

Investigation of the neuroprotective effect of Butein against scopolamine-induced Alzheimer's like symptoms in rats

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Abstract

Objectives: Neuroprotective effect of Butein towards Scopolamine (SCO) induced Alzheimer's disease (AD), experimental rat models. Oral dosage of SCO increases the deposition of A β and cognitive dysfunctions. Butein is an abundantly phytochemical constituent of *Dalbergia odorifera*, *Caragana jubata*, and *Rhus verniciflua* Stokes has numerous biological activities such as neuroprotective, antioxidant, and anti-inflammatory properties.

Materials and methods: To conduct the study, the Wistar rat received Scopolamine (1 mg/kg) through the oral route on an alternative day for 14 days. Rats were treated at 3 different doses of Butein (25, 50, and 100 mg/kg, orally) and donepezil (5 mg/kg, orally) for fourteen days. Every week, the behavioural impairments were assessed. Afterwards, rats were sacrificed for biochemical analysis (MDA, Nitrite, and GSH).

Results: Our result indicated that Butein reduced SCO, causing cognitive impairment and biochemical and neurobehavioral changes. Butein enhanced neuroprotective impact on cognitive impairment may be owing to its antioxidant and anti-inflammatory, according to the findings.

Conclusion: Together these findings suggest that Butein's antioxidant and anti-inflammatory properties may have contributed to its neuroprotective effect, making it a promising therapeutic candidate for the treatment of cognitive impairment and AD-like symptoms.

Keywords: Alzheimer's disease; Butein; Scopolamine, Neuroinflammation; Oxidative stress.

1. INTRODUCTION

Alzheimer's disease (AD) is a neurological disorder that causes cognitive and memory impairments. There are roughly 5.8 million AD patients in the United States alone, projected to be over 14 million by 2050 (1). However, currently no treatment for this debilitating neurological disorder. Although, it is the most significant health care problem and has a significant socioeconomic effect because it causes emotional and financial stress to the patient and his family. Cholinergic, synaptic, and cognitive deficits, as well as an increase in amyloid (A) protein in senile plaques and hyperphosphorylated Tau protein in neurofibrillary tangles, are the hallmarks of AD (2). Although the causes of most AD are unknown, various hypotheses have been offered to explain the disease's pathophysiology. Furthermore, mutations in the genes for presenilin (PS1, PS2), apolipoprotein E (ApoE), and the amyloid precursor protein (APP) have been linked to AD pathology in several investigations (2). Antagonizing muscarinic receptors, scopolamine impairs cholinergic neurotransmission and causes memory loss associated with Dementia of the Alzheimer type (DAT). The animal model of scopolamine-induced dementia is frequently used to study cognitive disorders. On the other hand, scopolamine-induced dementia enhanced NF-kB protein expression in the cerebral cortex and hippocampus, suggesting that a cognitive problem in experimental rats may be caused by an increased inflammatory response caused by oxidative stress (3).

In addition, chalcones have an open C-ring structure belonging to the flavonoid group. Various chalcones and their derivatives exhibit biological effects (4). One of the potent chalcones derivatives is Butein, a plant polyphenol isolated from the duramen of *Dalbergia odorifera*, *Caragana jubata*, and *Rhus verniciflua* Stokes (RVS). It has been shown to have anticancer, antioxidant, anti-inflammatory, & antibacterial properties (5-8). It has been reported that its antioxidant property inhibits lipid peroxidation and superoxide anion generation in rat hepatocyte microsomes and decreases free radical-induced cytotoxicity (9).

Additionally, glutamate-cysteine ligase expression and glutathione levels are increased by butein in rat primary hepatocytes to reduce oxidative damage (10). Butein's potential as an antioxidant in AD has not yet been studied; thus, we have emphasized studying the mechanism and its function in AD rats. Nrf2 regulates the inflammatory response and maintains the cellular redox equilibrium. Therefore, using Nrf2-related genetic and pharmacological approaches, drug development for AD has been suggested. The activation of Nrf2 protects against AD pathogenesis (11).

