RESEARCH PAPER

Comparative In vivo Assessment of Three Natural Compounds for Cognitive and Neuroprotective Efficacy in Alzheimer's Disease

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ABSTRACT

Background: Alzheimer's disease is a neurological disorder that progressively worsens over time and currently has no effective treatments.

Methods: In Swiss albino rats with scopolamine-induced cognitive impairment, the neuroprotective effectiveness of Zingerone, Picroside I, and Cadambine was assessed using behavioural, biochemical, and histological evaluations. OECD-423 criteria were used to evaluate acute oral toxicity.

Results: Each substance considerably enhanced cognitive function and restored oxidative stress indicators. Picroside I showed the highest efficacy, followed by Zingerone and Cadambine. There was no toxicity up to 300 mg/kg.

Conclusion: The investigated natural chemicals show strong neuroprotective effect, which lends credence to their potential as AD multi-target therapy options.

Keywords: Neuroprotection, Alzheimer's Disease, Picroside I, Cadambine, Zingerone, Oxidative stress.

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1. Introduction

Alzheimer's disease is the leading neurodegenerative condition and the predominant cause of dementia globally, with its prevalence rising steeply with advancing age. The neuropathological features of this degenerative disease include extracellular Amyloid-β, accumulation of intracellular tau neurofibrillary tangles and ongoing neuroinflammatory processes, which leads to synaptic dysfunction and neuronal loss. Amyloid plaques are created when beta and gamma secretases sequentially cleave the amyloid precursor protein. Though plaque burden in the brain is not linked to synapse loss or the advancement of dementia, dense plaques that stain with amyloid binding dyes like congo red and thioflavin S are linked to changes in the local neuropil, including substantial synapse loss. Rather, the work of numerous groups suggests that soluble forms of Aβ are harmful to cognition and poisonous to synapses. Soluble, oligomeric Aβ plays a significant part in synaptic degeneration. The development of truly disease-modifying treatments has been impeded by the intricate and multifaceted nature of AD pathophysiology, despite a great deal of study. Alternative therapy options are necessary because present treatment modalities, including newly licensed anti β amyloid immunotherapies, show poor clinical success and considerable adverse consequences [1,2]. Currently, acetylcholinesterase inhibitors for enhancing cognitive function and N-methyl-D-aspartate (NMDA) antagonists like memantine are the main clinical treatments for AD; however, these medications only decrease patients' symptoms and are unable to treat AD in its entirety. Therefore, it has become indispensable and critical to look for ways to lessen the escalation of these difficulties [3].

In this context, bioactive chemicals originating from plants provide a multi-targeted strategy because of their anti-inflammatory, antioxidant, anti-amyloidogenic, and neuroprotective properties. It has been shown that natural phenolic and antioxidant substances function in several

ways, reducing inflammation and general cognitive impairments, along with Aβ levels and oxidative stress. Numerous plant extracts and the active ingredients they contain have demonstrated a range of therapeutic pharmacological qualities as an AD anti-inflammatory therapy. These substances show promise as disease-modifying agents, particularly if given early in the course of the illness [4]. In contrast to the single-target action of the majority of medications currently being utilised to treat Alzheimer's disease, natural chemicals provide the advantage of a multitarget strategy, tagging many molecular locations in the human brain, since Alzheimer's disease is a multifactorial disease. By blocking pro-inflammatory NF-kB pathways and cytokines (TNF-α, IL-1β, and IL-6) to stop neuronal death, plant-based bioactives offer a multi-targeted approach. As strong antioxidants, phytonutrients protect mitochondrial ATP synthesis from oxidative stress, which would otherwise hasten synaptic loss. In order to reverse established neuropathological damage, natural extracts also promote neuroplasticity and nerve cell regeneration. This "green therapy" aims to revitalise biological processes with noticeably fewer side effects, making it a viable, disease-modifying substitute for symptomatic medications [5].

Neolamarckia cadamba, Picrorhiza kurroa, and Curcuma aromatica are three medicinal plants. Their neuroprotective qualities and rich phytochemical makeup have increased interest in them as potential Alzheimer's disease therapy agents. *C. aromatica* is referred to as "vanaharidra" in Ayurveda, wild turmeric in English. It is frequently utilised as a colouring and flavouring agent, in addition to its application in various traditional medicines across Southeast Asian nations [6]. Curcuminoids, Alkaloids, tannins, terpenoids and flavonoids found in C. aromatica's rhizomes are responsible for its purported antitussive, anti-inflammatory, antioxidant, wound-healing, antidiabetic, and neuroprotective qualities [7]. The main bioactive component, curcumin, has been found to control key inflammatory cytokines such as IL-6, TNF-α, and IL-1β, which contribute to neurodegenerative processes, and to have anti-inflammatory, antioxidant and anti-protein aggregation actions [8]. Curcumin has been reported to lower Aβ oligomer along with fibril production, downregulate Aβ-induced inflammation, prevent Aβ's neurotoxicity in the brain, and significantly lower IL-1β and iNOS levels in transgenic mice brains [9]. Natural plant-based chemicals have substantial therapeutic and medicinal potential, especially when a mixture of elements rather than a single isolated product yields the intended pharmacological effect. Picroliv, a glucoside combination made from the roots and rhizomes of the endangered herbal plant *P. kurroa*, is used extensively in Indian herbal preparations [10]. Numerous bioactive components, including as phenolic compounds, cucurbitacins, and iridoid glycosides like Picroside I and II, have been found by phytochemical studies of P. kurroa. The conservation status of P. kurroa has been endangered in a number of areas due to its widespread therapeutic usage, underscoring the necessity of sustainable use techniques [11]. The therapeutic effects of PK in AD might involve the inhibition of NLRP3 inflammasome-mediated microglia activation and reduction of the BACE1 expression. The twin effects of PK in inhibiting APP processing and NLRP3 inflammasome signals result in alleviating cognitive deficits [12].

