of DSIE on AD animals with Aβ-induced AD

The Y-maze test results showed that the total arm entries were significantly ($p<0.001$) higher in the Aβ group compared to the VC group, while those treated with DISE-low ($p<0.01$) and DISE-high dose ($p<0.001$) significantly reduced the total arm entries compared to the Aβ group. Donepezil significantly ($p<0.05$) reduced arm entries compared to Aβ. The Y-maze test results showed that spontaneous alterations of mice were significantly ($p<0.001$) decreased in the Aβ group compared to the VC group, while those treated with donepezil standard control ($p<0.001$), DISE-low ($p<0.001$), and DISE-high dose ($p<0.001$) mice increased the total arm entries compared to the Aβ group (Fig. 3).

DISE extract effects on the abilities of memory in Aβ-induced AD mice

The Morris water maze test was used to evaluate DSIE's memory effects on Aβ-induced AD animals. The water maze experiment measured mice's spatial learning using escape latency. The Aβ control group had significantly ($p<0.001$) longer escape latencies than the VC group ($p<0.01$), while those treated with DISE-low ($p<0.001$) and Dose-high ($p<0.001$) reduced escape latencies dose-dependently. Donepezil reduced escape latencies significantly ($p<0.001$) compared to Aβ.

In the experiment, time spent in the anticipated quadrant and number of crossings measure spatial memory retention. The Aβ control group spent significantly ($p<0.001$) less time in the target quadrant and crossed it less often than the VC group, while the donepezil ($p<0.001$) and DISE high dose ($p<0.01$) groups spent significantly more time in the quadrant and crossed it more often than Aβ control mice. DISE low dose ($p>0.05$) groups showed non-significant increases in time in the anticipated quadrant and crossings compared to Aβ control mice. Low-dose DISE animals showed no significant changes. Swimming time in the water maze test measures spatial memory retention in the experiment. The Aβ control group swam significantly slower ($p<0.001$) than the VC group, while the donepezil and DISE high dose groups swam significantly longer. The DISE low group swam slower than the VC group ($p<0.05$) (Fig.3).