Furthermore, Nrf2 activation protects cells from several diseases, including neurodegenerative disorders. The expression of the transcription factor Nrf2 (nuclear factor-erythroid 2-p45 derived factor 2) and its driving genes is reduced in AD brains as a change in Nrf2-related pathways. These data show that activating Nrf2 could be a new AD therapeutic. In addition, Nrf2 has also been demonstrated to interfere with several essential pathogenic processes in AD, including the A β and p-tau pathways, according to new research (12). The antioxidant defence system is controlled by the redox-sensitive Nrf2 (13). After that, Nrf2 activates heme oxygenase-1 (HO-1) in the nucleus, which safeguards the cell against oxidative stress (14-18). The previous investigation has linked oxidative and endoplasmic reticulum stress to an extracellular signal-regulated kinase (ERK). Importantly, Nrf2 is effectively activated by ERK1/2 (19). Based on the findings, it was postulated that butein might influence ERK/Nrf Signaling pathway-mediated oxidative stress injury in an AD rat model's cholinergic cells of the hippocampus. The experiments in this study were performed to how butein influenced oxidative stress injury in AD rats. Furthermore, we looked at the downstream pathway of butein in AD rats for the first time, which may offer new targets or a basis for comparison for improving AD outcomes.

2. Materials and Methods

2.1 Experimental Animals

2.1.1 Animals

All experimental protocols were approved by Institutional Animal Ethical Committee (IAEC), and experiments were carried out according to Indian National Science Academy (INSA) guidelines for the animal experiment. A total of 36 Wistar male rats (180-220gm) were obtained from Shambhunath Institute of Pharmacy, Prayagraj, Uttar Pradesh, India. Animals were housed in polyacrylic cages under standard laboratory conditions (room temperature 25°C \pm 2°C and 60 \pm 10% RH) with 12h light/dark cycle. The animal's diet was maintained with food and fresh water from commercial dry pallets. All behavioral parameters were measured between 09:00 hrs and 17:00 hrs, apart from those 3 animals housed in one cage.

2.1.2 Drugs and Chemical

The test drug Butein was purchased from TCI (India Pvt. Ltd.). All additional substances and reagents used in the investigation were of the highest analytical grade. The solution of scopolamine (Sigma-Aldrich, St. Louis, MO, United States) were prepared using 0.9% NaCl.

2.1.3 Experimental design and parameters

Alzheimer's disease was induced by the administration of Scopolamine (1mg/kg, oral) dissolved in normal saline and administered alternative day for 14 days. A total of 36 animals had randomly divided into 5 groups (n = 6). The test drug, i.e., Butein (25, 50, 100 mg/kg, p.o.) dissolved in normal saline, was administered for 14 days. It is well known neuroprotective and anti-inflammatory against AD. Before the protocol initiation, training was given to all rats for 6 days on Morris water maze, elevated plus maze, and Actophotometer. Behavioral parameters were assessed on per day basis. On day 22nd all the animals were sacrificed, and performed biochemical parameters which is mentioned on (Fig. 1).