The *cadamba Neolamarckia* Syn. (Family: Rubiaceae), often known as kadamba, holds a sacred place in Ayurveda, an Indian traditional medical system. Another name for it is Kadam [13]. Neolamarckia cadamba, member belonging to the Rubiaceae family, is cultivated because of its intrinsic economic worth and pharmacological properties in a number of ways. It is found throughout South China and Southeast Asia, and is a rapidly-growing tree species with significant commercial value [14]. The phytochemicals known as indole alkaloids, saponins, terpenes, lipids, steroids, reducing sugars, etc. are commonly found in many varieties of Neolamarckia indicus [15] .The cadama bark also contains an astringent and tannins smell, which may be ascribed to the presence of an acid resembling cincho-tannic acid. Quinovic acid, sitosterol, and cadambogenic acid can all be isolated from the stem bark of *Neolamarckia cadamba*. Numerous researchers have examined the steroidal and alkaloidal components of Neolamarckia indicus dry stem bark for their possible medical use. Isomers of dihydrocadambine and isodihydrocadambine, as well as cadambine, are found in the heartwood and leaves of this tree. The aerial regions of the tree are beneficial because of the aforementioned compounds [16,17]. The plant's polyphenolic components, including catechins and chlorogenic acid, are primarily responsible for its purported antioxidant, anti-inflammatory, antibacterial, hepatoprotective, and central nervous system stimulating properties [18].

These plants have pharmacological and traditional significance, but little is known about their

neuroprotective potential in AD. This work therefore attempts to examine and contrast the phytochemical, antioxidant, and neuroprotective profiles *of Picrorhiza kurroa, Neolamarckia cadamba*, and *Curcuma aromatica* as possible anti-Alzheimer's disease therapeutic agents.

2. Materials and Methods

2.1 Plant Material and Authentication

Fresh rhizomes of Curcuma aromatica (authentication number: NIScPR/RHMD/Consult/2023/4311-12-2), Picrorhiza kurroa roots (authentication number: NIScPR/RHMD/Consult/2023/4311-12-3), and Neolamarckia cadamba leaves (authentication number: NIScPR/RHMD/Consult/2023/4311-12-1) were gathered from the Raw Material Herbarium and Museum Department, a recognized national research institute. Microscopic and morphological methods were used for taxonomic authentication. The Indian Pharmacopoeia (2022 edition) was consulted in order to cross-check morphological characteristics including colour, texture, and odour. Transverse slices were stained with phloroglucinol-HCl for lignified tissues and iodine solution for starch granules for microscopic investigation. The sections were then seen at 40× magnification using a Leica DM500 microscope. Following purification by column chromatography, the recovered chemicals (Zingerone, Picroside I, and Cadambine) were characterised using spectroscopic techniques (^{1}H NMR and ^{13}C NMR).

2.2 Experimental Animals and Ethical Approval

Adult male Swiss albino rats (200–250 g) were acclimatized for 7 days under standard laboratory conditions with free access to standard pellet diet and water. All procedures adhered to CPCSEA guidelines and were approved by the Institutional Animal Ethics Committee**.** All experimental procedures were conducted in accordance with CPCSEA guidelines, Declaration of Helsinki and Good Clinical Practice guidelines.

2.3 Induction of Cognitive Impairment

Scopolamine hydrobromide was prepared in 0.9% saline and delivered via intraperitoneally (1 mg/kg body weight) to induce memory deficits.

2.4 Acute Oral Toxicity (OECD-423)

Plant extracts were given orally to Swiss albino rats (200–250 g) at dosages of 5, 50, 300, and 2000 mg/kg (n = 3 per dose) after the animals were starved overnight. The OECD technique was used to evaluate acute oral toxicity in order to derive GHS classification and LD_{50} cut-off values. Testing was stopped when two or more animals became moribund or died at a certain dose, validating the toxicity class. Animals were monitored sequentially [19].

2.5 Histopathological studies

Using an automated tissue processor, liver and kidney samples meant for histological analysis were dehydrated, cleared, and impregnated overnight. The specimens were embedded in paraffin blocks using an embedding station. A microtome was used to cut serial sections of 5 µm thickness, and hematoxylin and eosin was used to stain them. The sections were examined under a light microscope after drying, and a digital camera was used to capture images [20].

2.6 Behavioral Assessments

2.6.1 Morris Water Maze:

The Morris Water Maze was used to assess spatial reference memory in which rats were trained to locate a concealed circular platform in an opaque water pool with a diameter of 110 cm. The pool was divided into four quadrants based on the location of the platform. A triangular flag was used to indicate cued training for three days, and then the flag was removed for five days of acquisition training. Rats were exposed to the water in different positions throughout each session, and if they failed to locate the platform within a minute, they were led there. To evaluate the rats spatial memory without the platform, a probe trial was performed after acquisition training in which they swam from a new entry point. Throughout the trials, tracking software was employed to keep an eye on their swim habits and patterns [21].