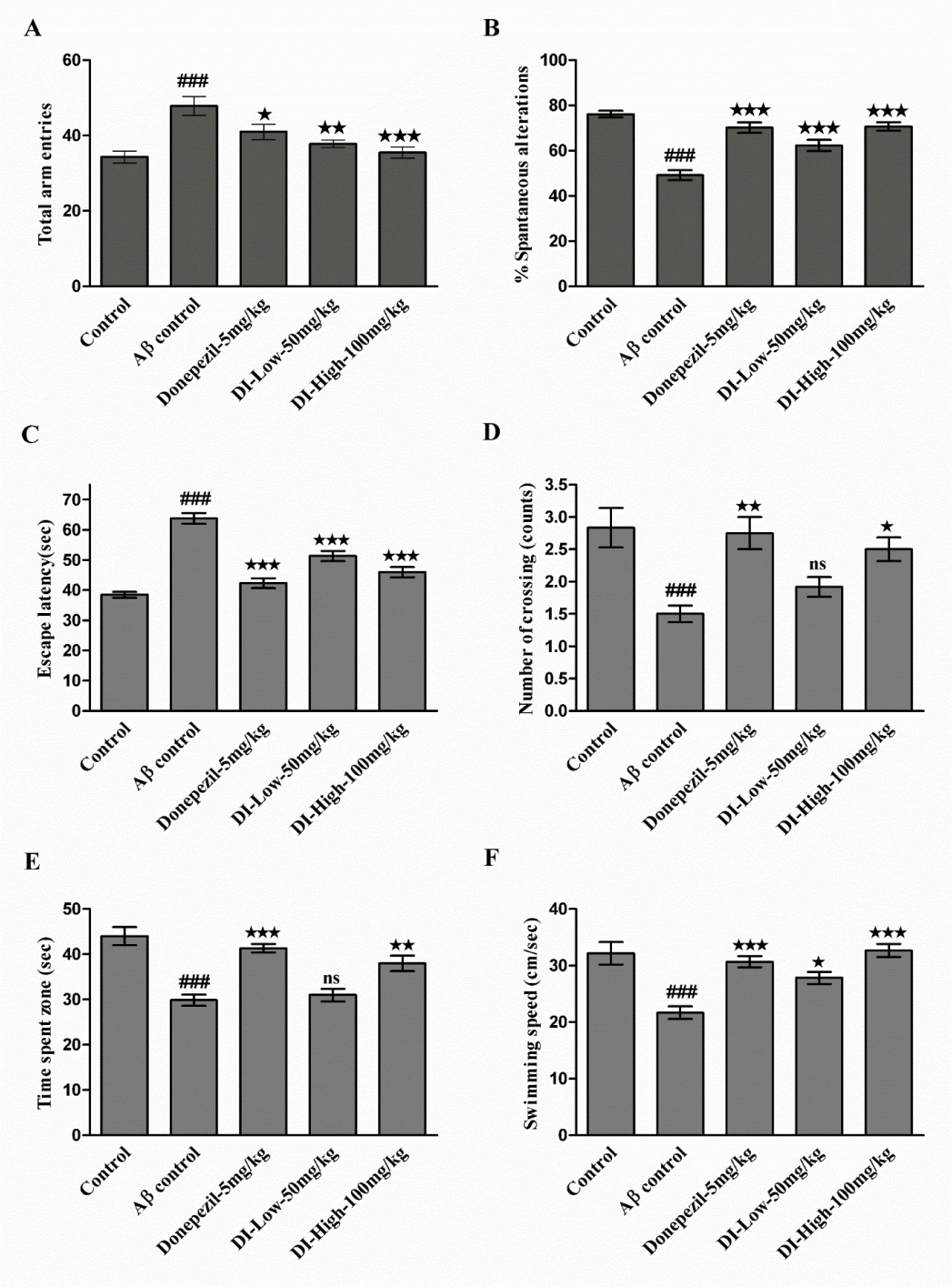

Fig. 3: DISE effect on Aβ-induced AD mice behavioural parameters: A) Arm entries B) Spontaneous alterations C) Escape latency, D) Number of crossings, E) Time spent zone and F) Swimming speed in the experimental animals

DISE effects on the oxidative stress in Aβ-induced AD mice

Based on results that indicate that the anti-oxidant enzyme quantities on Aβ induced AD mice, oxidative stress injury plays a major role in the pathogenesis mechanisms of AD. Fig. 4, shows that Aβ group significantly ($p<0.001$) reduced glutathione (GSH) levels, superoxide dismutase (SOD) activity, glutathione-S-transferase (GST) activity, and catalase (CAT) activity when compared to the VC group, whereas those treated with donepezil standard control ($p<0.001$), DISE-low ($p<0.001$) and dose-high dose ($p<0.001$) mice significantly reinstated the anti-oxidant enzyme quantities such as SOD and CAT in the brain tissue when compared to the Aβ group. The DISE low group also significantly alleviated oxidative damage in brain tissues, except for GSH and GST. Furthermore, donepezil-treated and DISE significantly restored the antioxidant quantities of GST when compared to Aβ control group. The donepezil-treated group and DISE high dose also significantly alleviated oxidative damage in brain tissues (Fig. 4).