Experimental design and Parameters

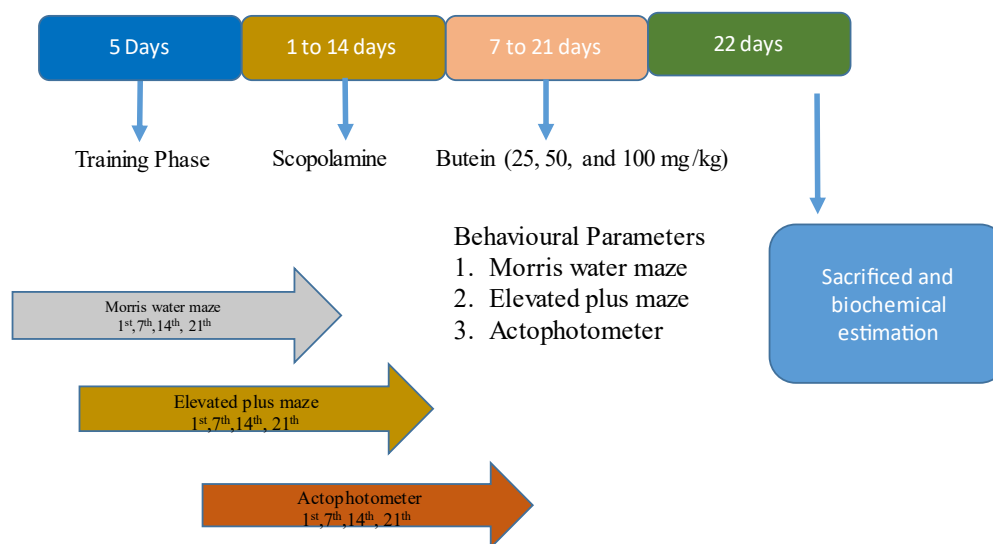


Fig.1. Study design

2.2 Behavioural Assessments

2.2.1 Morris Water Maze (MWM)

The MWM examined animals' spatial memory and learning (20). It comprises of a circular water tank with a height of 60 cm and a diameter of 180 cm, filled to a depth of 40 cm with water that is $(25 \pm 1^\circ\text{C})$. The water was made opaque by adding milk powder. At the centre of the pool, 4 quadrants were divided (North, south, east, and west) to make equally spaced locations around the edge of the pool. Below the 2 cm surface of the water, an escape platform (10 cm in diameter) pool was located. The animals' training sessions were taken for 4 days (days 1, 7, 14, and 21), with ongoing 4 trials per session (once from each beginning point), every test taking a maximum time of 120 seconds & a test break roughly 30 seconds (21). Afterward ascending onto the buried stage, the animals continued there aimed at 30 seconds previously the beginning of the following trails. After 24 hours of the acquisition phase, a probe test (day 12) was done for 60 seconds, and the % time spent in the target quadrant's earlier buried stage was noted. After the acquisition trail, time spent in the target quadrant indicated the degree of memory consolidation.

2.2.2 Actophotometer

All animals monitored their Spontaneous locomotor activity (SLA) on days 1, 7, 14, and 21. The ambulatory activity was recorded using a digital Actophotometer (INCO, India). They were individually placed in the Actophotometer chamber for 3 minutes habituation period before the locomotor task. The locomotor activity was measured for 5 minutes using an Actophotometer equipped with IR light-sensitive photocells, and data were presented in terms of count per minutes (22).

2.2.3 Elevated Plus Maze

The transfer latency time (acquisition memory) process was evaluated by applying the elevated plus maze (EPM). EPMs were conducted on the 1st, 7th, 14th, and 21st. The time it took for rats to travel from the open arm to either of the enclosed arms with all four legs crossing to the enclosed arm was recorded in order to calculate the acquisition transfer latency time. The rats were placed at the end of the open arm, facing away from the central platform. The rat did not enter the arm's enclosure in the 19s. The rat was given the 20s to go around the plus maze with its arms open and closed after the transfer latency time measurements. The maze was cleaned after every rat experiment (23).

2.3 Biochemical Analysis

On the 22nd day, all the biochemical parameters were examined in the brain tissue homogenate.

2.3.1 Brain homogenate preparation

Decapitated rats were sacrificed, and the brains were extracted and rinsed with ice-cold isotonic saline. In ice-cold 0.1 M

phosphate buffer, brain cortical and hippocampal tissue samples were homogenised 10 times (w/v) (pH 7.4). Supernatant aliquots were isolated and used for biochemical assessment after the tissue homogenate was centrifuged at 10,000 g for 15 minutes.

