

Evaluation of In Vivo Anti Alzheimer's Activity of Neolamarckia cadamba using Beta Amyloid Induced Neurotoxicity in Rats

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Research Article

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Abstract

Background:

Alzheimer's disease (AD) is a progressive neurodegenerative disorders characterized by amyloid-beta ($A\beta$) deposition, oxidative stress, and neuroinflammatory cascades leading to cognitive dysfunction. Current cholinesterase inhibitors provide only symptomatic relief and often produce adverse effects. The rubiaceae family membrane *Neolamarckia cadamba* (Roxb). Bosser is abundant in bioactive metabolites with anti-inflammatory and antioxidant qualities, including cadambine. Cadambagenic acid, quinovic acid, quinovic acid, and β -sitosterol. In an $A\beta_{142}$ -induced rat model, the current study sought to assess the ethanolic extract of *N. cadamba*'s in vivo anti- Alzheimer's potential.

Methods:

Adult wistar rats received intracerebroventricular injections of $A\beta_{142}$ (5 μ L, 1 mg/mL) to induced experimental AD. For 21 days, the animals were treated orally with 200 and 400 mg/kg of *N. cadamba* extract. Cognitive performance was evaluated using the radial Arm Maze and passive Avoidance Test Lipid peroxidation (LPO), nitric oxide (NO), superoxide dismutase (SOD), Catalase (CAT), and glutathione (GSH), levels were measured through biochemical assays. ESLIA was used to determine the levels of pro-inflammatory cytokines (IL-1 β and TNF- α).

Results:

Rats administered $A\beta_{1-42}$ demonstrated significant deficits in learning and memory, including diminished antioxidant enzyme levels (SOD, CAT and GSH), heightened oxidative stress indicators (LPO, NO), and enhanced neuroinflammatory cytokines. *N. cadamba* extract therapy significantly improved cognitive performance by decreasing reference memory error (RME), correct working memory errors (CWME), and incorrect working memory errors (IWME). The extract effective suppressed the expression of the TNF- α and IL-1 β , reduced lipid peroxidation and nitric oxide levels, and reinstated antioxidant enzyme activity.

Conclusion:

The research provides substantial evidence that *Neolamarckia cadamba* employs synergistic anti-inflammatory and antioxidant pathways to elicit neuroprotective benefits. The control of oxidative and inflammatory pathways, along with histological protection, underscores its therapeutics promise as a natural option for preventing or deceleration the progression of Alzheimer's disease.

Introduction

Nearly 3.7 billion individuals worldwide suffered from a nervous system ailment in 2025, according to a recent estimate from the global burden of aliment, injuries, Risk factors study (GBD)[1]. These included stress, depression, headaches, migraines, sleeplessness, parkinson's diseases, epilepsy, and Alzheimer's disease (AD) is one of them. Amyloid plaques accumulate and neurotransmitters such as acetylcholine

(ACh), which is critical for memory and cognition, are depleted in this chronic, progressive neurodegenerative disease. [2, 3].

The start and progression of Alzheimer's disease are believed to be significantly influenced by oxidative stress [4]. A significant drop in ACh levels is one of the disease's key molecular features. The primary enzymes that break down ACh are acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which results in cholinergic deficits and issues with neurotransmission [5]. Thus, one of the most important clinical therapy strategies for Alzheimer's disease and other neurodegenerative illnesses is to inhibit these enzymes. This technique aims to restore normal ACh levels in the brain, which will enhance cholinergic signaling and cognitive functions. Individuals routinely assess the effectiveness of this type of treatment and if ACh-mediated cognitive processes are improving [6].

Because it acts on nicotinic and muscarinic receptors, ACh is crucial for memory and learning [7]. By suppressing cholinesterase rather than employing direct receptor agonists, treatment methods seek to maintain steady natural ACh levels. Donepezil is approved by the Food and Drug Administration (FDA) in the United States. This second-generation cholinesterase inhibitor is used to treat the symptoms of Alzheimer's disease since it is very selective for the central nervous system [8]. In order to slow down the breakdown of ACh and enhance its availability in the synaptic cleft, it briefly blocks AChE and BChE. It has been demonstrated that this improves cholinergic signaling and helps people with Alzheimer's disease with their cognitive and neuropsychiatric symptoms [9, 10].

Seek for natural remedies:

The search for plant-based molecules with cholinesterase inhibition and antioxidant activity has accelerated due to the disadvantages and adverse effects of synthetic antioxidants, including potential liver toxicity and carcinogenic qualities[11, 12].

There is still much to learn about many of the physiologically active secondary metabolites present in plants. For medication development, these metabolites which include flavonoids, alkaloids, terpenoids, and phenolics offer safer alternatives [13].

Conventional medicine continues to be the primary treatment option for neurological diseases, particularly in developing countries. Ethnobotanical knowledge has played an important role in the development of neuroprotective drugs [14]. One significant achievement is the conversion of galantamine, an alkaloid obtained from *Galanthus nivalis*, into a therapeutically authorized acetylcholinesterase (AChE) inhibitor for the management of Alzheimer's disease [15].

Neurodegenerative illnesses like Alzheimer's disease are associated with a variety of pathogenic processes, including oxidative stress, neuroinflammation, cholinergic impairments, amyloid- β buildup, and mitochondrial dysfunction [16, 17]. In regions around amyloid plaques and neurofibrillary tangles, the activities of AChE, butyrylcholinesterase (BChE), and reactive oxygen species (ROS) are elevated above physiological levels [18]. Cholinesterase hyperactivity and amyloid- β toxicity combine to decrease

acetylcholine synthesis and release, which interferes with cholinergic neurotransmission and results in neurotoxicity, particularly in the cerebral cortex and hippocampus [19].

Furthermore, microglial activation is one of the primary causes of neuroinflammation in Alzheimer's disease [20]. When neurons are damaged, microglia, the immune cells that dwell in the central nervous system, release mediators that are both inflammatory and neurotrophic. Notwithstanding the initial protective impact, chronic activation results in elevated cholinesterase activity and an excess of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α [21]. Neuronal degeneration and cognitive decline are caused by oxidative stress being exacerbated by reactive metabolites such hydrogen peroxide and peroxynitrite, as well as ROS generated by microglia [22].

In physiological contexts, microglia maintain neuronal homeostasis and aid in cognitive functions. However, persistent oxidative stress overwhelms their antioxidant defenses, resulting in progressive cellular damage [23].

Antioxidants and Oxidative Stress

When the body's antioxidant capacity is exceeded by the production of free radicals, oxidative stress results. Important biomolecules like proteins, lipids, and DNA can be harmed by reactive oxygen species (ROS), which are produced by regular metabolic redox reactions [24]. Elevated ROS during disease states can affect the body's enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (vitamins C, E, glutathione) antioxidant systems, which can lead to neurodegenerative, cardiovascular, metabolic, and oncological disorders [25].

Due to their antioxidant potential, medicinal plants have been shown in numerous studies to have protective effects against oxidative damage [26]. Fruits and vegetables are rich in phenols and flavonoids, which have potent metal-chelating and free radical-scavenging capabilities [27]. In order to neutralize ROS, restore redox homeostasis, and slow the progression of disease, current research focuses on substituting natural compounds derived from plants for synthetic antioxidants [28, 29].

Neolamarckia cadamba as a Possible Treatment

Because of its diverse pharmacological potential and rich phytochemical profile, *Neolamarckia cadamba*, a well-known member of the Rubiaceae family, has a prominent place in herbal medicine [30]. The various secondary metabolites of *N. cadamba*, such as β -sitosterol, quinovic acid, cadambagenic acid, cadamine, and cadambine, are responsible for its remarkable biological and therapeutic activities [31, 32].

With its diuretic, sedative, and antipyretic qualities, this plant offers a natural substitute for synthetic drugs in the treatment and prevention of chronic conditions like diabetes, epilepsy, hypertension, and insomnia [33–34]. Due to the lack of effective translational models, only a small percentage ($\leq 2\%$) of

known plant-derived bioactive compounds have found commercial or clinical use despite a century of intensive phytochemical research [35].

N. cadamba is valued as a sacred and multipurpose medicinal plant in traditional medicine systems throughout South and Southeast Asia, in addition to its pharmacological potential [36–38].

Goal of the Study:

The current study aimed to examine the phytochemical composition of *Neolamarckia cadamba* and assess its anticholinesterase and antioxidant effects via in vitro assays. Furthermore, the study sought to evaluate its potential neuroprotective and anti-Alzheimer's properties in vivo utilising a rat model. This study aims to offer scientific evidence for the conventional application of *N. cadamba* and investigate its therapeutic significance in the treatment of neurodegenerative illnesses, specifically Alzheimer's disease.

Materials and methods

Drugs and chemicals:

We purchased Beta Amyloid_{1–42} from Sigma-Aldrich, as well as its substrates, potassium phosphate buffer (pH 8.0) and acetylthiocholine iodide. Additional reagents and chemicals of analytical grade were sourced from Merk Pvt. Ltd., New Delhi.

Collecting, identifying, and extracting plant material:

People in Gwalior gathered leaves from a *Neolamarckia cadamba*. Medicaps University's Head of Agriculture, Dr. Upadhyay, confirmed and authorized the plant. The leaves were carefully washed and then left to dry in the shade for fifteen days. After the leaves had dried, they were ground up with a machine. For three days, 500 g of the powdered material was extracted using Soxhlet in 80% ethanol, with regular checks. Thereafter, the mixture was first put through muslin cloth and then through Whatman filter paper. A desiccator was employed to turn the resulting filtrate into a semisolid substance. This semisolid mass had a final yield of 65 g after it solidified in the open air. Then, a solvent-solvent extraction method was employed to break up the crude ethanolic extract into smaller pieces, starting with a solvent that had low polarity and moving up to one that had high polarity. The yields for the fractions were 12% n-hexane, 16% chloroform, 11% ethyl acetate, and 35% water.