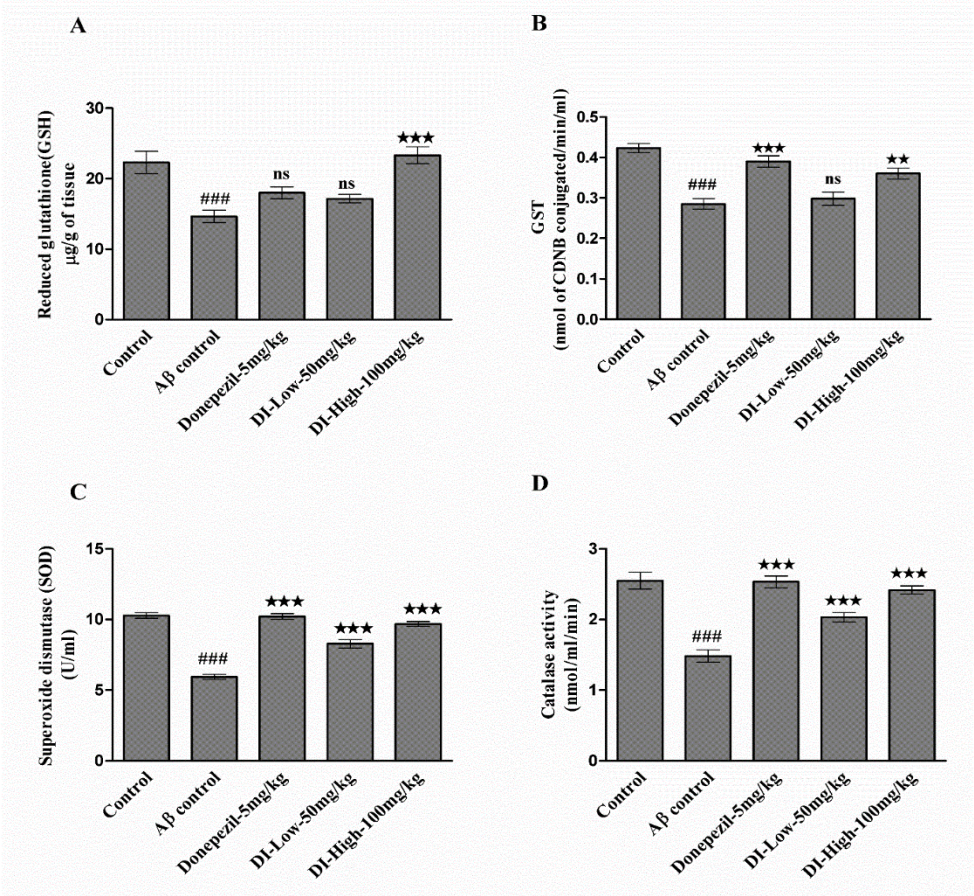

Fig. 4: Effect of DISE treatment on biochemical markers of antioxidants in Aβ-induced brain tissue (A) Reduced glutathione (B) Glutathione-S transferase (GST) (C) Superoxide dismutase (SOD) activity (D) Catalase activity in brain tissue.

DISE extract effects on the myeloperoxidase, nitrassative stress and lipid peroxidation in Aβ plaques induced AD mice

Based on consequences, MPO is the hallmark of infiltration neutrophils in the disease pathogenesis, Nitric oxide and MDA are the oxidative stress markers in the disease pathogenesis. When compared to the VC group, the quantities of MPO, NO, and MDA were significantly ($p<0.001$) higher in the mice that had been exposed to Aβ. When compared to the mice exposed to Aβ, the MPO, NO and MDA levels in the donepezil-alone group of animals were significantly lower than those oxidative stress markers. In comparison to the group of mice that had been given Aβ, the quantities of MPO, NO, and MDA were significantly ($p<0.01$) reduced in the treatment groups with DISE low and DISE high doses (Fig. 5).