2.6.2 Novel Object Recognition Test:

The device is a soundproof box with a 25 cm × 25 cm × 25 cm plexiglass box inside of which a video camera records rat behaviour. Four stages make up the experimental process: pre-habituation, habituation, training, and testing. Rat are acclimated for half an hour on the first day before spending five minutes exploring in

an empty box. The empty box must be habituated to for 20 minutes on the second and third days. Two identical objects are put in the box for ten minutes on the fourth day of the training trial. After that, one object is changed out for a new one during the testing trial. Although the objects are identical in size and shape, they are fastened to prevent movement; cleaning after trials removes scent clues. The amount of time the rat's nose is within two centimetres of an object is known as exploration time. As engagement measures, location preference and recognition index (RI) are calculated during the training session, with location preference kept at 50%. Analysis does not include rat that explore for less than three seconds throughout testing [22].
(DI), calculated as:

Discrimination Index = Time with novel − Time with familiar / Total exploration time.

2.6.3 Radial Arm Maze :

Eight-arm radial maze served as the apparatus for assessing behaviour had an octagonal central platform and eight radial arms, each of which held a feeding well. Rats were used in the experiment, which was carried out in a closed space with visual signals. The rats were given drug for three weeks and then instructed to abstain from food. Over the course of five days, the maze was familiarised with incentive pellets strewn throughout. Reference memory errors (RME), total errors and correct memory errors were used to measure memory function over the five weeks that each rat underwent two daily trials [23].

2.6.4 Y-Maze Test:

The plexiglas holding cage known as the Y-maze measured 40 cm (length), 30 cm (height), and 15 cm (breadth). For an observation time of eight minutes, rats were free to explore the maze. 40 dB of masking noise was produced by air circulation equipment running continuously. Alternation was characterized as overlapping triplet sets with sequential entry into the three arms. Calculating the ratio of actual to potential alternations yielded the alternation percentage [24].
Spontaneous Alternation (%) = [(Number of actual alternations) / (Total arm entries − 2)] × 100. Higher alternation percentage indicated better memory performance .

2.6.5 Passive Avoidance Test:

On the first day, each animal was given two minutes to get used to the shuttle box. The rats were given 0.6 mA foot shock for one second after entering the dark compartment on the 2nd and 3rd days after being put in the light compartment box. The process was the same as on the second day on the 4th and 5th days, with the exception of foot shock. After 120 seconds, the rats were deemed to have learnt if they did not move to the dark compartment in the third, fourth, and fifth trial sessions [21].

2.7 Biochemical Analysis

2.7.1 Tissue Preparation

Rats were beheaded, and thereafter, the entire brain was swiftly dissected, rinsed with isotonic saline, desiccated, and weighed. The sample was promptly homogenised to achieve 10% (w/v) homogenate in an ice-cold solution comprising 50 mmol/l Tris-HCl (pH 7.4) and 300 mmol/l sucrose. The homogenised tissue sample were centrifuged at 3000 rpm for 10 minutes. Biochemical examination was conducted subsequent to the separation of the supernatant. The procedures were conducted at 4°C [25].

2.7.2 MDA Assay

All of the chemicals used in the MDA assay, such as trichloroacetic acid (TCA; Cat. No. GRM1081) and thiobarbituric acid (TBA; Cat. No. RM276), were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and used in accordance with the manufacturer's instructions. This study assessed a spectrophotometric approach for the precise measurement of thiobarbituric acid reactive compounds (TBARS) with enhanced sensitivity. MDA is a recognised indicator of oxidative state inside a biological system [26]. Estimation of malondialdehyde, a marker of lipid peroxidation was conducted utilising the 2-thiobarbituric acid reaction technique. A 5% brain homogenate was produced in cooled 150 mM KCl, incubated aerobically at 37°C for 2 hours, and the lipid peroxidation was terminated with cold trichloroacetic acid. Samples at zero time were obtained for comparative analysis. Subsequent to centrifugation, the supernatant was treated with TBA, subjected to boiling, cooled, diluted, and the absorbance of the samples was quantified

at 535 nm with a spectrophotometer, with findings reported in nmol of MDA [27].

2.7.3 GSH Assay

GSH was determined using the following chemicals, all of which were purchased from Sigma-Aldrich: 5,5′-dithio-bis-2-nitrobenzoic acid (Sigma-Aldrich, Cat. No. D8130), EDTA (Sigma-Aldrich, Cat. No. E9884), NaCl (Sigma-Aldrich, Cat. No. S9888), Na_2HPO_4 (Sigma-Aldrich, Cat. No. S7907). 3.6 mL of double-distilled water (DDW) and 0.4 mL of homogenised brain tissue were combined for the test. 0.6 mL of precipitating reagent, which included the aforementioned compounds and was built up to 100 mL with DDW, was added to this. The mixture was centrifuged for ten minutes at 600 × g. Then, 0.25 mL of 0.4% DTNB in 1% sodium citrate and 2 mL of 0.3 M Na_2HPO_4 were added to 0.3 mL of the supernatant. After using DDW to adjust the final volume to 3 mL, the optical density was measured at 412 nm against a blank. GSH concentrations were measured in µg of reduced glutathione for every µg of protein. [28].