Phytochemical Screening:

Preliminary Phytochemical Screening:

Initial phytochemical screening was conducted using various extracts prepared from the leaves of *Neolamarckia cadamba*,

Evaluation of Anti-Alzheimer's Activity:

The anti-Alzheimer's potential of the leaf extract from *N. cadamba* was assessed in rats using an intracerebroventricular (ICV) injection model of Amyloid- β ($A\beta_{1-42}$). According to OECD Guideline 423 and acute toxicity studies, a dose of 200 mg/kg (or one-tenth of 2000 mg/kg) was chosen for the pharmacological screening of Alzheimer's disease.

Animals Used in Experiments:

Wistar albino rats weighing 175–200 g were purchased from Shriram College of Pharmacy's Animal House in Banmore, Morena. The animals were kept under typical laboratory conditions, which included a 12-hour light/dark cycle, a controlled temperature of $23 \pm 2^\circ\text{C}$, and a relative humidity of $60 \pm 5\%$. The animals were given free access to water and allowed to acclimate and fast for 12 to 18 hours before operation. Every experimental operation was carried out from 9:00 a.m. to 5:00 p.m.

In compliance with globally recognised standards for the moral use of lab animals, the experimental procedure was authorised by the Institutional Animal Ethics Committee (IAEC) of Shriram College of Pharmacy, Banmore, and Morena.

Making the $A\beta_{1-42}$ Solution:

Intracerebroventricular injection was carried out using artificial cerebrospinal fluid (aCSF). The composition of the freshly made CSF was as follows: 2.2 mM dextrose, 2.9 mM KCl, 1.6 mM MgCl_2 , 1.7 mM CaCl_2 , and 147 mM NaCl. To promote peptide aggregation, the $A\beta_{1-42}$ peptide was dissolved in aCSF and incubated for 72 hours at 37°C . Each rat was given a 2.0 μL (5 $\mu\text{g}/\mu\text{L}$) ICV injection of $A\beta_{1-42}$ into each lateral ventricle on both sides of the bregma under stereotaxically guided guidance.

Making a Plant Extract:

To create oral dosages of 100 mg/kg and 200 mg/kg for administration, the ethanolic extract of *Neolamarckia cadamba* leaves was precisely weighed and suspended in 0.3% carboxymethyl cellulose (CMC).

Experimental Grouping and Treatment

The study consisted of six groups, each comprising six rats (Table 1). All animals underwent behavioural assessments to evaluate learning, memory, and cognitive performance following treatment.

The study's design

The investigation was performed on experimental animals over a duration of 21 days. Day 0 was the day that $A\beta_{1-42}$ was given. The mice had a week to recover following the first $A\beta_{1-42}$ ICV injection. From Day 8 onward, extract treatments were given to each group once a day, and the procedure continued every 24

hours until Day 21. The animals were put through habituation trials for the radial arm maze (RAM) test on Days 15 to 17 and for the step-through avoidance test (PAT) on Days 18 and 19. After these tests, the animals were tested with PAT on Day 20 to see how long it took them to start (IL) and on Day 21 to see how long it took them to step through (STL). After the PAT test on Day 21, the animals were tested again with the RAM test. Following behavioural evaluations, the animals were euthanised, and brain specimens were obtained. Micro-dissection of the hippocampus was performed for neurobiochemical investigations, encompassing cytokines, neurotransmitters, and histological assessment.[39, 40]

Table 1
Experimental Grouping and Treatment

S.No	Experimental Animals	Groups	Treatment Description	Number Of Animals
1	Wister albino Rat (170-200g)	Group 1	Control	6
2		Group 2	Sham operated (SO)(ICV-2µl a CST/ Site)	6
3		Group 3	Amyloid-β (Aβ1–42) (ICV- 10µg/2µl aCSF/site	6
4		Group 4	Amyloid-β (Aβ1–42) + Donepezil (5mg/kg p.o)	6
5		Group 5	Amyloid-β (Aβ1–42) + N.cadamba extract (100mg/kg p.o)	6
6		Group 6	Amyloid-β (Aβ1–42) + N.cadamba extract (200mg/kgp.o)	6
7		Group 7	Amyloid-β (Aβ1–42) + N.cadamba extract (400mg/kg p.o)	6

Experimental Induction of Alzheimer’s disease (ICVAβ1–42) model

Surgical Procedure for Induction of Alzheimer’s Model

Rats were anaesthetised using ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Once anaesthesia was established, Amyloid-β_{1–42} (Aβ_{1–42}) was administered intracerebroventricularly (ICV) using a stereotaxic apparatus. The scalp was carefully incised, and the head was positioned such that the bregma and lambda were aligned on a horizontal plane. A small burr hole was drilled into the skull, and Aβ_{1–42} solution was injected bilaterally into the hippocampal region at the following stereotaxic coordinates: anteroposterior – 3.8 mm from bregma, mediolateral ± 2.2 mm, and dorsoventral – 2.7 mm.

Each rat received an injection of 2.0 µL (5 µg/µL) Aβ_{1–42} per side through a Hamilton microsyringe fitted with a stainless-steel cannula. The injection was performed over five minutes, and the syringe was left in

place for an additional two minutes to ensure proper diffusion of the peptide. SHAM-operated control rats received an equal volume of sterile normal saline.[39, 40]

Following surgery, the incision site was cleaned, and mupirocin antibiotic ointment was applied daily for seven days to prevent infection and facilitate wound healing. After a recovery period, animals were subjected to behavioural and neurochemical assessments.

Cognitive Behavioural Studies

Radial Arm Maze (RAM) Test

Rats' working memory, reference memory, and spatial learning were evaluated using the Radial Arm Maze. The device was made up of eight equal-length, four-foot arms that extended from a central, round platform that was 90 cm above the ground. Each arm had walls that were 2.5 cm high to prevent falls and a tiny feeding cup at the end that served as a food reward Zone.

In order to increase the animals' motivation for the food rewards, they were fasted for the entire night before the experimental trials. For three days in a row, they spent fifteen minutes each getting used to the maze. Food pellets were used to bait specific arms during testing in a predetermined pattern for the duration of the investigation. The rats had ten minutes to look through all of their arms for the food. While entry into an unbaited arm was recorded as a reference memory error, repeated entries into previously visited baited arms were counted as working memory faults.

Scent cues were removed from the maze by thoroughly cleaning it with 70% ethanol after each trial. To evaluate cognitive performance, the amount of time it took to get to the goal box was timed during both the acquisition and retention phases. [41]

Passive Avoidance (Step-Through) Test:

The passive avoidance test was used to assess memory retention after the Radial Arm Maze challenge. The device included a guillotine door connecting a brightly illuminated chamber to a dark chamber, the dark side of which included a grid floor for minor foot shocks.

Rats were habituated for two minutes in the lit chamber during the training period. Animals instinctively approached the dark compartment after the door was opened, where they were given a minor electric foot shock. Rats were not allowed to continue testing if it took them more than 60 seconds to enter the dark container.

A retention test without shock administration was conducted twenty-four hours later. Step-through latency, or the amount of time it took for each rat to enter the dark chamber, was measured, with a maximum cut-off of 60 seconds. Longer latencies reflected better memory retention. [42]

Neurobiochemical Evaluations :

Brain Dissection and Sample Preparation:

After the behavioural tests were completed, the rats were euthanised and their brains were quickly removed. The hippocampus was carefully cut up, cleaned with cold saline, and kept at -8°C for later study. To prepare brain homogenates for biochemical testing, we used 10% ice-cold KCl (100 μL per 10 mg tissue). [43]

Lipid Peroxidation (LPO) Test

We changed the thiobarbituric acid reactive substances (TBARS) method from Ohkawa et al. (1979) to find out how much lipid peroxidation there was. The reaction mixture was made up of 1.5 mL of 0.9% thiobarbituric acid, 0.2 mL of 8% sodium dodecyl sulfate, and 0.5 mL of the sample. After 30 minutes in a boiling water bath, we allowed the mixture to cool before separating it using 5 millilitres of n-butanol: pyridine (15:1, v/v). The organic layer was centrifuged at 4000 rpm for 10 minutes, and its absorbance at 532 nm was measured using a UV spectrophotometer. 1,1,3,3-Tetraethoxypropane (80–240 nmol) was the standard. [44]

Estimating Nitric Oxide (NO)

To determine the amount of nitric oxide in the sample, we employed the Griess reagent method. 1.8 mL of saline, 0.4 mL of 35% sulfosalicylic acid, and 0.2 mL of a 10% brain homogenate sample were mixed. For ten minutes, the mixture was spun at 4000 rpm. Next, 2 mL of freshly prepared Griess reagent containing sulfonamide, orthophosphoric acid, and naphthylethylenediamine was combined with 1 mL of the supernatant. The absorbance at 540 nm was measured after 20 minutes in the dark. Sodium nitrite at 200–1000 mg/mL served as the calibration standard [45].

