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Butin attenuates behavioral disorders *via* cholinergic/BDNF/Caspase-3 pathway in scopolamine-evoked memory deficits in rats

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Abstract. – OBJECTIVE: Recent research suggests that butin may also exert neuroprotective effects. However, its influence on cognitive performance and, specifically, its potential to mitigate scopolamine-induced memory impairment remains unexplored. The aim of the study is to investigate the effects of butin on the cognitive and behavioral performance of rats with scopolamine-induced memory impairment.

MATERIALS AND METHODS: Scopolamine-injected memory-impediment model in rats was used to determine the efficacy of butin in higher and lower doses (10 and 20 mg/kg) for 14 days. Y-maze, along with Morris water, was used to assess the ability to recall spatial and working information. Biochemistry-related functions such as acetylcholinesterase, choline acetyltransferase, superoxide dismutase, glutathione transferase, malonaldehyde, catalase, nitric oxide and neurotransmitters levels were estimated as indicators of free radical damage. Furthermore, we evaluated neuro-inflammatory responses by assessing tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin-6 (IL-6), brain-derived neurotrophic factor (BDNF) and caspase-3 immuno-reactive proteins.

RESULTS: When assessed through behavioral paradigms, the butin-treated group enhanced the spatial and working memory of rodents. Scopolamine caused a substantial alteration in biochemical-related parameters, neuronal enzymatic, inflammation responses and apoptosis markers prominently restored by butin.

CONCLUSIONS: This study concludes that butin protects scopolamine-injected rats from behavioral impairments and neuronal damage by reducing apoptosis and neuroinflammation.

Key Words:
Antioxidants, Butin, Cytokines, Memory impairment, Neuroinflammatory, Scopolamine.

Introduction

Dementia refers to the loss of personal, intellectual, and behavioral functioning as a result of Alzheimer's disease (AD), a type of neurodegenerative disorder influencing older individuals. This type of condition results from the shrinkage of cortical areas comprising the decomposition of β-amyloid protein in the form of senile plaques and the generation of neurofibrillary triangles with a reasonable lack of cholinergic activities in the brain, as well as affecting other neurotransmitters. However, the pathophysiology of cholinergic injury is poorly understood, despite the idea that it has a significant role in cognitive impairment[1]. A decline in acetylcholine (ACh) levels can cause the dropping of cholinergic neurons in the hippocampus and other brain regions due to the loss of these neurons[2,3].

Scopolamine (SCOP) is commonly used to cause cognitive impairments with muscarinic cholinergic receptor antagonists, which act by obstructing muscarinic cholinergic receptors found in the brain. According to the scientific literature, SCOP, a non-selective muscarinic receptor block-

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er, impairs both the cognitive function of humans and animals due to its inhibition of the uptake of ACh at the postsynaptic level. As a nonselective muscarinic antagonist, SCOP is a tropane alkaloid isolated from plants. Both humans and animals heavily rely on the central neurotransmitter system for learning and memory functions[4]. Deficiencies in the memory of the old person result from a drop in cholinergic neurons and deprived ACh levels in the brain. This is in accordance with the hypothesis of the cholinergic system. As a result of blocking cholinergic signaling, this type of amnesia has been used as a method in pharmacological studies related to AD[5].

Previous studies[6,7] have proposed that SCOP-injected memory impairment is caused by blockage of the muscarinic receptors, and SCOP-triggered memory impairment is mediated by many neurotransmitter systems, including glutamatergic, adrenergic, dopaminergic, serotonergic, and histaminergic neurotransmitters, which can be improved by stimulating or blocking these neurotransmitters. This suggests that SCOP-induced memory impairment may be caused by these substances as well. Furthermore, SCOP triggers free radical damage, programmed cell death, and inflammatory responses in the brain[6]. As a result of flavonoids' ability to modulate several cellular corridors, including the mitogen-activated protein kinase and phosphoinositide 3-kinase/Akt signaling pathways, flavonoids have been shown to exert their effects. Further, through protein kinase inhibition, which is responsible for signal transduction, they also modulate nuclear factor-kappa B transcription factor, and by enhancing the expression of brain-derived neurotrophic factor (BDNF), they aid in survival and progress of neurons and synapses[4,8,9].