2.3.2 Protein estimation

The Lowry method and the Folin phenol reagent were used to quantify the protein (24). A protein's phenolic group of tyrosine and tryptophan residues (amino acids) generated a blue-purple colour complex when combined with the Folinocalteu reagent, which is made up of sodium tungstate, molybdate, and phosphate. An ELISA reader was used to measure absorbance at 660 nm (Model 680 Microplate Reader, Bio-Rad, Japan)

2.3.3 Assessment of nitrite levels

Brain nitrite levels were estimated by Giustarini et al., with some modifications. To assess nitrite levels, incubate the 96 well plates at 37°C for 30 mins after adding equal volumes of brain supernatant and Griess reagent (mixture of phosphoric acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride). The nitrate levels were measured using sodium nitrite as a standard, and the results were expressed in μM .

2.3.4 Estimation of malondialdehyde (MDA)

The MDA product of lipid peroxidase was evaluated in brain cortex and hippocampus tissue homogenates using the method of (K. Maryam et al.) (25). An ELISA reader determined the quantity of MDA after its reaction with thiobarbituric acid at 532nm (Model 680 Microplate Reader, Bio-Rad, Japan). MDA concentrations were measured using a standard curve and expressed as nmol/mg protein.

2.3.5 Estimation of reduced glutathione (GSH)

Hippocampal homogenate reduced glutathione levels were calculated using the technique described by Ellman et al. (26). 1 mL of supernatant was precipitated with 1 mL of 4% sulfosalicylic acid and cold digested for 1 hour at 4 °C. For 15 minutes, the sample was centrifuged at 1200 rpm, and 1 ml of supernatant was mixed with 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5' dithiobis (2-nitro benzoic acid) (DTNB). Utilizing an ELISA reader, the formed yellow colour was determined at 412nm (Model 680 Microplate Reader, Bio-Rod, Japan). Using a standard curve, the amount of glutathione in the supernatant was determined and expressed as mol/mg protein.

3. Statistical Analysis

The obtained data were expressed as mean \pm SD. A two-way variance analysis (ANOVA) was used to evaluate the behavioural and biochemical parameters, which were then analysed with Bonferroni's multiple comparison test. However, the biochemical and neuroinflammatory analyses were carried out using one way ANOVA and Post-hoc Tukey's test. It was concluded that the 0.05 p-value was statistically significant.

4. Results

4.1 Effect of Butein treatment on Morris water maze activity in scopolamine-treated rats

Scopolamine-treated rats showed significantly decreased escape latency time at the end of the 21st day compared to the normal control group ($p < 0.001$). Administration of Butein low (25 mg/kg, 50 mg/kg; orally) and high dose (100 mg/kg; orally) starting from the 7th to 21 days had suggestively enhanced the escape latency time activity as compared with the SCO treated group ($p < 0.05$). Furthermore administration of Butein (100 mg/kg) substantially increased the escape latency time as compared to Donepezil (5mg/kg) ($p < 0.05$) (Fig.2).