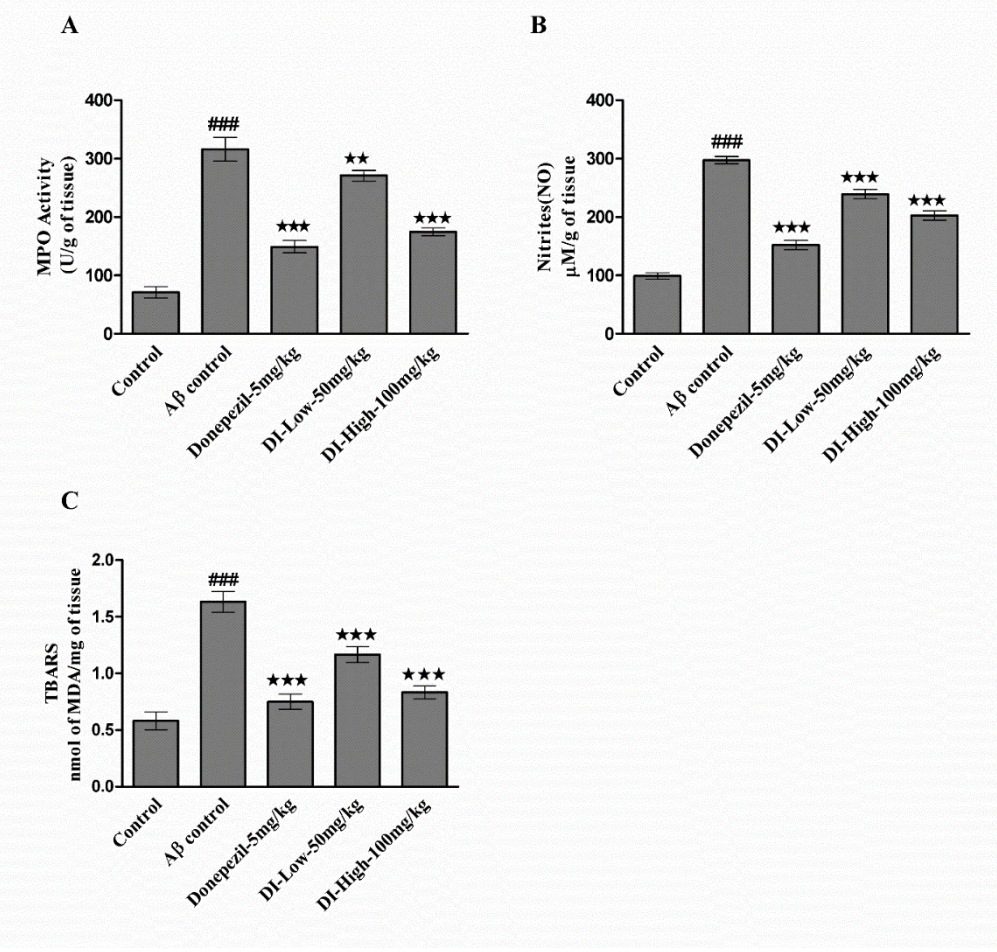

Fig. 5: Effect of DISE treatment on biochemical markers of infiltration of neutrophils in Aβ-induced brain tissue (A) Myeloperoxidase activity (MPO) activity (B) nitrosative stress: Nitric oxide (NO); and (C) Lipid peroxidation stress markers:TBARS.

DISE extract effect on histological changes in the brain tissue of Aβ-induce mice

According to histological analysis, the VC mice's cerebral cortex and hippocampus had normal morphology. Aβ-induced mice had many tiny necrosis and apoptosis foci in the red arrowed hippocampal region. Cerebral cortex also had apoptotic neurons. The donepezil control group had normal cerebral cortex morphology, but frontal gliosis (red arrow). DISE-Low-dose treated mice had significant hippocampal neuron hyperplasia, while the cerebral cortex was normal. DISE-high-dose treated animals had normal frontal cortex, but prefrontal cortex gliosis (red arrow).

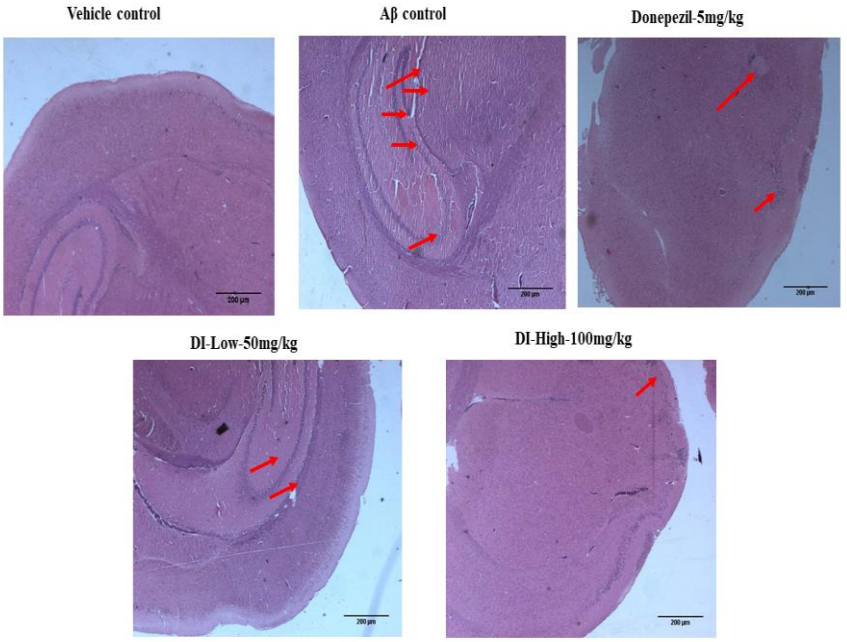

Fig. 6: Effect of DISE treatment on histology in Aβ-induced brain Alzheimer's disease. Representative photomicrograph of brain tissues stained with H&E.