2.7.4 SOD Assay

Assay chemicals from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) included sodium pyrophosphate buffer (Cat. No. S6422), phenazine methosulfate (PMS; Cat. No. P9625), and NADH (Cat. No. N8129).The manufacturer's instructions were followed when performing the process. 0.1 ml of phenazine methosulphate (186 µmol), 1.2 ml of sodium pyrophosphate buffer (0.052 mmol; pH 7.0), and 0.3 ml of the supernatant following homogenate centrifugation (1500 × g for 10 min and 10,000 × g for 15 min) made up the reaction mixture for this procedure. 0.2 millilitres of NADH (780 µmol) were used to start the enzyme process, and one millilitre of glacial acetic acid was used to stop it after one minute. The amount of chromogen produced was determined by measuring the colour intensity at 560 nm. The results are expressed in units per milligramme of protein [29].

2.7.5 Catalase Assay

For the catalase test, Merck Life Sciences Pvt. Ltd. (India) provided the hydrogen peroxide ($H2O_2$, 30%; Cat. No. 107209) and phosphate buffer components (Cat. No. 106346), which were employed in compliance with standard manufacturer instructions. The Aebi method, which is based on H2O2 breakdown at 240 nm at room temperature, was used to quantify the catalase activity.Immediately before to the test, In 50 mM phosphate buffer (pH 7), each sample was diluted to 1: 500. To start the reaction, 2 ml of the diluted sample and 60 mM H2O2 were added to the reaction mixture, which had a total volume of 3 ml. With the exception of the buffer and sample, the blank was empty. Catalase activity was determined using a first-order process' rate constant (k) [30].

3. Results

3.1 Acute Oral Toxicity and Histopathological Evaluation

Acute oral toxicity studies of the isolated compounds—Zingerone, Picroside I, and Cadambine—were performed in Swiss albino rat as per OECD guidelines. No mortality or severe clinical signs were observed up to the maximum tested dose of 2000
mg/kg. At the 300 mg/kg dose (10× the therapeutic dose), animals displayed only transient lethargy, which resolved spontaneously within a few hours. Body weight changes remained within normal physiological limits, and relative organ weights (liver and
kidneys) showed no significant deviations, suggesting the absence of systemic toxicity (Table 1). Figures 1 and 2 display representative histological images of liver and kidney sections after 2000 mg/kg was administered.

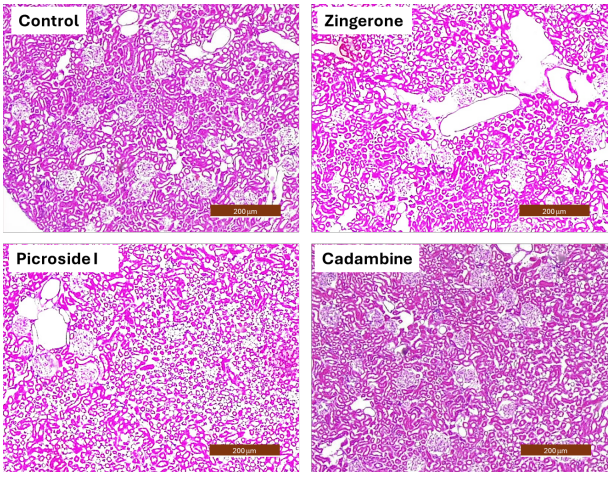

Figure 1 Following acute oral administration of the test medications at 2000 mg/kg, typical photomicrographs of kidney sections stained with haematoxylin and eosin from

treated and control rats show normal renal architecture (magnification: 5×).

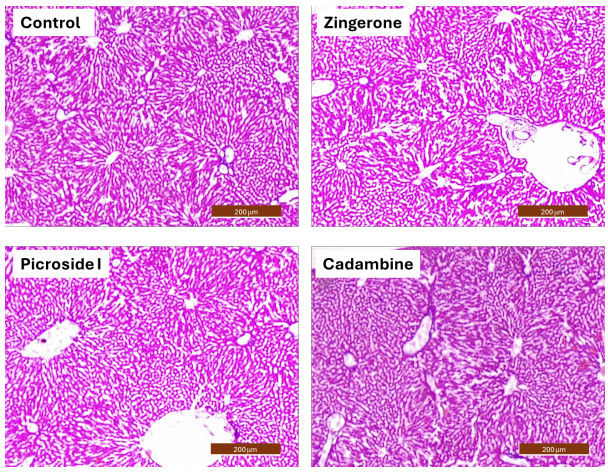

Figure 2 Following acute oral administration of the test medicines at 2000 mg/kg, typical photomicrographs of liver sections stained with haematoxylin and eosin from control and treated rats show preserved hepatic architecture (magnification: 5×).