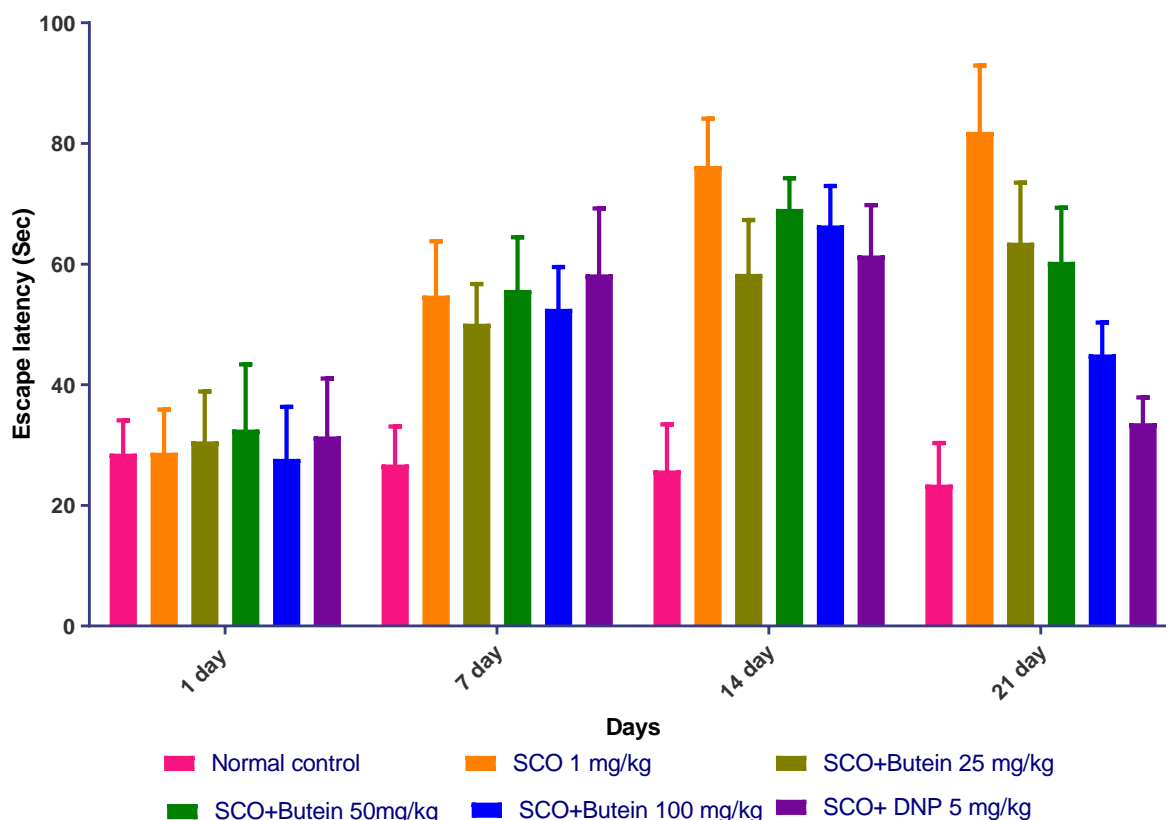


Fig.2. Effect of Butein treatment on Morris water maze activity in scopolamine-treated rats

Fig 2 Effect of Butein on escape latency time in SCO treated rats. Data are expressed as Mean \pm S.D. There was a significant change in escape latency time in Butein treatment doses (25, 50,100). Data were analyzed by two-way ANOVA followed by Bonferroni's multiple comparisons. NC, normal control; SC, sham control; SCO, Scopolamine

4.2 Effect of Butein treatment on Elevated plus Maze activity in Scopolamine-treated rats

SCO-treated rats showed a significant decrease in the transfer latency time at the end of the 21st day in contrast to a typical control group ($p < 0.001$). Administration of Butein low (25 mg/kg, & 50 mg/kg; orally) and high dose (100 mg/kg; orally) initially from the 7th to 21 days had substantially enhanced the transfer latency time activity as compared with the SCO treated group ($p < 0.05$). Moreover, administration of Butein (100 mg/kg) significantly increased the transfer latency time as compared to Donepezil (5 mg/kg) ($p < 0.05$) (Fig.3).