DISE extract effects on the proinflammatory cytokines in Aβ-induced AD mice

Preceding studies have associated the secretion of pro-inflammatory cytokines such as TNF-α, IL-6 and anti-inflammatory cytokine like IL-10 with AD. The ELISA technique was used to assess the concentrations of TNF-α, IL-6 and IL-10 in brain tissues. According to Fig. 7, when compared to the VC group, the TNF-α and IL-6 pro-inflammatory cytokine quantities in the Aβ-induced group were significantly ($p<0.001$) higher. The DISE-treated group at both the dosages showed a significant reduction in the dose-dependent way, as well as the mice that received donepezil also had noticeably lower levels of TNF-α and IL-6 ($p<0.01$). When compared to the animals in the VC group, the mice in the Aβ induced group had substantially lower levels of IL-10. However, dose-dependently

administered DISE was able to counter these reductions. Additionally, reinstated in the donepezil-treated mice was the quantities of IL-10 ($p<0.01$) Fig.7.

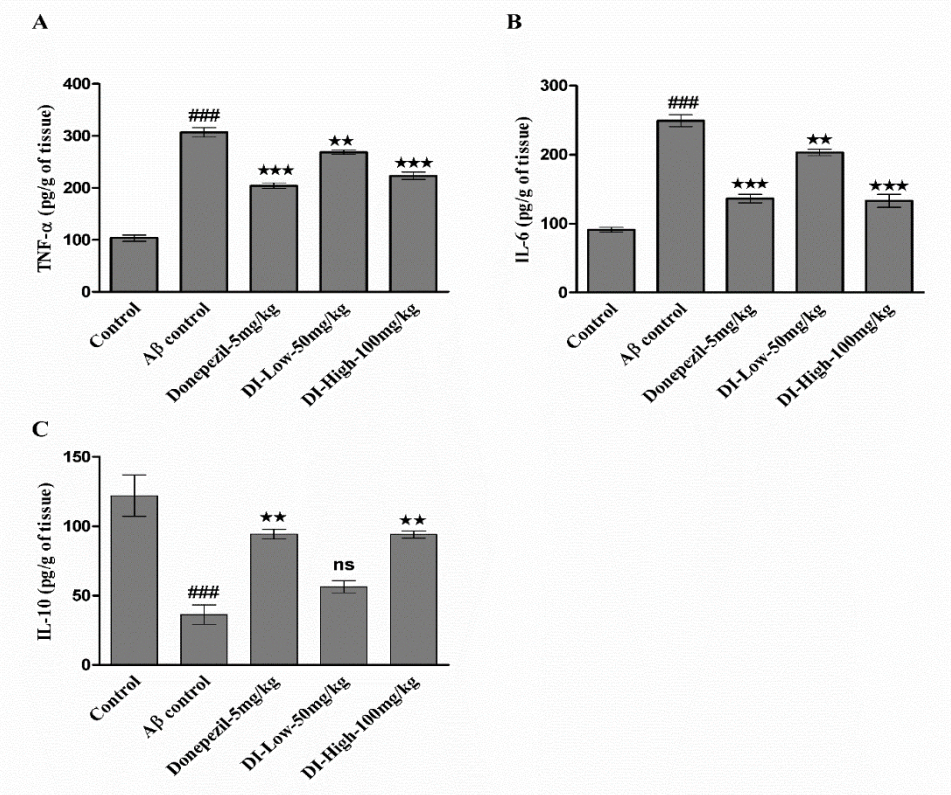

Fig. 7: Effect of DISE on proinflammatory cytokine in Aβ induced brain tissue. Proinflammatory cytokines (A) TNF-α and (B) IL-6 (C) IL-10.