Compound	*Dose (mg/kg)*	*Mortality (%)*	*Clinical Signs*	*Body Weight Change (%)*	*Relative Organ Weight (% of Body Weight)*
Cadambine	*5*	*0*	*None*	*+2.0 ± 0.4*	*Liver: 4.1 ± 0.2; Kidneys: 1.5 ± 0.1*
	50	*0*	*None*	*+1.9 ± 0.3*	*Liver: 4.4 ± 0.3; Kidneys: 1.6 ± 0.1*
	300	*0*	*Transient lethargy*	*+1.6 ± 0.3*	*Liver: 4.3 ± 0.2; Kidneys: 1.7 ± 0.2*
	2000	*0*	*None*	*+1.3 ± 0.2*	*Liver: 4.2 ± 0.3; Kidneys: 1.6 ± 0.1*
Picroside I	*5*	*0*	*None*	*+2.0 ± 0.4*	*Liver: 4.2 ± 0.2; Kidneys: 1.5 ± 0.1*
	50	*0*	*None*	*+1.9 ± 0.3*	*Liver: 4.5 ± 0.3; Kidneys: 1.6 ± 0.1*
	300	*0*	*Transient lethargy*	*+1.6 ± 0.3*	*Liver: 4.1 ± 0.2; Kidneys: 1.7 ± 0.2*
	2000	*0*	*None*	*+1.3 ± 0.2*	*Liver: 4.4 ± 0.3; Kidneys: 1.6 ± 0.1*
Zingerone	*5*	*0*	*None*	*+2.0 ± 0.4*	*Liver: 4.1 ± 0.2; Kidneys: 1.5 ± 0.1*

	50	***0***	***None***	***+1.9 ± 0.3***	***Liver: 4.6 ± 0.3; Kidneys: 1.6 ± 0.1***
	300	***0***	***Transient lethargy***	***+1.6 ± 0.3***	***Liver: 4.4 ± 0.2; Kidneys: 1.7 ± 0.2***
	2000	***0***	***None***	***+1.3 ± 0.2***	***Liver: 4.3 ± 0.3; Kidneys: 1.6 ± 0.1***

3.2 Behavioral Assessments

3.2.1 Morris Water Maze (MWM) Performance

When compared to the normal control group, scopolamine administration significantly impaired spatial memory as seen by higher escape latency and less time spent in the target quadrant. Treatment with Zingerone, Picroside I, and Cadambine significantly reduced escape latency and lengthened target quadrant time in comparison to the disease control group. Of all the medications under investigation, Picroside I showed the greatest improvement and was comparable to donepezil. (Figure 3)

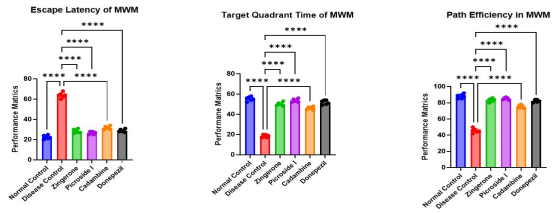

*Figure 3 shows the effect of Zingerone, Picroside I, and Cadambine affect memory and spatial learning in the Morris Water Maze test in scopolamine-induced cognitive impairment. The mean ± SEM (n = 6) is the data expression. One-way ANOVA and Dunnett's multiple comparisons test were used in the statistical study. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 in relation to the disease control group.*

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3.2.2 Novel Object Recognition Test

Reduced exploration of the novel object and a negative discriminating index were signs of decreased recognition memory in the illness control group. When compared to the illness control group, treatment with the separated chemicals greatly enhanced the discriminating index and novel object exploration. Among the treated groups, Picroside I caused the most noticeable improvement (Figure 4).

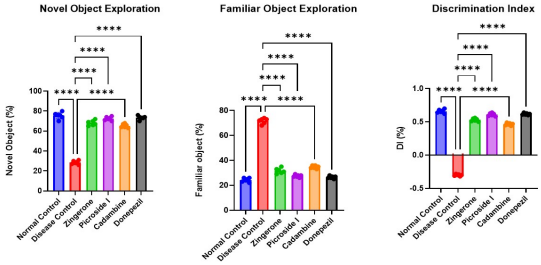

*Figure 4 shows the effects of zingerone, picroside I, and cadambine on recognition memory in rats given scopolamine as measured by the Novel Object Recognition test. The data are displayed as mean ± SEM (n = 6). Dunnett's multiple comparisons test was used after a one-way ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 in comparison to disease control.*

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3.2.3 Radial Arm Maze

Scopolamine-induced cognitive impairment was demonstrated by a decrease of correct arm entries and a marked increase in total and reference memory errors. When compared to the disease control group, the administration of Zingerone, Picroside I, and Cadambine dramatically reduced memory errors and boosted correct arm entries. Picroside I showed the biggest gains in reference and working memory metrics (Figure 5).

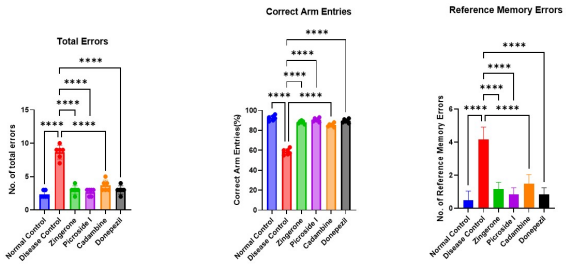

*Figure 5: Zingerone, Picroside I, and Cadambine's effects on working and reference memory parameters in rats given scopolamine during the Radial Arm Maze test. The values are given as mean ± SEM (n = 6). One-way ANOVA and Dunnett's post-hoc test were used for statistical comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 in comparison to disease control.*

3.2.4 Y-Maze

Spontaneous alternation % was considerably reduced in the disease control group of rats, suggesting that their working memory was compromised. In comparison to the disease control group, treatment with the isolated chemicals dramatically improved spontaneous alternation; Picroside I demonstrated the greatest improvement (Figure 6).