Activity of Superoxide Dismutase (SOD) :

0.05 mL of brain homogenate, 0.3 mL of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.025 mL of PMS (186 μM), and 0.075 mL of NBT (300 μM) were combined to determine the level of SOD activity. 0.075 mL of NADH was added to begin the reaction, which was maintained at 30°C for 90 seconds. 0.25 mL of glacial acetic acid was added to halt the reaction. After that, the mixture was spun for a minute at 4000 rpm and extracted using 2 mL of n-butanol. We measured the absorbance of the organic phase at 560 nm. [46]

Catalase (CAT) Action:

To determine the catalase activity, we combined 2.25 mL of 65 mM potassium phosphate buffer (pH 7.8) with 100 μL of brain homogenate. The reaction was initiated by adding 650 μL of 7.5 mM hydrogen peroxide. For two to three minutes, the decrease in absorbance at 240 nm was observed using a UV spectrophotometer (Shimadzu, Japan). [47]

Lowered Glutathione (GSH) Estimation :

We used the method of Jollow et al. (1974) to find out how much GSH there was. To put it simply, 0.25 mL of brain homogenate was combined with 0.25 mL of cold 5% trichloroacetic acid (TCA) and spun at 4000 rpm for 10 minutes. We added 0.25 mL of 0.2 M phosphate buffer (pH 8.0) and 0.5 mL of DTNB (0.6 mM) to 1 mL of supernatant. A spectrophotometer was used to detect absorbance at 412 nm. [48]

Estimating Inflammatory Cytokines :

We mixed up hippocampal tissue in a solution that had 0.1% BSA, 81 mM Na₂HPO₄, 19 mM NaH₂PO₄, 50 mM NaCl, and 0.1% Triton X-100. We used ELISA kits (Quantikine and Invitrogen, USA) to measure the levels of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, according to the manufacturer's instructions. [49]

Estimating the Amount of Glutamate:

The hippocampus was mixed with 0.1 N HCl made with 80% ethanol (200 μ L per 10 mg of tissue) and spun at 4500 rpm for 20 minutes at 25°C. We used High-Performance Thin Layer Chromatography (HPTLC, CAMAG, USA) to measure the amount of glutamate in the supernatant. The stationary phase was silica gel GF254, and the mobile phase was a mixture of n-butanol, glacial acetic acid, and water (65:15:25 v/v). We used 0.2% ninhydrin in acetone to find the sample, and we measured the absorbance at 486 nm. We established a standard curve with L-glutamic acid (20–200 ng/spot). [49]

Statistical Analysis:

Mean $\pm$ SEM was used to show each data point. We utilized GraphPad Prism (version 5.01) to do the statistical analyses. We utilized Tukey's post hoc multiple comparison test after one-way ANOVA to find out the overall significance. Differences were deemed statistically significant at $p < 0.05$.

Results

Two safe and efficient doses of the *Neolamarckia cadamba* ethanolic leaf extract 100 mg/kg (1/20th of 2000 mg/kg) and 200 mg/kg (1/10th of 2000 mg/kg) were chosen for pharmacological evaluation in the intracerebroventricular (ICV) amyloid-beta (A β) rat model of Alzheimer's disease based on the results of the acute toxicity studies.

Cognitive Behavioural Assessment

Radial Arm Maze (RAM) Test

The number of reference memory errors (RME) was significantly higher ($p < 0.05$) in rats that received intracerebroventricular injections of A β_{1-42} than in the sham-operated (SO) group, suggesting significant cognitive impairment. The neuroprotective effectiveness of donepezil (DPZ, 5 mg/kg) was confirmed by the substantial reduction in the RME score ($p < 0.05$) that followed treatment. *N. cadamba* extract also

showed promise in improving spatial learning and memory and reducing A β -induced cognitive deficits by producing dose-dependent improvements in memory performance at 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.05$), and 400 mg/kg ($p < 0.05$).

Groups: Control, Sham, A β_{1-42} (disease), A β_{1-42} + Donepezil, A β_{1-42} + NC (100, 200, 400 mg/kg). *N. cadamba* reduced RME significantly ($p < 0.05$, $p < 0.01$) vs A β_{1-42}

Correct Working Memory Error (CWME)

When compared to the sham-operated (SO) group, the A β_{1-42} treated group showed a significant increase in working memory errors (CWME) ($p < 0.05$), indicating significant cognitive impairment. The neuroprotective effect of donepezil (DPZ, 5 mg/kg) was confirmed by the significant reduction in CWME scores ($p < 0.05$) following treatment. Likewise, administering *Neolamarckia cadamba* extract led to a dose-dependent enhancement in cognitive performance, evidenced by significant reductions in CWME at doses of 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.05$), and 400 mg/kg ($p < 0.05$), suggesting its capacity to enhance working memory and mitigate A β -induced cognitive impairments.

Incorrect Working Memory Error (IWME)

When A β_{1-42} was injected intracerebroventricularly (ICV), initial working memory errors (IWME) significantly increased ($p < 0.05$) in comparison to the sham-operated (SO) group, suggesting a deficit in short-term memory and learning capacity. Donepezil (DPZ, 5 mg/kg) treatment significantly decreased IWME scores ($p < 0.05$), demonstrating its protective effect on cognitive function. *Neolamarckia cadamba* extract administration also significantly and dose-dependently reduced IWME at 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.05$), and 400 mg/kg ($p < 0.05$), indicating that working memory was improved and cognitive performance was effectively restored.

Step through the Passive Avoidance Test

Initial Latency

When compared to the sham-operated (SO) group, the A β_{1-42} treated group's initial latency (IL) time increased significantly ($p < 0.05$), suggesting a decline in learning and memory retention. The considerable decrease in IL duration ($p < 0.05$) after therapy with donepezil (DPZ, 5 mg/kg) proved its cognitive-enhancing impact. In a similar way, giving *Neolamarckia cadamba* extract at 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.05$), and 400 mg/kg ($p < 0.05$) led to a dose-dependent decrease in IL time. This suggests that it improves memory retention and reverses cognitive decline caused by A β .

Step Through Latency

Intra-cerebroventricular (ICV) infusion of A β_{1-42} significantly reduced the step-through latency (STL) duration ($p < 0.05$) compared to the sham-operated (SO) group, demonstrating considerable impairment in memory retention. Donepezil (DPZ, 5 mg/kg) treatment significantly prolonged the STL period ($p < 0.05$), thereby validating its memory-enhancing capabilities. Similarly, the administration of *Neolamarckia cadamba* extract significantly and dose-dependently restored STL duration 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.05$), and 400 mg/kg ($p < 0.05$) indicating a notable enhancement in memory retention and a protective effect against A β -induced cognitive deficits.

Neurobiochemical Parameters

Lipid Peroxide (LPO)

A β_{1-42} significantly increased ($p < 0.05$) brain LPO levels. Treatments with DPZ and *N.cadamba* extract significantly reduced LPO ($p < 0.05$), indicating antioxidant potential.

Nitric Oxide (NO)

A β_{1-42} increased brain NO ($p < 0.05$), while DPZ and *N.cadamba* extract treatments significantly reduced NO levels ($p < 0.01$ to $p < 0.05$), showing antioxidant effects.

Superoxide Dismutase (SOD)

A β_{1-42} decreased ($p < 0.05$) SOD activity. DPZ and *N. cadamba* extract significantly increased SOD levels ($p < 0.05$), reflecting enhanced antioxidant defence.

Catalase (CAT)

A β_{1-42} significantly reduced CAT ($p < 0.05$). DPZ and *N.cadamba* extract elevated CAT ($p < 0.05$), indicating free radical scavenging activity.

Glutathione (GSH)

A β_{1-42} reduced ($p < 0.05$) brain GSH. DPZ and *N.cadamba* extract significantly increased GSH ($p < 0.05$), suggesting restoration of antioxidant capacity.

Brain Cytokines

IL-1 β

A β_{1-42} significantly elevated ($p < 0.05$) IL-1 β . DPZ and *N.cadamba* extract significantly reduced IL-1 β ($p < 0.05$), demonstrating anti-inflammatory potential.

TNF- α

A β_{1-42} markedly increased ($p < 0.05$) TNF- α . DPZ and *N.cadamba* extract significantly reduced TNF- α ($p < 0.05$), indicating anti-neuroinflammatory activity.

Discussion

Dementia is an escalating global health issue, requiring the immediate identification of strategies to avert or mitigate cognitive decline [50]. A defining feature of Alzheimer's disease (AD) is the aggregation of amyloid-beta (A β) peptides, especially A β_{1-42} , which causes neuronal injury, synaptic impairment, and oxidative stress [51, 52]. The A β_{1-42} -injected rat model used in this study closely resembles the main clinical features of AD, making it easier to evaluate potential neuroprotective medications [53]. Our findings showed that the ethanolic extract of *Neolamarckia cadamba* significantly reduced the cognitive deficits brought on by A β_{1-42} , as demonstrated by behavioral assessments using the Radial Arm Maze & Passive Avoidance Test. A notable reduction in Reference Memory Errors (RME), Correct Working Memory Errors (CWME), & Incorrect Working Memory Errors (IWME) suggests that the extract enhances cognitive function [54].

According to neurobiochemical studies, A β injection led to a significant increase in markers of oxidative stress like nitric oxide (NO), lipid peroxidation (LPO) and also lets to a significant decrease of activity of antioxidant enzymes like SOD, CAT, and GSH. These values returned to normal after treatment with *N. cadamba* extract, indicating its strong antioxidant potential [55]. To protect neurons from oxidative damage, enzymatic and non-enzymatic defense mechanisms must function more effectively, as seen by increased SOD, CAT, and GSH activities [56].

Additionally, rats given A β had noticeably higher levels of TNF- α and IL-1 β a pro-inflammatory cytokines, underscoring the crucial part neuroinflammation plays in Alzheimer's disease. Cytokine levels were significantly reduced with administration of *N. cadamba* extract, suggesting strong anti-inflammatory and neuroprotective effects [57, 58].

These results are consistent with recent studies showing that phytochemicals from plants offer neuroprotection through anti-inflammatory and antioxidant pathways [59–60]. The bioactive components of *N. cadamba*, such as triterpenoids, flavonoids, and phenolic compounds, are believed to synergistically regulate inflammatory pathways, neutralize reactive oxygen species, and inhibit amyloid aggregation [61].

Neolamarckia cadamba's neuroprotective, anti-inflammatory, and antioxidant properties highlight its potential therapeutic benefit in reducing the progression and underlying pathophysiology of Alzheimer's

disease [62].