According to previous research, butin protects against intracerebral hemorrhage edema[10], has a ferroptotic effect through an antioxidant pathway[11], and improves conditions such as Huntington's disease produced by 3-nitropropionic acid[12], arthritis induced by Freund's adjuvant[13], and memory impairment induced by streptozotocin[14]. Therefore, butin is useful in conditions like AD or memory deficits evoked by SCOP. In a recent study[15], the effect of butin in memory deficit rats was studied with the estimation of probable mechanisms and controlling the associated complications. This study evaluates the protective effect of butin on behavioral and biochemical parameters in rats injected with SCOP. The biochemical parameters include cholinesterase (AChE) activity, choline acetyltransferase (ChAT) activity, gamma-aminobutyric acid (GABA) content, dopamine (DA) content, 5-hydroxytryptamine (5-HT) content, norepinephrine (NE) content, malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, glutathione (GSH) content, catalase (CAT) activity, interleukin-1β (IL-1β) content, interleukin-6 (IL-6) content, tumor necrosis factor-α (TNF-α) content, nuclear factor kappa B (NF-κB) activity, brain-derived neurotrophic factor (BDNF) content, nitric oxide (NO) content, and caspase-3 activity.

Materials and Methods

Animals

We housed male Wistar rats (150-180 g, 10-12 weeks old) under standard laboratory conditions encompassing (n=6) 12:12 h light: dark cycle modules with 24°C and 50-60% humidity controlled. Diets containing 60% fat were administered to rats, and they were free to drink water at will. No previous procedure was performed on the animals included in the study. For rats, standard laboratory conditions were used for acclimatization for seven days. The approval for conducting this experiment was permitted by the Institutional Animal Ethics Committee (IAEC/TRS/PT/023/028), India, and research was conducted as per the criteria mentioned in ARRIVE[16].

Chemicals

SCOP (Sigma-Aldrich, St. Louis, MO, USA) was used in the current protocol. IL-6, IL-1β, TNF-α, and caspase-3 were analyzed by ELISA kit (MSW Pharma, Chandrapur, Maharashtra, India). The other consumables utilized for the experiments were of analytical standards.

Experimental

We used simple randomization to assign 30 rats to each of the following groups (n=6), starting in January-May 2023. Rats were administered a fresh solution of SCOP (1 mg/kg) intraperitoneally (i.p.) in saline (pH 7.4) to induce memory impairment[17,18].

Group I- Control
Group II- SCOP-injected
Group III- SCOP-injected + butin 10 (mg/kg)
Group IV- SCOP-injected + butin 20 (mg/kg)
Group V- Butin 20 (mg/kg)

Rats were orally administered butin one hour before SCOP administration for 14 days in morn-

ing sessions. Morris water maze and Y-maze tests were performed 2.0 hours after SCOP administration on days 14-19 and days 14-15, respectively[19,20].

Behavioral Y-Maze Test

This test signifies the working memory. It records the random rearrangements that occur during the test. A wooden maze with three independent arms staggered by 120° on each side was used to construct a maze that measured 40 cm in length, 35 cm tall, and 12 cm wide. In addition, the walls of each tower were decorated with diverse designs and were referred to as X, Y, and Z. Each branch of the maze was divided into sections, so rats could explore them at their leisure and find their way out. Five minutes were dedicated to counting the number of visits to each arm during the experiment. As soon as the exercise was over, the device was wiped down with 10% ethanol to mitigate the smell generated by the device. An alternation of three sequential entries in three separate arms is considered random. Examples include XYZ, ZXY, and YZX. Thus, spontaneous alternation (SAP) percentages and total arms entries were calculated based on spontaneous alternation performance[18,21].

Morris Water Maze Test (MWMT)

There were four exactly equal quadrants or zones in the MWMT round tank. During the initial four days, an escape platform was located 1 cm beneath the liquid in each quadrant. There was a scatter of small white particles across the liquid's surface. In each of the three events of the learning exercise, one animal was randomly placed in a randomly sampled spot in the tank. To conduct the experiment, an animal was placed in a tank. We calculated the average escape latency every time the animal covered and stepped onto the platform. There was a maximum exposure time of 60 seconds; latency was noted if the animal did not reach the platform within 60 seconds after being initially pushed onto it. It was left on the stand between sessions for 20 seconds. The animals were delicately wiped and placed in their respective homes as soon as the tests were completed at all three entry points. The rats' memory of the position of the concealed platform for 60 seconds was assessed on the fifth day of the study. During this phase, the platform was removed from the tank. In addition, the time spent in the compartment and the latency time to find the proper quadrant were measured[22].