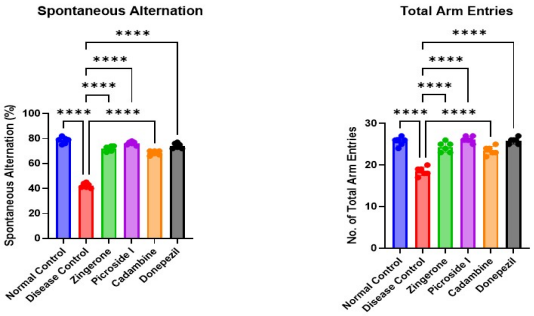

*Figure 6: In scopolamine-induced cognitive impairment, the effects of zingerone, picroside I, and cadambine on spontaneous alternation behaviour in the Y-maze test. The mean ± SEM (n = 6) is the resultant expression. Dunnett's multiple comparisons test was employed after a one-way ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 in comparison to the illness control.*

3.2.5 Passive Avoidance Test

Rats given scopolamine demonstrated a marked reduction in step-through latency, which is indicative of poor retention memory. In comparison to the control disease group, treatment with Zingerone, Picroside I, and Cadambine considerably lengthened the latency period. Among the substances tested, picroside I showed the greatest delay enhancement (Figure 7).

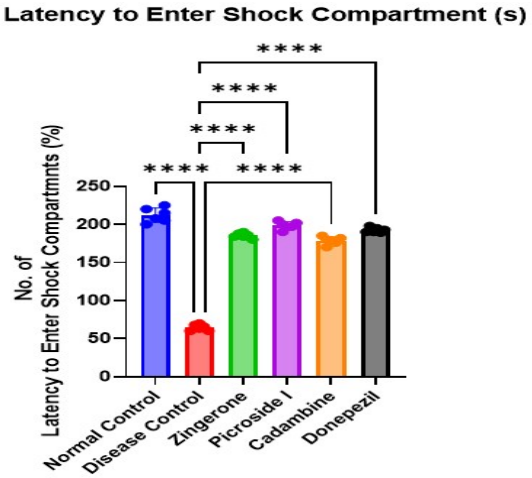

Figure 7: Impact of Cadambine, Picroside I, and Zingerone on step-through latency in the Passive Avoidance test in rats given scopolamine. Mean ± SEM is used to express the data (n = 6). One-way ANOVA and Dunnett's multiple comparisons test were used for the statistical analysis. In comparison to disease control, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

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3.3 Biochemical analysis

3.3.1 Oxidative Stress Markers

In rat brain homogenates, scopolamine treatment significant increase in malondialdehyde levels and decreased glutathione, superoxide dismutase, and catalase activity.In comparison to the disease control group, treatment with Zingerone, Picroside I, and Cadambine dramatically decreased MDA levels and restored antioxidant enzyme activity. Picroside I demonstrated the highest degree of oxidative stress parameter normalisation among the treatment groups (Figure 8).

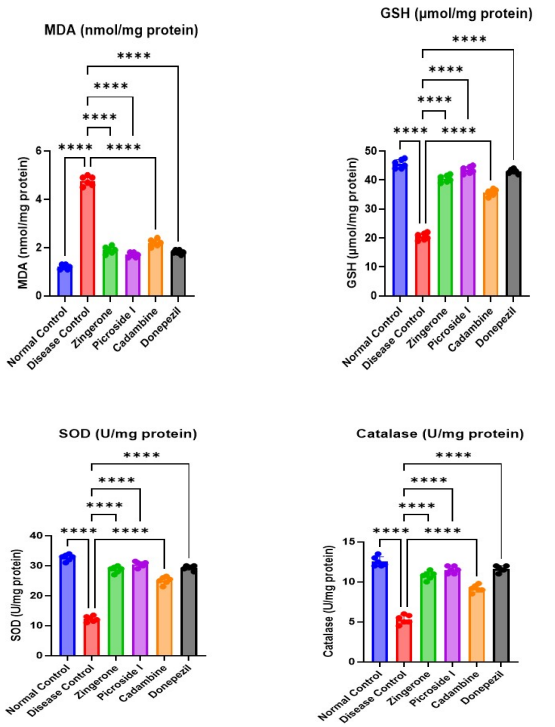

Figure 8: Zingerone, Picroside I, and Cadambine's effects on oxidative stress indicators in brain homogenates of rats given scopolamine. Superoxide dismutase, reduced glutathione, malondialdehyde, and catalase were measured. The results are shown as mean ± SEM (n = 6). For statistical analysis, a one-way ANOVA and Dunnett's multiple comparisons test were employed. In comparison to disease control, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

3.4 Statistical Analysis

The data is expressed using the mean ± standard error of the mean (SEM), with six animals in each group. The normality of the data distribution was assessed using the Shapiro-Wilk test, and the homogeneity of the variance was confirmed using Levene's test. Dunnett's multiple comparisons test was employed after statistical group comparisons using one-way analysis of variance (ANOVA). Statistical significance was defined as a difference of less than 0.05. All statistical analyses were conducted using GraphPad Prism (version 9.0).