Discussion

Neurodegenerative diseases are a major concern for the elderly in developed nations, both working and retired. Neurodegenerative illnesses are frequently caused by oxidative stress in today's lifestyle(25). Oxidative stress contributes to neurodegenerative diseases by increasing ROS and RNS. These compounds damage proteins, lipids, and nucleic acids(26). Because of their high oxygen consumption, poor antioxidant capacity, iron content, and fatty acid content, brain tissues are particularly vulnerable to oxidative stress(27).

Extreme oxidative stress on the hippocampus and amygdala can impair hippocampal synaptic plasticity, causing memory deficits(28, 29). According to reports, phenolics, flavonoids, steroids, alkaloids, saponins, and terpenoids in plants have medical uses(30). Phenolic chemicals can delay AD's onset and progression(31). Alzheimer's impairs thinking, behaviour, and memory (2). This study focused on DISE extract's ability to restore memory deficits because DISE is associated with behavioural and memory problems. Aβ-induced mice showed cognitive impairment and pathological changes similar to AD(32). DISE extract

was tested in an Aβ-induced AD mouse model. Using the MWM and EPM, the effects of DISE on an Aβ-induced AD mouse model's memory and learning were evaluated. In this study, $A\beta_{25-35}$ was injected intravenously to create neurotoxicity and evaluate DISE's cognitive effects. After Aβ-induction, behavioural and biochemical measures declined, which DISE treatment reversed. Step-down inhibitory avoidance, water maze spatial learning, and open field exploring for habituation memory were used in the behavioural study. Behaviour improved after DISE treatment, suggesting a neuroprotective effect. AD and other neurodegenerative disorders damage the hippocampus and its afferent neural system. Cholinergic neuronal cell grafting improved hippocampal cognition and spatial memory. Increased muscarinic receptors affect learning and behaviour, supporting this(33). The amount of AChE in the parasympathetic nuclei, globus pallidum, and dentate gyrus of the hippocampus is related to spatial working memory on water mazes. Higher AChE levels reduce water maze spatial working memory recall(34). DISE extract reduced Aβ-induced memory impairment by reducing water maze escape latency. This decrease in water maze evaluation time illustrates the anti-amnesiac effect of behavioural parameters and intensifies hippocampus memory. AD cortex and hippocampus contain more lipids, proteins, and nucleic acid oxidation products than normal(35). According to reports, AD patients have lower levels of GPx, SOD, and CAT, antioxidants that neutralise free radicals(36). SOD, CAT, GSH, and GST levels increased in this study, indicating better brain antioxidant activity. DISE reduced Aβ-induced neurotoxicity in mice, which is linked to its antioxidant properties that benefit AD cognition. DISE may also restore the histopathological architecture of the cerebral cortex and hippocampus and treat Aβ-induced neuro damage. Aβ-induced AD mice had tiny necrosis and apoptosis foci in the hippocampal region and cerebral cortex. The role of neuroinflammation and the immunological effects of DISE were also studied by inhibiting pro-inflammatory cytokines TNF-A, IL-6, and IL-10 in the brain after Aβ-induced AD. DISE treatment reduced IL-6 and TNF- while restoring IL-10, suggesting that it may treat via the neuro-immune-inflammatory system. Learning and memory are affected by the brain's biogenic amine turnover. Efferent vagus nerve activates CNS acetylcholine-secreting neurons, which connect with macrophage nicotinic acetylcholine receptors to inhibit cytokine release and control inflammation(37). According to this study, pro-inflammatory cytokines regulate excitatory neurotransmission and the anti-amnesic effect by inhibiting cholinesterase and preventing Aβ aggregation. This suggests DISE could treat Aβ-induced AD. Which could reduce proinflammatory and anti-inflammatory cytokines.