Biochemical Estimation

Brain homogenate

The brains of the animals were isolated and subjected to washing with ice-cold saline solution. These samples were added to a buffer solution with pH 7.0 and subjected to centrifugation at 1,000 rpm. The duration was 10.0 mins. A variety of parameters were evaluated using the supernatant.

Determination of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT)

AChE and ChAT activities in the brain were measured using commercial kits (MSW Pharma, Chandrapur, Maharashtra, India).

Determination of gamma amino butyric acid (GABA)

In this study, we applied the standard method of determining brain GABA content. In the presence of glutamate, GABA reacts with ninhydrin at alkaline pH to produce a fluorescent product. The fluorescence obtained was estimated at a wavelength of 380 nm with a Shimadzu spectrofluorometer (rf-1501 Shimadzu Japan)[18].

5-Hydroxy tryptamine (5-HT), Dopamine (DA), and Nor-epinephrine (NE) estimation

The ascertainment of 5-HT, DA, and NE was estimated using HPLC (Thermo Fisher Scientific, Mumbai, Maharashtra, India)[4].

Superoxide dismutase (SOD) estimation

SOD activity was tested by reducing nitroblue tetrazolium (NBT) by photochemical means to measure the activity of the enzyme. An additional 1.5 mL supernatant was added to each 1.5 mL reaction mixture containing 100 mM Tris/HCl (pH 7.8), 75 mM NBT, 2 M riboflavin, 6.0 mM EDTA, and 200 L of the reaction mixture. Detection of blue formazan was done by analyzing the change in absorbance at 560 nm (UV-1800 Shimadzu Japan) as a result of the formation of formazan. The SOD was quantified in U/mg[23,24].

Determination of glutathione (GSH)

GSH estimation was carried out using tissue homogenate produced in 0.1 M phosphate buffer pH 7.4. A 20% solution of trichloroacetic acid containing 1.0 mM EDTA was added to the homogenates to precipitate the proteins. After centrifuging the mixture, it was allowed to stand for 5.0 minutes. The supernatant (200 μL) was

then transferred to a freshly cleaned set of test tubes, and 1.8 ml of Ellman's reagent (5, 5'-dithio bis-2-nitrobenzoic acid) (0.1 mM) was prepared in 0.3 M phosphate buffer with 1 % sodium citrate solution). Then, all the test tubes were filled to a volume of 2.0 mL. After completion of the total reaction, the solutions were measured at 412 nm against a blank (UV-1800 Shimadzu Japan). Absorbance values were compared with a standard curve generated from a standard curve of known GSH. The concentration was measured as U/mg[25].

Catalase (CAT) estimation

Among the components of the reaction mixture were 150 µL buffer (phosphate) (0.01 M, pH 7.0), and 100 µL supernatant. The reaction was commenced by adding 250 µL of H_2O_2 0.16 M at 37 °C for 1.0 minute; the reaction was stopped by adding 1.0 mL of dichromate acetic acid reagent. The tubes were immediately kept in a boiling water bath for 15 mins. A spectrophotometer (UV-1800 Shimadzu Japan), 570 nm was used to measure the green color developed during the reaction. Parallel processing was also carried out with tubes devoid of enzymes. Catalase activity was calculated using the difference in absorbance per unit to measure catalase activity in U/mg[26].

Malonaldehyde (MDA) estimation

The test tubes were filled with 500 ml of homogenate and the control tube with 500 µl Tris-HCl buffer (50 mM, pH 7.4). In each tube, 250 ml of trichloroacetic acid (TCA) 20% and 500 µL of TBA 0.67% was added. Tubes were incubated for 10 minutes in a water bath at 90°C after sealing with a glass stopper. Tubes were cooled at room temperature and centrifuged for 15 minutes at 3000 rpm. The concentration was determined at 530 nm against a control (UV spectrophotometer) (UV-1800 Shimadzu Japan). The amounts of MDA were estimated as (nmol/L)[27].

Determination of neuroinflammatory cytokines

The quantification of IL-1β, IL-6, and TNF-α was achieved by standard ELISA kits and as per the standard procedures. The amounts of IL-1β, IL-6, and TNF-α were estimated as pg/mL and NF-κB was calculated as ng/mg.