Conclusion

According to the article's findings, rats with $A\beta_{1-42}$ induced Alzheimer's disease exhibit notable neuroprotective benefits from *N. cadamba* ethanolic extract. The extracts reduced oxidative stress, reduced neuroinflammation, and improved memory function.

The phytochemicals in the extracts that combat oxidation and inflammation may be the cause of the beneficial effects. Thus, the study supports these plants' potential as natural remedies for cognitive decline and Alzheimer's disease. To prove their translational significance, however, more studies concentrating on the extraction of bioactive compounds, the elucidation of molecular mechanisms, and clinical validation are required.

Declarations

Author Contribution

A- Ravindra Mishra is actually a research scholar, so the data and other things are evaluated by him only. He did the original work of this research article. B- Dr. Jeetendra Kumar Gupta, mentor of Ravindra Mishra, guided him and finally checked the article and approved it for sending publication.

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Figures

Number of Reference Memory Errors

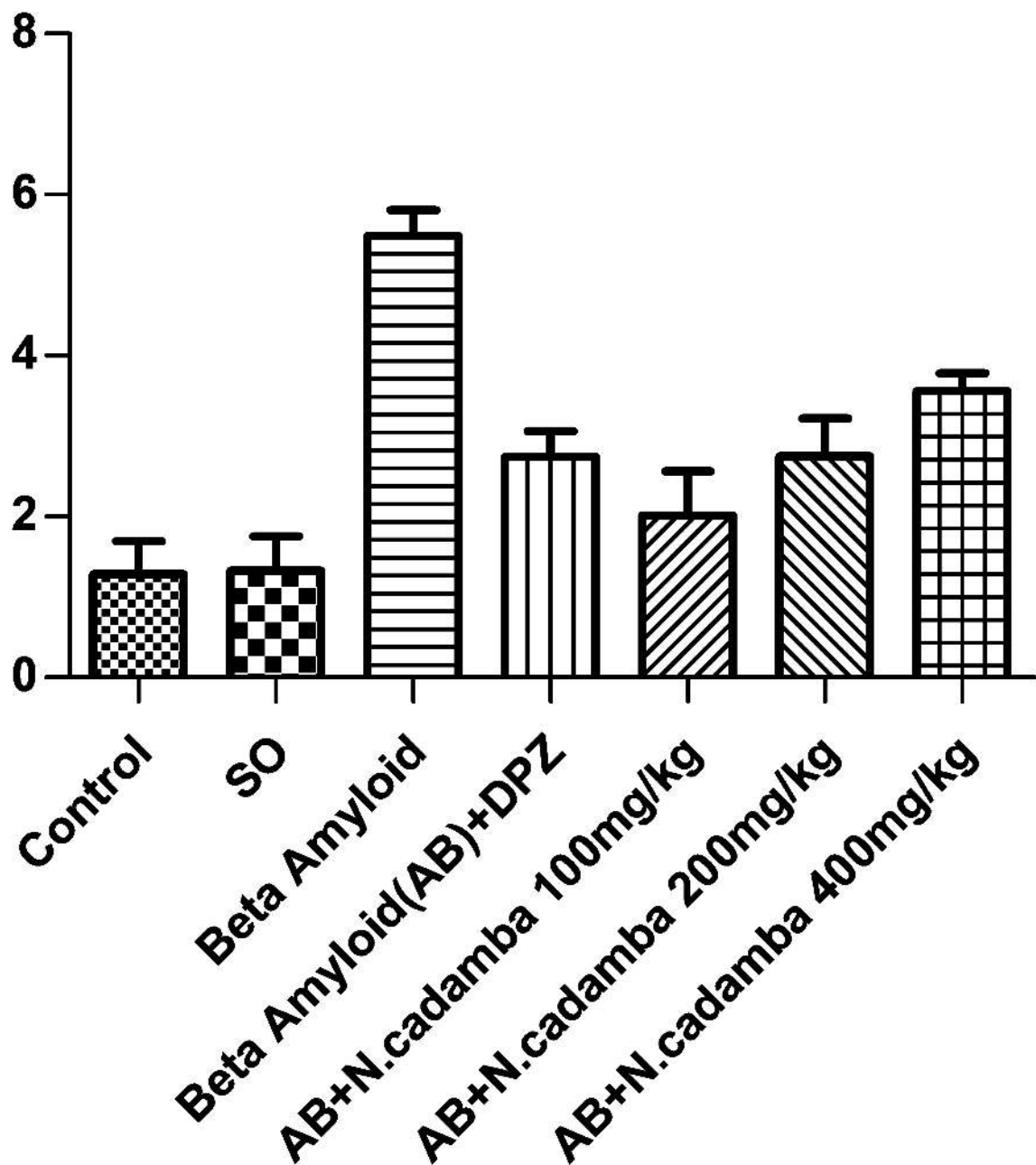


Figure 1

Effect of *Neolamarckia cadamba* on reference memory errors in radial arm maze.

Groups: Control, Sham, $A\beta_{1-42}$ (disease), $A\beta_{1-42}$ + Donepezil, $A\beta_{1-42}$ + NC (100, 200, 400 mg/kg). *N. cadamba* reduced RME significantly ($p < 0.05$, $p < 0.01$) vs $A\beta_{1-42}$

Number of Correct Working Memory Errors

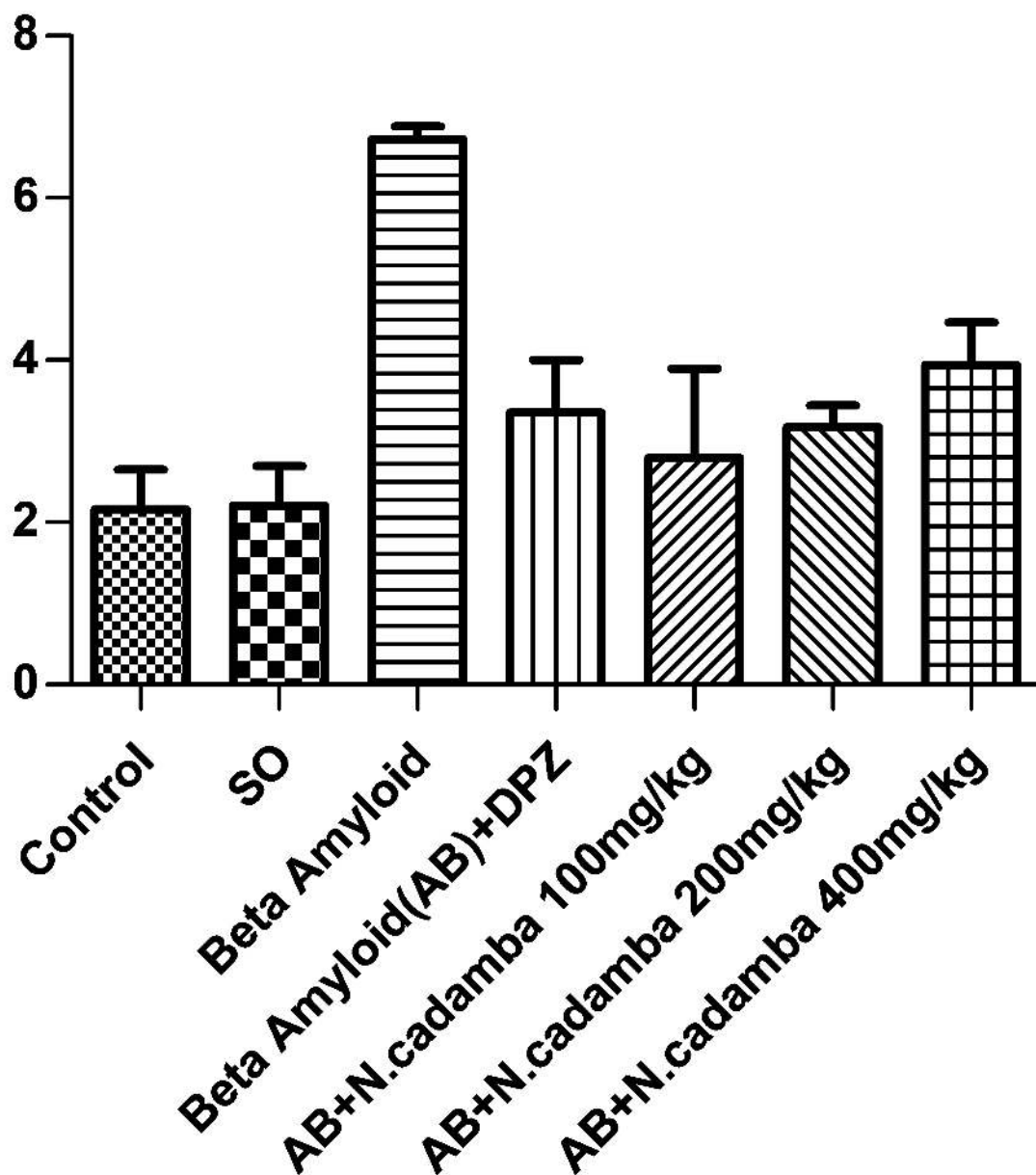


Figure 2

Correct working memory errors across experimental groups. $A\beta_{1-42}$ increased CWME; *N. cadamba* (200–400 mg/kg) and donepezil improved working memory ($p < 0.05$).