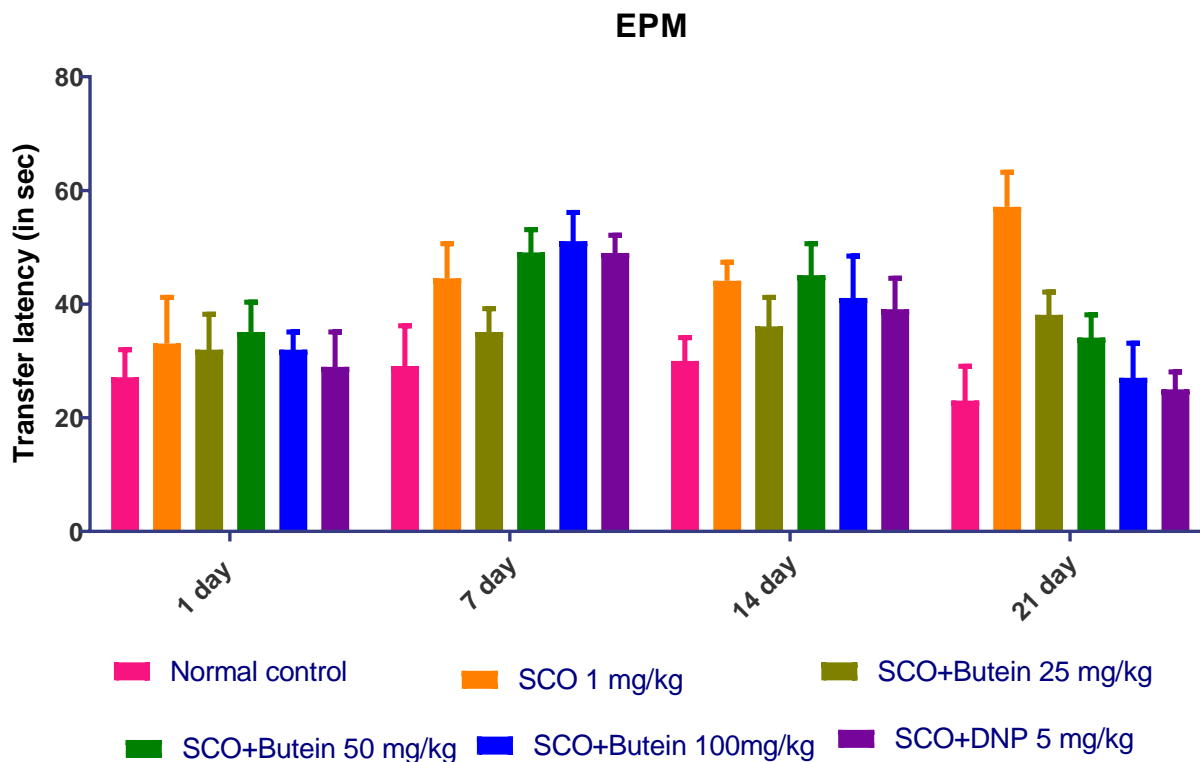


Fig.3. Effect of Butein treatment on Morris water maze activity in scopolamine-treated rats

Fig 3 Effect of Butein on transfer latency time in SCO treated rats. Data are expressed as Mean \pm S.D. There was a significant change in transfer latency time in Butein treatment doses (25, 50,100). Data were analyzed by two-way ANOVA followed by Bonferroni's multiple comparisons. NC, normal control; SC, sham control; SCO, Scopolamine.

4.3 Effect of Butein treatment on Actophotometer activity in SCO-treated rats

SCO-treated rats showed a remarkable decrease in the beam cross time at the end of the 21st day evaluated compared to the normal control group ($p < 0.001$). Administration of Butein with two doses (25 mg/kg, 50 mg/kg and 100 mg/kg; p.o.) starting from the 7th to 21st days significantly enhanced the beam cross-time activity as compared with the SCO treated group ($p < 0.05$). Additionally, administration of Butein (100 mg/kg) substantially increased the beam cross activity time as evaluated by comparing it to Donepezil (5 mg/kg mg) ($p < 0.05$) (Fig.4).