4. Discussion

This study showed that in experimental models of Alzheimer's disease, zingerone, picroside I, and cadambine greatly reduced oxidative stress and cognitive impairment. Significant deficits in spatial, working, recognition, and associative memory were caused by the administration of scopolamine and amyloid-β. These deficits were reliably alleviated after treatment with the investigated substances across a variety of behavioural paradigms.

Significant alterations in oxidative stress indicators in brain tissue coincided with behavioural recovery. The disease control animals' elevated lipid peroxidation and decreased antioxidant enzyme activity were considerably reversed in the treatment groups, suggesting that the redox equilibrium had been restored. Picroside I outperformed Zingerone and Cadambine in terms of both cognitive function and antioxidant status among the three substances.

Histopathological findings, which demonstrated tissue architecture preservation in treated mice when compared to disease controls, provided additional evidence for the compounds' neuroprotective properties. A favourable safety profile was also demonstrated by the acute oral toxicity study, which verified that all three drugs were well tolerated with no mortality or significant side effects.

Overall, these results show that Zingerone, Picroside I, and Cadambine have neuroprotective potential in vivo, with Picroside I showing the best efficacy of the substances examined.

5. Conclusion

In experimental models of Alzheimer's disease, the current study shows that Zingerone, Picroside I, and Cadambine considerably enhance cognitive function and lower oxidative stress. Picroside I was the most effective of the investigated substances in terms of behavioural, biochemical, and histological evaluations; Zingerone and Cadambine were next in line. In acute toxicity testing, every chemical was well tolerated, suggesting a good safety profile. These results corroborate the natural chemicals' potential for neuroprotection in Alzheimer's disease.

References

1. Zhang J, Kong G, Yang J, Pang L, Li X. Pathological mechanisms and treatment progression of Alzheimer's disease. *Eur J Med*

Res. 2025;30(1). doi:10.1186/s40001-025-02886-9

2. Crimins JL, Pooler A, Polydoro M, Luebke JI, Spires-Jones TL. The intersection of amyloid beta and tau in glutamatergic synaptic dysfunction and collapse in Alzheimer's disease. *Ageing Res Rev*. 2013;12(3):757-763. doi:10.1016/j.arr.2013.03.002
3. Mo JJ, Li JY, Yang Z, Liu Z, Feng JS. Efficacy and safety of anti-amyloid-β immunotherapy for Alzheimer's disease: a systematic review and network meta-analysis. *Ann Clin Transl Neurol*. *Wiley-Blackwell*. 2017;4(12):931-942. doi:10.1002/acn3.469
4. Sharman MJ, Verdile G, Kirubakaran S, et al. Targeting Inflammatory Pathways in Alzheimer's Disease: A Focus on Natural Products and Phytomedicines. *CNS Drugs*. *Adis*. 2019;33(5):457-480. doi:10.1007/s40263-019-00619-1
5. Bhat BA, Almilaibary A, Mir RA, et al. Natural Therapeutics in Aid of Treating Alzheimer's Disease: A Green Gateway Toward Ending Quest for Treating Neurological Disorders. *Front Neurosci*. *Frontiers Media S.A.* 2022;16. doi:10.3389/fnins.2022.884345
6. Umar NM, ivam TP, Aminu N, Toh SM. Phytochemical and pharmacological properties of Curcuma aromatica Salisb (wild turmeric). *J Appl Pharm Sci*. 2020;10(10):180--194. doi:10.7324/JAPS.2020.1010018
7. Kanase V, Khan F. An overview of medicinal value of curcuma species. *Asian Journal of Pharmaceutical and Clinical Research*. *Innovare Academics Sciences Pvt. Ltd*. 2018;11(12):40-45. doi:10.22159/ajpcr.2018.v11i12.28145
8. Otsuka S, Kawamura M, Fujino S, et al. Coronarin D, a Metabolite from the Wild Turmeric, Curcuma aromatica, Promotes the Differentiation of Neural Stem Cells into Astrocytes. *J Agric Food Chem*. 2022;70(10):3300-3309. doi:10.1021/acs.jafc.2c00020
9. Liu ZJ, Li ZH, Liu L, et al. Curcumin attenuates beta-amyloid-induced neuroinflammation via activation of peroxisome proliferator-activated receptor-gamma function in a rat model of Alzheimer's disease. *Front Pharmacol*. 2016;7(AUG). doi:10.3389/fphar.2016.00261
10. Verma PC, Basu V, Gupta V, Saxena G, Rahman LU. *Pharmacology and Chemistry of a Potent Hepatoprotective Compound Picroliv Isolated from the Roots and Rhizomes of Picrorhiza Kurroa Royle Ex Benth. (Kutki)*. Vol 10. 2009.
11. Masood M. Picrorhiza kurroa: An ethnopharmacologically important plant species of Himalayan region. *Pure and Applied Biology*. 2015;4(3):407-417. doi:10.19045/bspab.2015.43017
12. Kim N, Do J, Ju IG, Jeon SH, Lee JK, Oh MS. Picrorhiza kurroa Prevents Memory Deficits by Inhibiting NLRP3 Inflammasome Activation and BACE1 Expression in 5xFAD Mice. *Neurotherapeutics*. 2020;17(1):189-199. doi:10.1007/s13311-019-00792-7
13. Dubey A, Nayak S, Goupale DC. A Review on Phytochemical, Pharmacological and toxicological studies on Neolamarckia cadamba. www.scholarsresearchlibrary.com
14. Huang T, Long J, Liu SW, et al. Selection and Validation of Reference Genes for mRNA Expression by Quantitative Real-Time PCR Analysis in Neolamarckia cadamba. *Sci Rep*. 2018;8(1). doi:10.1038/s41598-018-27633-5
15. Tairin I, Abhijit D, Kumar BS, Palash K, Shafiul I, Mohammad MS. Evaluation of membrane stabilizing, anthelmintic, antioxidant activity with phytochemical screening of methanolic extract of Neolamarckia cadamba fruits. *Journal of Medicinal Plants Research*. 2015;9(5):151-158. doi:10.5897/jmpr2014.5720
16. Singh M, Kumar P, Singh H, Kumar A, Kumar A, Kumar R. Neolamarckia cadamba: A Comprehensive review on its Physiological, Ecological, Phytochemical and Pharmacological Perspectives. *Ecology, Environment and Conservation*. 2023;29:241-250. doi:10.53550/eec.2023.v29i02s.042
17. Pandey A, Negi PS. Traditional uses, phytochemistry and pharmacological properties of Neolamarckia cadamba: A review. *J Ethnopharmacol*. *Elsevier Ireland Ltd*. 2016;181:118-135. doi:10.1016/j.jep.2016.01.036
18. Kareti SR, Subash P. In silico exploration of anti-Alzheimer's compounds present in methanolic extract of Neolamarckia cadamba bark using GC–MS/MS. *Arabian Journal of Chemistry*.