Number of Incorrect Working Memory Errors

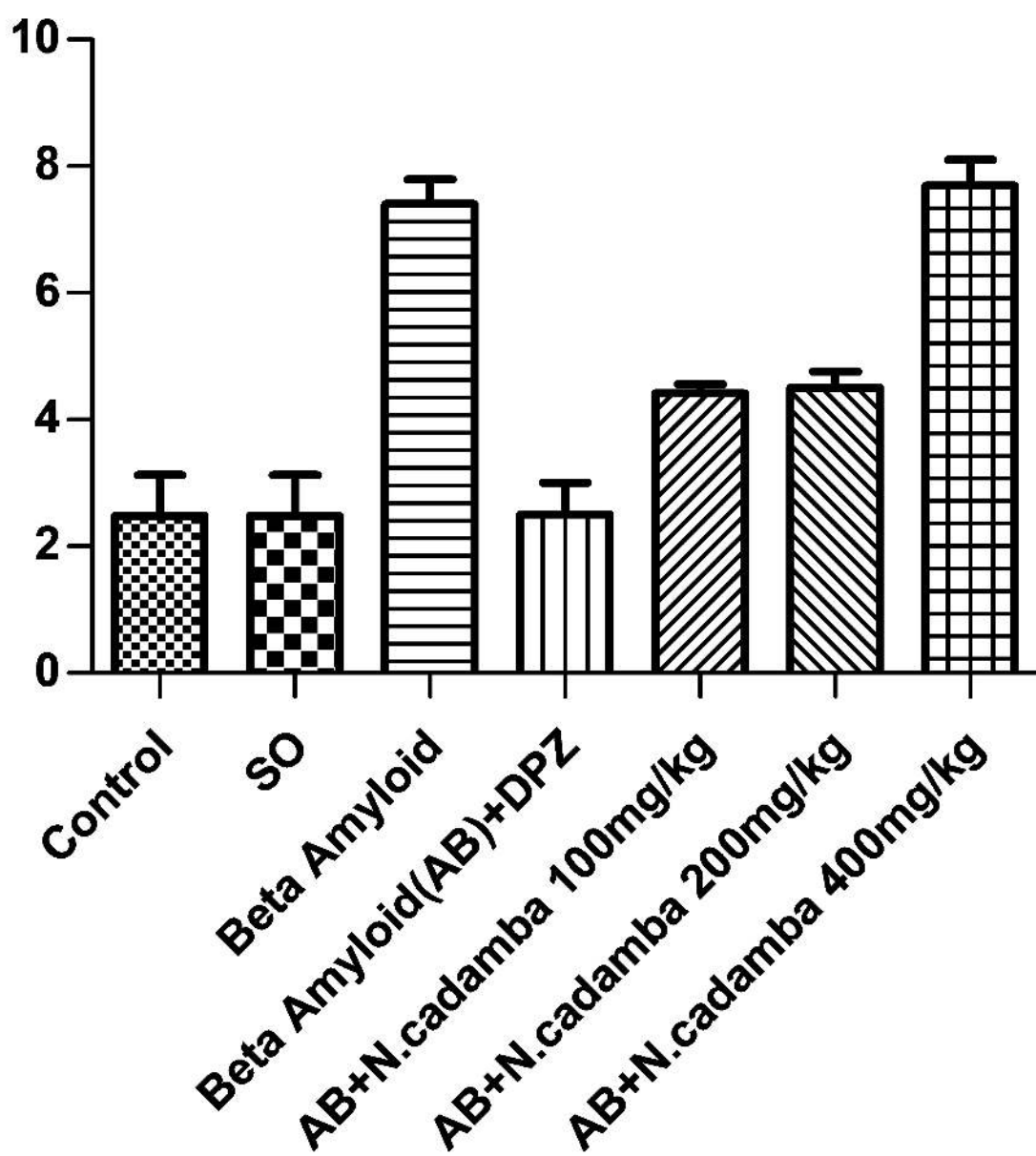


Figure 3

Incorrect working memory errors in $A\beta_{1-42}$ induced rats. High IWME in disease group; *N. cadamba* reduced errors dose dependently, comparable to donepezil ($p < 0.05$).

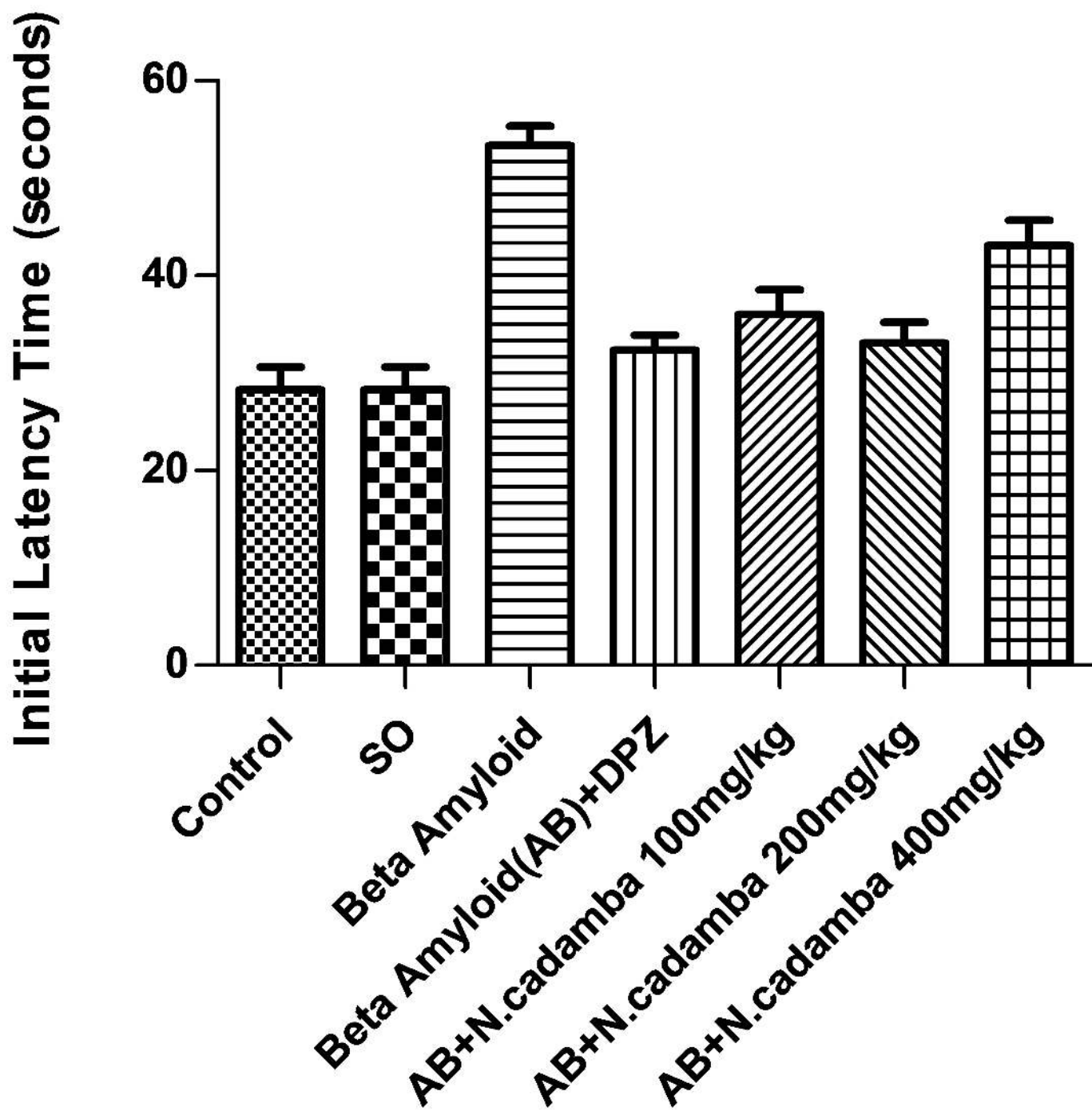


Figure 4

Initial latency (IL) in passive avoidance test. $A\beta_{1-42}$ rats showed prolonged IL; *N. cadamba*(200–400 mg/kg) shortened IL indicating improved learning ($p < 0.05$).

Step-Through Latency Time (seconds)