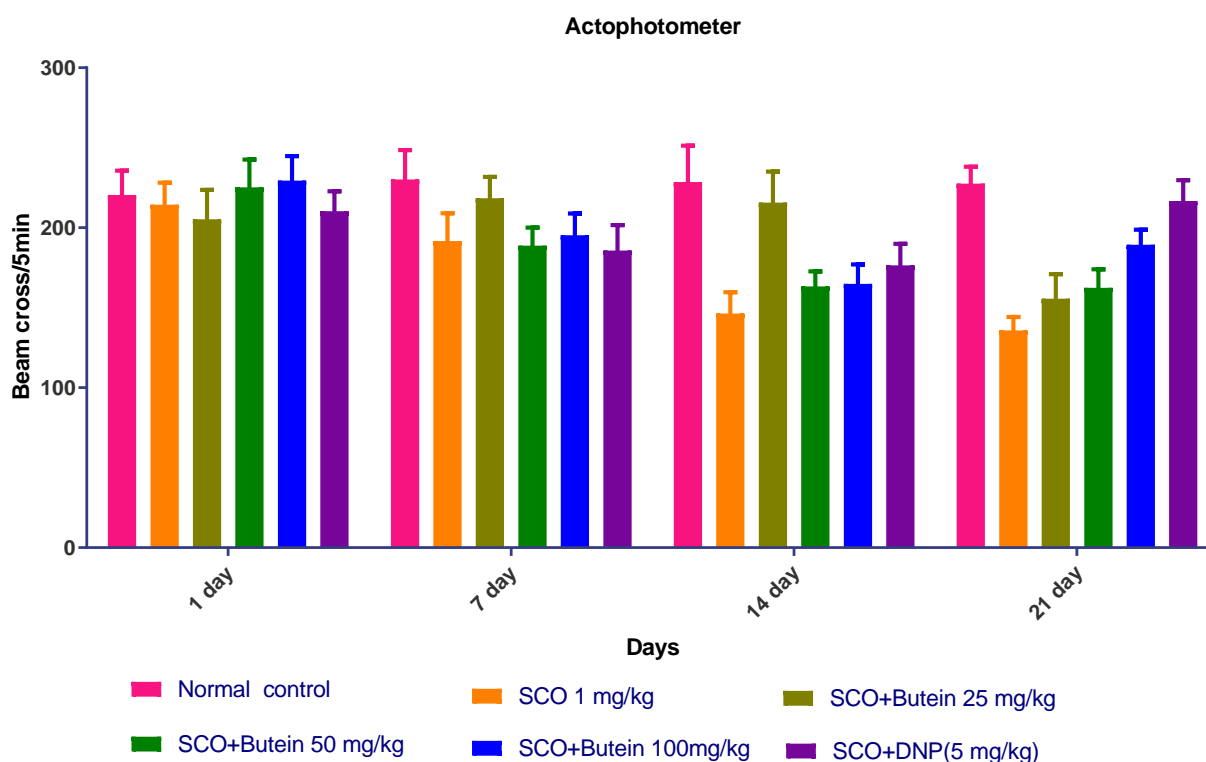


Fig.4. Effect of Butein treatment on Morris water maze activity in scopolamine-treated rats

Fig. 4 Effect of Butein on significantly enhanced the beam cross-time activity in SCO treated rats. Data are expressed as Mean \pm S.D. There was a significant enhanced the beam cross-time in Butein treatment doses (25, 50, 100). Data were analyzed by two-way ANOVA followed by Bonferroni's multiple comparisons. NC, normal control; SC, sham control; SCO, Scopolamine.

4.4 Protective effect of Butein on MDA, Nitrite, and GSH in SCO-treated rats

The level of MDA and nitrite was increased significantly with a reduction of GSH level in SCO treated i.p. compared to a normal control group. Further, treatment with two doses of Butein (25 mg/kg, 50 mg/kg and 100 mg/kg; p.o.) starting from 7 to 21 days substantially decreased MDA, nitrite, and GSH levels as compared to SCO Treated group. Moreover, administration of Butein (100 mg/kg; p.o.) had substantially decreased MDA, nitrite levels, and increased GSH levels as evaluated by comparing to Donepezil (5mg/kg) ($p < 0.05$) (Table 1).