Conclusion

In this investigation, DISE extract demonstrated an improvement in learning and memory in a dose-dependent manner. According to this research, Derris indica seed aqueous methanolic extract may be effective in treating Alzheimer's and other neurological diseases. The traditional usage of Derris indica for problems of the nervous system or the memory is also supported by this investigation. The results of the Alzheimer's study on behavioural markers revealed right away that DISE therapy increased hippocampal memory. The activation of antioxidant enzymes and regulation of free radical scavenging and decreased pro-inflammatory cytokines prevents oxidative stress, lipid peroxidation, and nitric

oxide stress, demonstrating anti-Alzheimer's activity. These DISE could be a useful source of therapeutic ingredients in order to control neurodegenerative diseases.

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References

1. Alavala S, Nalban N, Sangaraju R, Kuncha M, Jerald MK, Kilari EK, et al. Anti-inflammatory effect of stevioside abates Freund's complete adjuvant (FCA)-induced adjuvant arthritis in rats. Inflammopharmacology. 2020;28(6):1579-97.
2. Arshad N, Rashid N, Absar S, Abbasi MS, Saleem S, Mirza B. UV-absorption studies of interaction of karanjin and karanjachromene with ds. DNA: Evaluation of binding and antioxidant activity. Central European Journal of Chemistry. 2013;11(12):2040-7.
3. Belagihally SM, Rajashekhar S, Jayaram VB, Dharmesh SM, Thirumakudalu SKC. Gastroprotective properties of karanjin from Karanja (Pongamia pinnata) seeds; Role as antioxidant and H+, K+-ATPase inhibitor. Evidence-Based Complementary and Alternative Medicine. 2011;2011.
4. Beuscher L, Beck C. A literature review of spirituality in coping with early-stage Alzheimer's disease. Journal of clinical nursing. 2008;17(5a):88-97.
5. Bose M, Chakraborty M, Bhattacharya S, Mukherjee D, Mandal S, Mishra R. Prevention of arthritis markers in experimental animal and inflammation signalling in macrophage by karanjin isolated from Pongamia pinnata seed extract. Phytotherapy Research. 2014;28(8):1188-95.
6. Bubser M, Byun N, Wood MR, Jones CK. Muscarinic receptor pharmacology and circuitry for the modulation of cognition. Muscarinic receptors. 2012:121-66.
7. Candelario-Jalil E, Al-Dalain SM, Castillo R, Martínez G, León Fernández OS. Selective vulnerability to kainate-induced oxidative damage in different rat brain regions. Journal of Applied Toxicology: An International Journal. 2001;21(5):403-7.
8. Canevelli M, Lacorte E, Cova I, Zaccaria V, Valletta M, Raganato R, et al. Estimating dementia cases amongst migrants living in Europe. European Journal of Neurology. 2019;26(9):1191-9.
9. Cheignon Cm, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox biology. 2018;14:450-64.
10. Chellammal HSJ, Veerachamy A, Ramachandran D, Gummadi SB, Manan MM, Yellu NR. Neuroprotective effects of 1δ-1-acetoxyeugenol acetate on Aβ (25-35) induced cognitive dysfunction in mice. Biomedicine & Pharmacotherapy. 2019;109:1454-61.