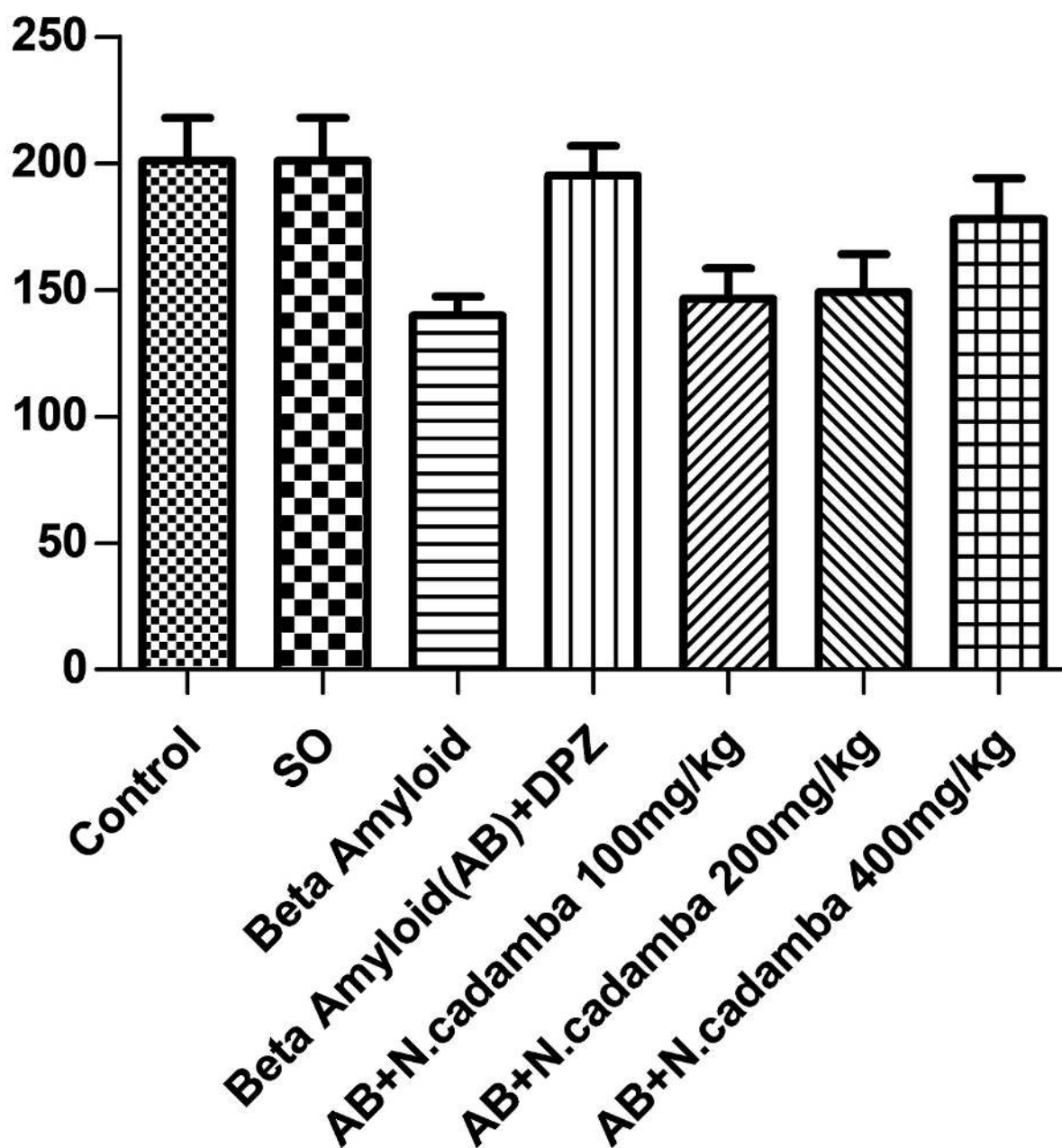


Figure 5

Step-through latency (STL) in passive avoidance test. Memory-impaired rats showed reduced STL; *N. cadamba* treatment prolonged STL ($p < 0.05$) similar to donepezil.

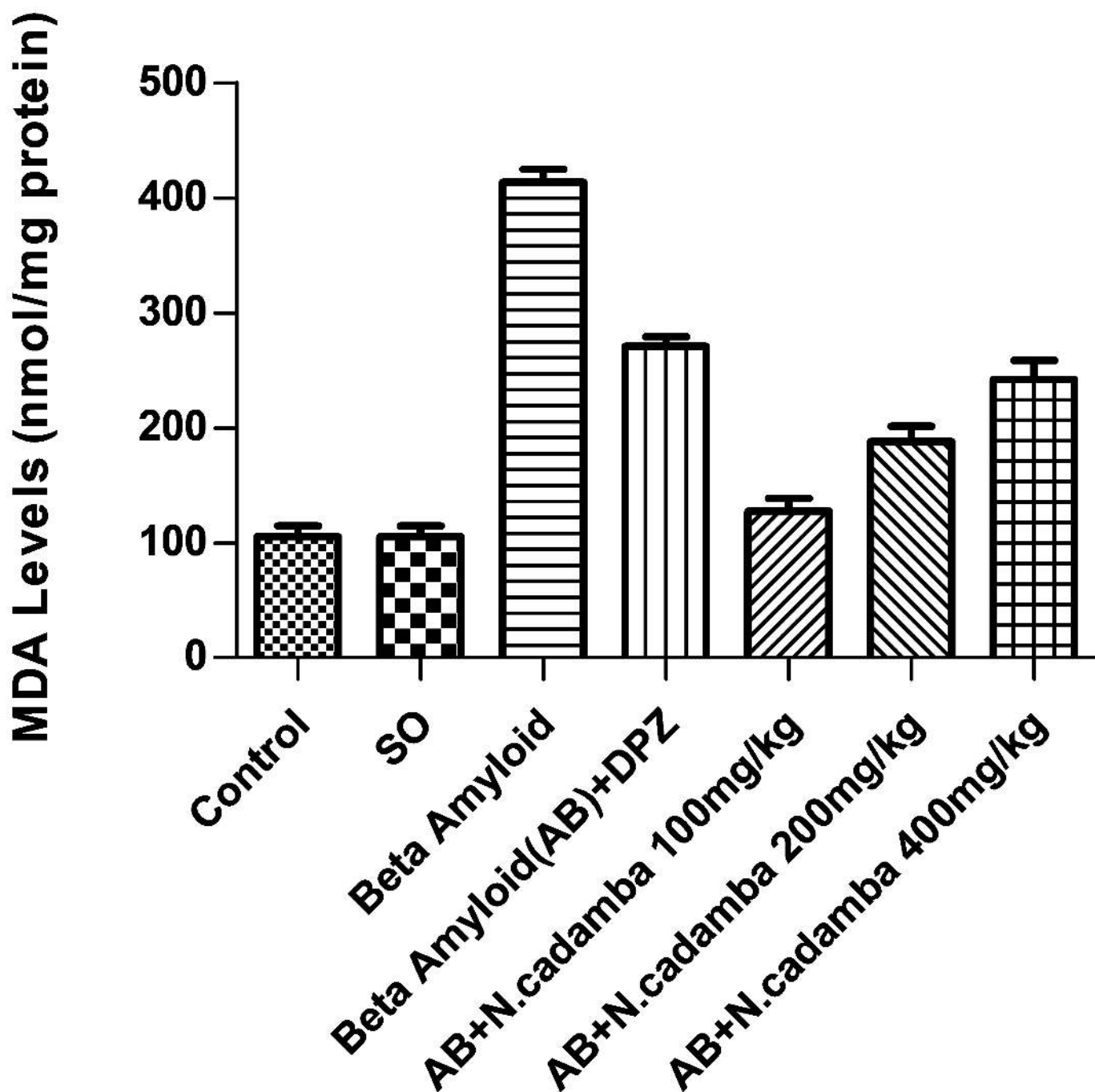


Figure 6

Lipid peroxidation (MDA levels) in brain tissue. $A\beta_{1-42}$ increased MDA; *N. cadamba* and donepezil reduced peroxidation ($p < 0.05$) restoring redox balance.

Nitrite Concentration ($\mu\text{mol}/\text{mg}$ protein)

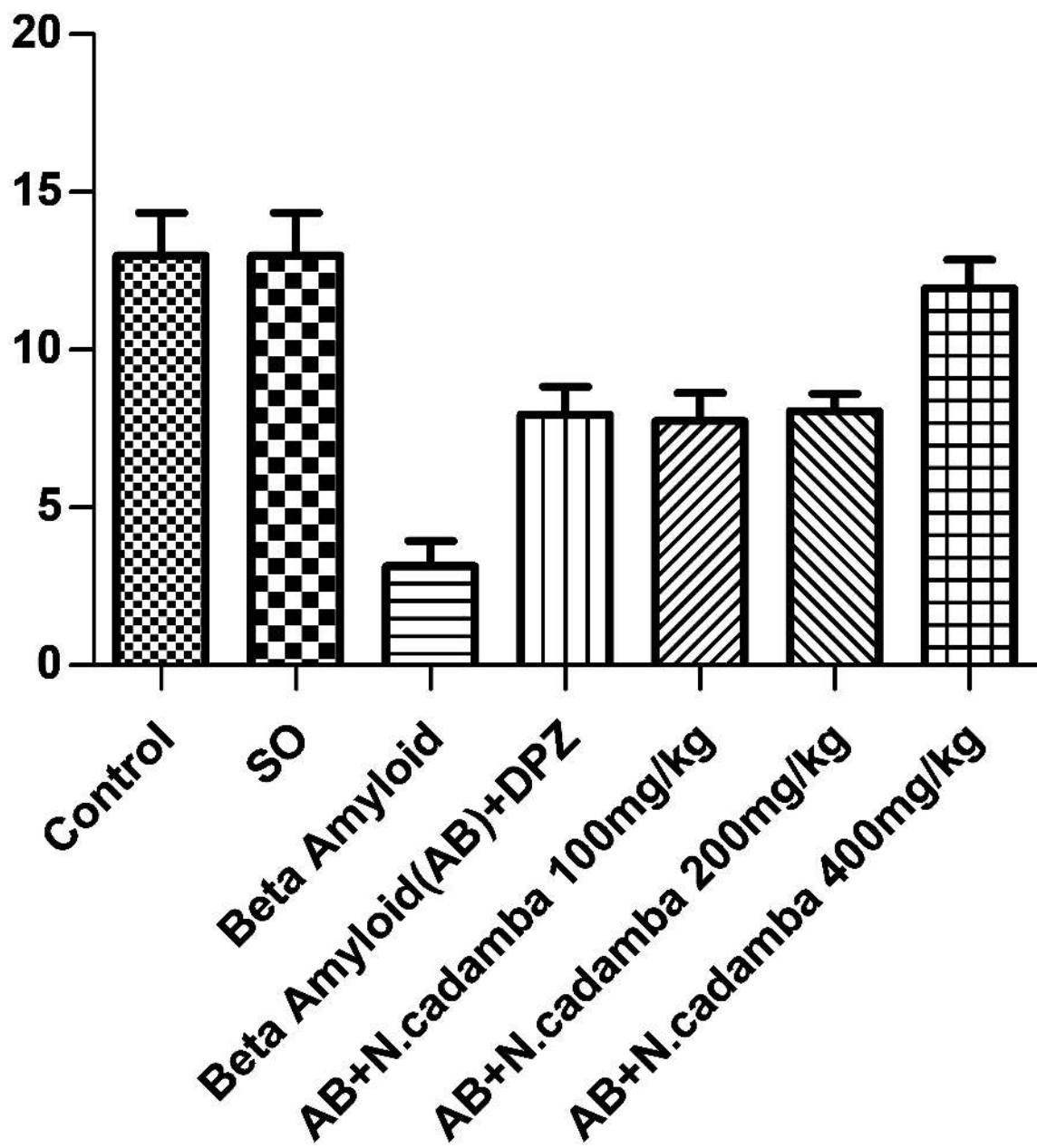


Figure 7

Nitric oxide (NO) levels in different groups. $\text{A}\beta_{1-42}$ raised nitrite levels; *N. cadamba* decreased NO concentration ($p < 0.05$) dose-dependently.