2020;13(7):6246-6255. doi:10.1016/j.arabjc.2020.05.035

19. Rameshwar GS, Venkatrao PU, Vithalrao NA, Pramodrao AM. OECD GUIDELINES FOR ACUTE ORAL TOXICITY STUDIES: AN OVERVIEW. *Int J Res Ayurveda Pharm*. 2023;14(4):137-140. doi:10.7897/2277-4343.1404130
20. Kadhim A, Ben Slima A, Alneamah G, Makni M. Assessment of Histopathological Alterations and Oxidative Stress in the Liver and Kidney of Male Rats following Exposure to Aluminum Chloride. *J Toxicol*. 2024;2024. doi:10.1155/2024/3997463
21. Taherianfard* M, Ahmadijokani S. The passive avoidance task ameliorate the toxic effects of bisphenol A on dopamine D1 receptor density in hippocampus, amygdala, and cerebellum of male rats. *Brain Behav*. 2023;13(4). doi:10.1002/brb3.2942
22. Zhang R, Xue G, Wang S, Zhang L, Shi C, Xie X. Novel object recognition as a facile behavior test for evaluating drug effects in AβPP/PS1 Alzheimer's disease mouse model. *Journal of Alzheimer's Disease*. 2012;31(4):801-812. doi:10.3233/JAD-2012-120151
23. Gamoh S, Hashimoto M, Hossain MdS, Sugioka K, Misawa Y, Masumura S. The effect of Docosahexaenoic acid (DHA) on radial maze performance in young rats. *Jpn J Pharmacol*. 1997;73:273. doi:10.1016/s0021-5198(19)45595-9
24. Nobakht M, Hoseini SM, Mortazavi P, et al. *Neuropathological Changes in Brain Cortex and Hippocampus in a Rat Model of Alzheimer's Disease*. Vol 15. 2011. http://IBJ.pasteur.ac.ir
25. Beigom Hejaziyan L, Hosseini SM, Taravati A, et al. Effect of Rosa damascena Extract on Rat Model Alzheimer's Disease: A Histopathological, Behavioral, Enzyme Activities, and Oxidative Stress Study. *Evidence-based Complementary and Alternative Medicine*. 2023;2023. doi:10.1155/2023/4926151
26. Fauziah PN, Maskoen AM, Yuliati T, Widiarsih E. Optimized steps in determination of malondialdehyde (MDA) standards on diagnostic of lipid peroxidation. *Padjadjaran Journal of Dentistry*. 2018;30(2):136-139. doi:10.24198/pjd.vol30no2.18329
27. Rehman S, Chandra O, Abdulla M. *Evaluation of Malondialdehyde as an Index of Lead Damage in Rat Brain Homogenates*. Vol 8. 1995.
28. Zargar S, Wani TA. Protective role of quercetin in carbon tetrachloride induced toxicity in rat brain: Biochemical, spectrophotometric assays and computational approach. *Molecules*. 2021;26(24). doi:10.3390/molecules26247526
29. Khan RA, Khan MR, Sahreen S. Brain antioxidant markers, cognitive performance and acetylcholinesterase activity of rats: Efficiency of Sonchus asper. *Behavioral and Brain Functions*. 2012;8. doi:10.1186/1744-9081-8-21
30. Nakhaee A, Bokaeian M, Akbarzadeh A, Hashemi M. Sodium tungstate attenuate oxidative stress in brain tissue of streptozotocin-induced diabetic rats. *Biol Trace Elem Res*. 2010;136(2):221-231. doi:10.1007/s12011-009-8537-0