Table 1. Effect of Butein on MDA, Nitrite, and GSH in SCO-treated rats

S. No	Groups	MDA ($\mu\text{g/ml}$)	GSH ($\mu\text{mol/mg}$)	Nitrite($\mu\text{g/ml}$)
1	Normal control	2.546 \pm 1.2498	29.567 \pm 1.8420	9.567 \pm 1.5942
2	Sham control	3.568 \pm 2.2692	26.769 \pm 2.9320	10.769 \pm 1.9300
3	SCO (1mg/kg)	14.903 \pm 1.0538 ^b	11.186 \pm 2.17800 ^b	31.876 \pm 2.14300 ^b
4	SCO+ Butein(50mg/kg)	10.1740 \pm 1.0987 ^{cd}	17.1740 \pm 2.1760 ^{cd}	23.2300 \pm 2.1500 ^{cd}
5	SCO+ Butein (100mg/kg)	5.8767 \pm 0.7654 ^{ce}	23.1767 \pm 1.7654 ^{ce}	17.7860 \pm 1.3120 ^{ce}
6	Donepezil	3.143 \pm 0.5854 ^{cf}	28.567 \pm 2.1760 ^{cf}	13.654 \pm 1.8100 ^{cf}

5. Discussion

This study showed that Butein has a neuroprotective effect against SCO-induced AD in rats. AD is a neurodegenerative

condition marked by cognitive decline brought on by protein buildup and excessive phosphorylation in the brain. (27-29). Similar pathological alterations were found when rats were given an i.p. injection of SCO and gradually lost memory, learning, and cognitive skills (30). The current investigation verified the experimentally produced SCO AD model regarding cognitive, and neuroinflammatory characteristics.

Furthermore behavioral tests, including the elevated plus maze, Actophotometer, and Morris water maze, were used to assess cognitive damage in SCO-treated rats. The SCO model showed a substantial reduction in actophotometer beam cross time, in the elevated plus-maze, transfer latency is increased, and increased escape latency in the Morris water maze in our study. These findings corroborated the learning and memory deficits in rats with SCO-induced AD. These alterations in behavioral factors are consistent with earlier research. On the other hand, Butein boosted learning and memory in rats in a dose-dependent manner.

According to studies, oxidative stress is a pathophysiological sign of AD (31, 32). An imbalance of antioxidants (like GSH), reactive nitrogen species, and reactive oxygen species have been linked to oxidative stress. Furthermore, ROS causes lipid peroxidation when it combines with polyunsaturated fatty acids. As a result, lipid peroxides create powerful chemicals like malondialdehyde (MDA), accelerating oxidant production.

Furthermore, ROS interacts with NO to form RNS, which are also involved in neuronal death (33). ROS has been linked to cholinergic system failure and cognitive impairment in AD (34). In the SCO model, the level of ROS has also been found to increase, along with a decrease in antioxidant levels (35). Similarly, in the current investigation, SCO administration increased MDA and nitrite levels while decreasing GSH, levels in rats. On the other hand, Butein's significant antioxidant properties have a neuroprotective effect. In addition, increasing GSH and SOD levels while lowering MDA and nitrite levels lessens neuronal damage.

6. Conclusion

In this work, the neuroprotective impact of butein against SCO-induced AD in rats was investigated. In the rat hippocampus, Butein reduced SCO, causing cognitive impairment and biochemical and neurobehavioral changes. Butein enhanced neuroprotective impact on cognitive impairments may be owing to its anti-inflammatory and antioxidant, according to the findings. The current investigation showed that butein has neuroprotective properties and may be used as a therapeutically drug to treat AD. However, understanding additional Butein molecular pathways or approaching the drug from different perspectives may yield beneficial results.

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Ethics Committee Approval: Approval received.

Authorship Contributions

Virendra Kushwaha was done the experiment, writing and data collection; Kuldeep Singh and Manoj Kumar Mishra were done the project design, data analysis and interpretation.

Conflict of Interest: No conflict of interest was declared by the authors.

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