11. Dong H, Csernansky CA, Martin MV, Bertchume A, Vallera D, Csernansky JG. Acetylcholinesterase inhibitors ameliorate behavioral deficits in the Tg2576 mouse model of Alzheimer's disease. Psychopharmacology. 2005;181(1):145-52.
12. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959;82(1):70-7.
13. Ghosh A, Mandal S, Banerji A, Kar M, Banerji J. A new chalcone from Pongamia pinnata and its antioxidant properties. Natural Product Communications. 2009;4(2):1934578X0900400207.
14. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-Transferases: THE FIRST ENZYMATIC STEP IN MERCAPTURIC ACID FORMATION. Journal of Biological Chemistry. 1974;249(22):7130-9.
15. Halliwell B. Reactive oxygen species and the central nervous system. Journal of neurochemistry. 1992;59(5):1609-23.
16. Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. Neurology. 2013;80(19):1778-83.
17. Ismail MS, Brand C, Martin K. Benefits of early Pharmacological treatment in Alzheimer Disease. Psychiatric Times. 2007;24(4):49-.
18. Katekhaye S, Kale M, Laddha K. A simple and improved method for isolation of karanjin from Pongamia pinnata Linn. seed oil. 2012.
19. Kumar GP, Anilakumar K, Naveen S. Phytochemicals Having Neuroprotective Properties from Dietary Sources and Medicinal Herbs. Pharmacognosy Journal. 2015;7(1).
20. Manigauha A, Patel S, Monga J, Ali H. Evaluation of anticonvulsant activity of Pongamia pinnata Linn in experimental animals. Int J Pharm Tech Res. 2009;4:1119-21.
21. Marynets, N., Marynchenko, H., Vynnychuk, R., Voloshchuk, H., & Voropayeva, T. (2022). Education of humanitarian specialists during COVID-19 pandemic. *International Journal of Health Sciences*, *6*(1), 244–254. https://doi.org/10.53730/ijhs.v6n1.3907
22. Maurice T, Lockhart BP, Privat A. Amnesia induced in mice by centrally administered β-amyloid peptides involves cholinergic dysfunction. Brain research. 1996;706(2):181-93.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8.
24. Osborn GG, Saunders AV. Current treatments for patients with Alzheimer disease. Journal of Osteopathic Medicine. 2010;110(s98):16-26.
25. Pasinetti G, editor Role of polyphenols in promotion of healthy brain aging and Alzheimer's disease preventative initiatives. ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY; 2016: AMER CHEMICAL SOC 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
26. Peterson DM, Emmons CL, Hibbs AH. Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. Journal of Cereal Science. 2001;33(1):97-103.
27. Prince MJ, Wimo A, Guerchet MM, Ali GC, Wu Y-T, Prina M. World Alzheimer Report 2015-The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends. 2015.
28. Ratnawati, I. G. A. A., Sutapa, G. N., & Ratini, N. N. (2018). The concentration of radon gas in air-conditioned indoor: Air quality can increase

the potential of lung cancer. *International Journal of Physical Sciences and Engineering*, *2*(2), 111–119. https://doi.org/10.29332/ijpse.v2n2.169
29. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 1999;26(9-10):1231-7.
30. Rosas-Ballina M, Tracey K. Cholinergic control of inflammation. Journal of internal medicine. 2009;265(6):663-79.
31. Schneider LS, Insel PS, Weiner MW, Initiative AsDN. Treatment with cholinesterase inhibitors and memantine of patients in the Alzheimer's Disease Neuroimaging Initiative. Archives of neurology. 2011;68(1):58-66.
32. Serrano F, Klann E. Reactive oxygen species and synaptic plasticity in the aging hippocampus. Ageing research reviews. 2004;3(4):431-43.
33. Sharma A, Parikh V, Singh M. Pharmacological basis of drug therapy of Alzheimer's disease. Indian journal of experimental biology. 1997;35(11):1146-55.
34. Sikarwar MS, Patil M. Antidiabetic activity of Pongamia pinnata leaf extracts in alloxan-induced diabetic rats. International journal of Ayurveda research. 2010;1(4):199.
35. Singleton V, Orthofer R, Lamuela-Raventós R. Analysis of total phenols and 569 other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in 570 Enzymology, 299 (1974), 152–178. 1998.
36. Smith M. Rott amp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochim Biophys Acta. 2000;1502(1):139-44.
37. Sribuhoma T, Saraphona C, Decharchoocharta P, Boonyaratb C, Yenjai C. Acetylcholinesterase inhibition and cytotoxicity of flavonoids and chalcones from Derris indica. ScienceAsia. 2016;42(4):247-51.
38. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, *5*(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937
39. Vural H, Demirin H, Kara Y, Eren I, Delibas N. Alterations of plasma magnesium, copper, zinc, iron and selenium concentrations and some related erythrocyte antioxidant enzyme activities in patients with Alzheimer's disease. Journal of Trace Elements in Medicine and Biology. 2010;24(3):169-73.
40. Xing Z, He Z, Wang S, Yan Y, Zhu H, Gao Y, et al. Ameliorative effects and possible molecular mechanisms of action of fibrauretine from Fibraurea recisa Pierre on d-galactose/AlCl 3-mediated Alzheimer's disease. Rsc Adv. 2018;8(55):31646-57.