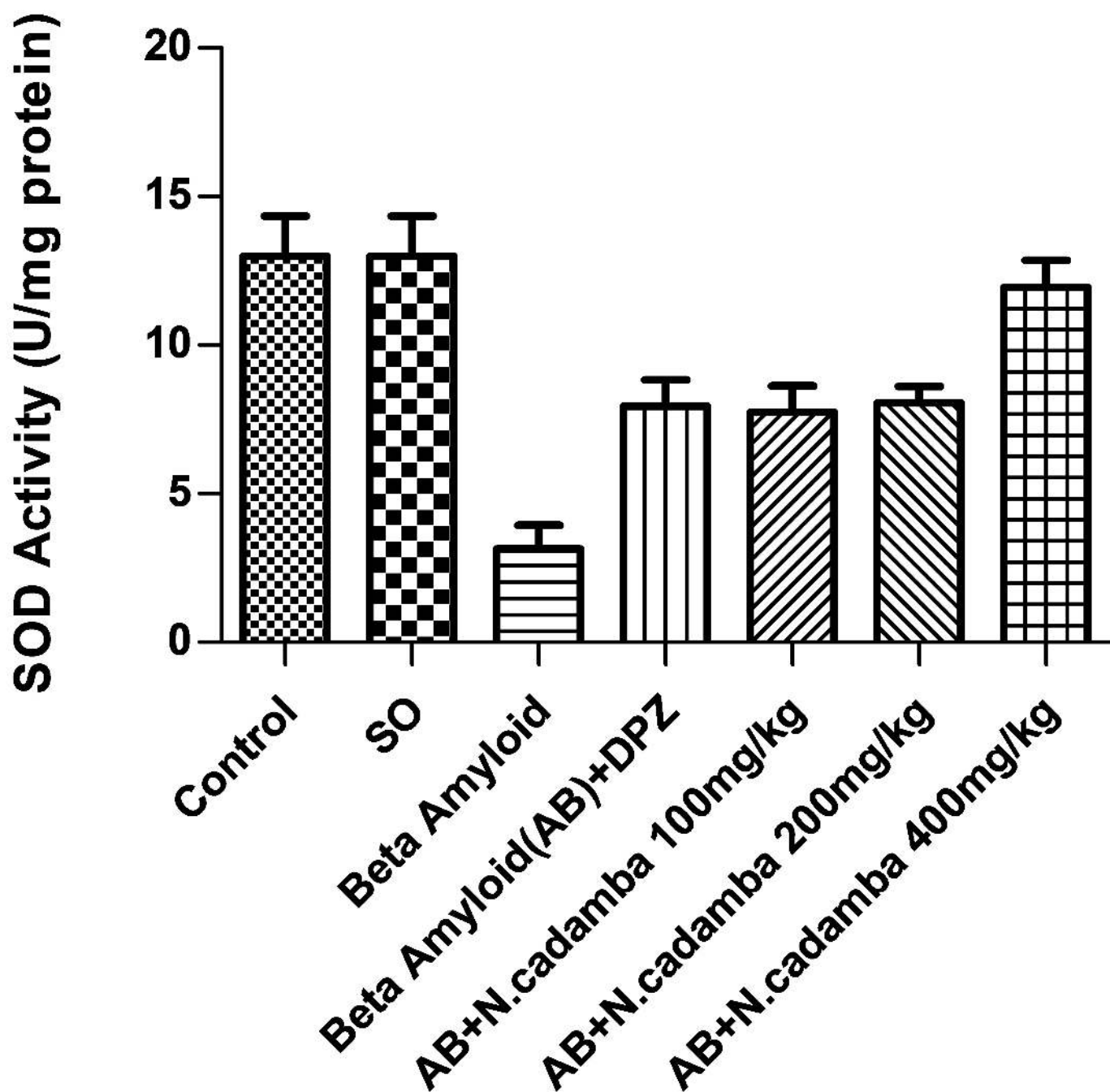


Figure 8

Superoxide dismutase (SOD) activity. Reduced SOD in $A\beta_{1-42}$ group improved after *N. cadamba* treatment ($p < 0.05$).

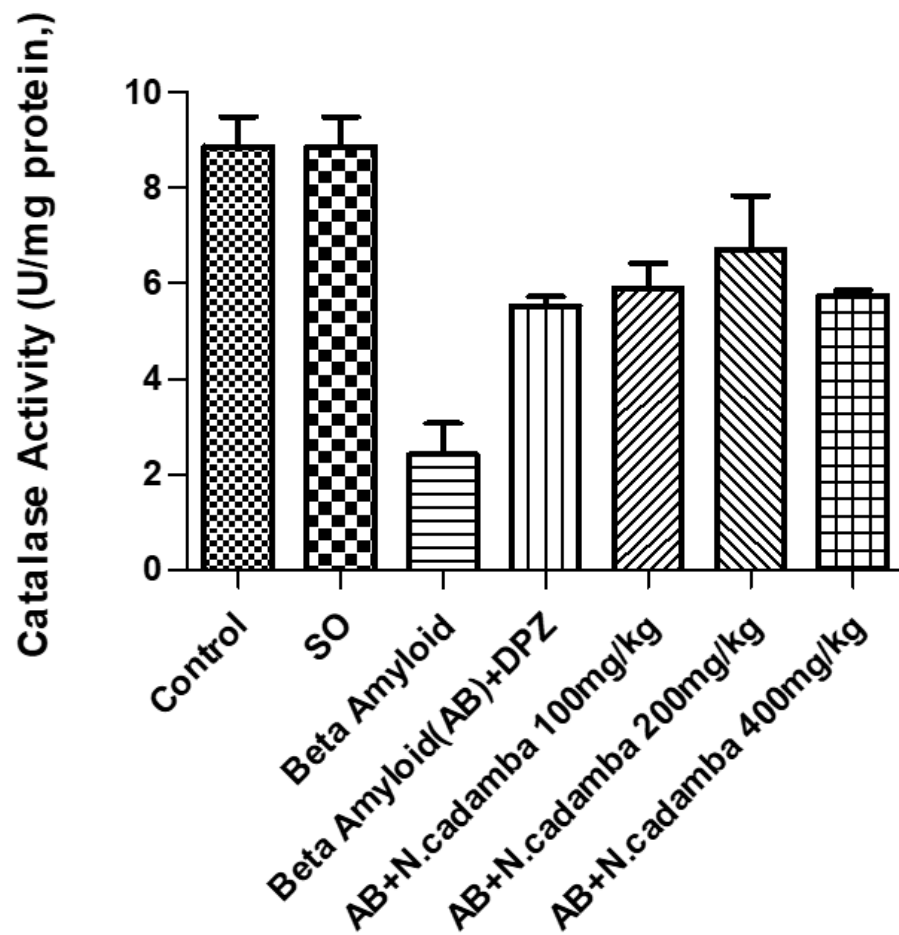


Figure 9

Catalase (CAT) activity in rat brain homogenate. $A\beta_{1-42}$ lowered CAT; *N. cadamba* restored activity ($p < 0.05$) in a dose-dependent manner.

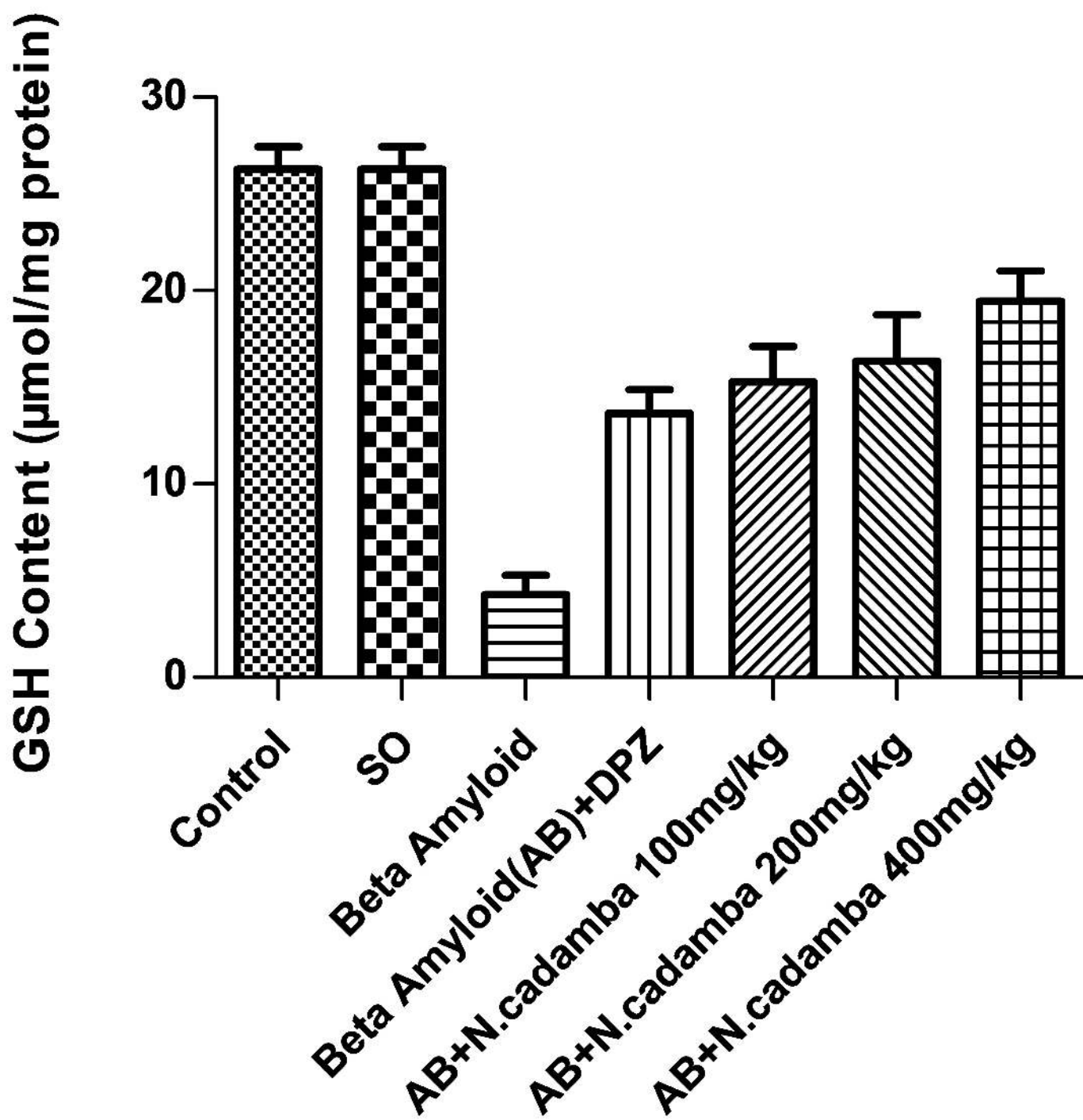


Figure 10

Reduced glutathione (GSH) levels. Depleted GSH in $\text{A}\beta_{1-42}$ rats elevated after *N. cadamba* administration ($p < 0.05$).

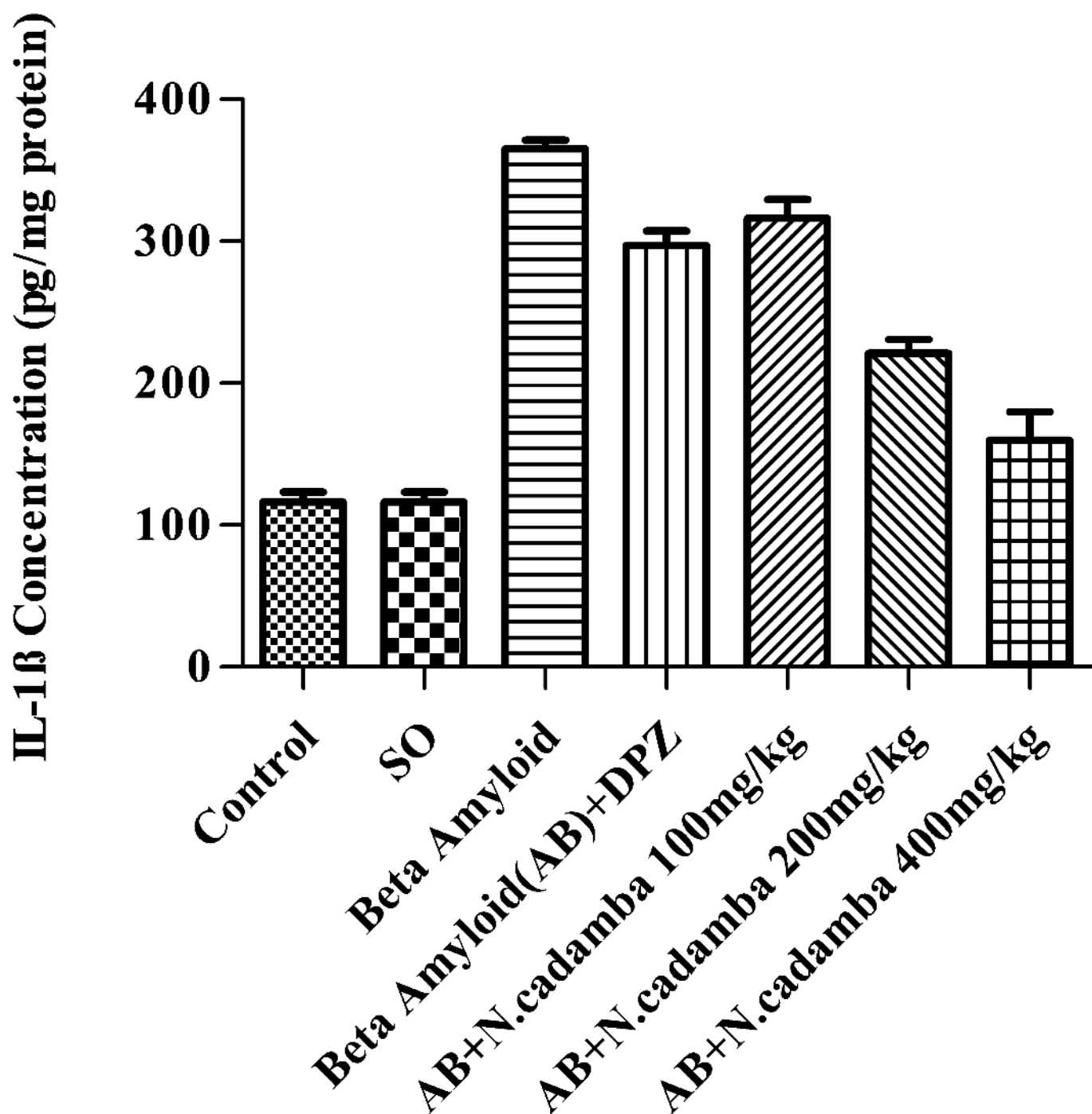


Figure 11

Interleukin-1 β (IL-1 β) levels. Neuroinflammatory rise in A β_{1-42} group decreased with *N. cadamba* (200-400 mg/kg) ($p < 0.05$).

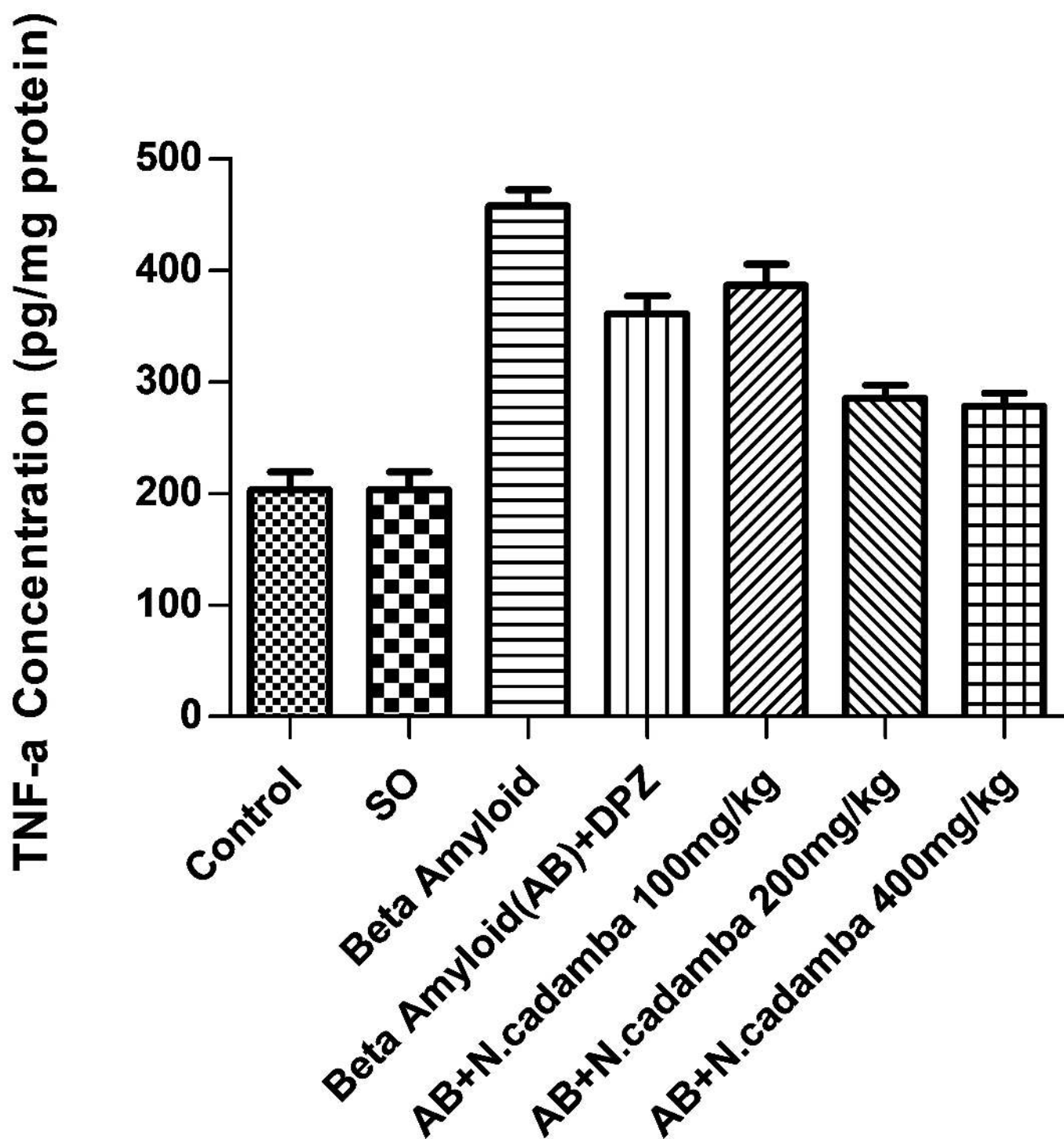


Figure 12

Tumor necrosis factor- α (TNF- α) levels. $A\beta_{1-42}$ significantly elevated TNF- α ; *N. cadamba* reduced cytokine levels dose-dependently ($p < 0.05$).