Nitric oxide (NO) estimation

Oxidation of NO results in nitrite formation, which is used to calculate NO. An assay for nitrite formed after oxidizing NO was conducted, as mentioned in diazotization reactions[28].

Brain derived neurotrophic factor (BDNF) and caspase-3 estimation

The BDNF and caspase-3 activity was estimated by employing a biochemical test with the help of an ELISA kit.

Statistical Analysis

GraphPad Prism software (Version 8.0, San Diego, CA, USA) was used to perform the analysis, which was displayed as standard error mean (SEM). Shapiro-Wilk normality test was used to confirm the validity and distribution of the data, using a One-way analysis of variance (ANOVA). One-way ANOVA followed by Tukey's post-hoc test was applied to groups for data analysis. Statistical significance was determined by a p-value lower than 0.05.

Results

Y-Maze Test

As depicted in Figure 1A-B, it was found that SCOP-injected rats decreased the SAP as well as total arm entries to a substantial extent $p<0.01$ in association with to control group. The butin treatment significantly increased the SAP and total arm entries in rats F (4, 25) = 7.564, (p=0.0004)]. The butin 20 group has no significant effect compared to the control group.

Morris Water Maze Test (MWMT)

The MWMT assessed cognitive function. As a result of the 20 learning trials, all groups of trained rats experienced a decrease in their escape latency. On days 2, 3, 4, and 5, consecutively, SCOP-injected rats displayed a substantial difference from control rats ($p<0.001$). However, treatment significantly affected escape latency ($p<0.0001$) compared to SCOP-injected rats. Post-hoc tests disclose that SCOP-injected rodents after butin treatment exited significantly [F (4, 125) = 190.7, ($p<0.0001$)] (Figure 2A). The butin 20 group did not exhibit any effect.

It was explored from Figure 2B that the SCOP-injected rats exhibited a substantial drop ($p<0.001$) in time spent in the target quadrant in context to control, whereas butin treated rats exerted a significant increase in time spent in target quadrant [F (4, 25) = 36.94, ($p<0.0001$)]. Butin 20 group was free from any effect.

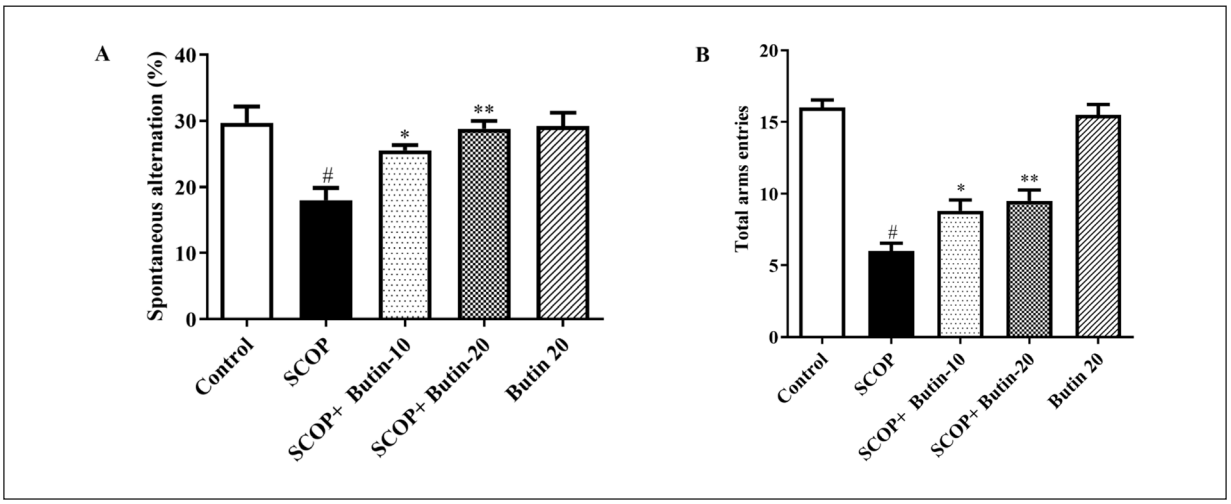

Figure 1. Shows the effect of butin (**A**) SAP (**B**) Total arm entries. Mean ± S.E.M. (n = 6). One-way ANOVA followed by Tukey's post analytic test. $^{\#}p < 0.001$ *vs*. normal, $^{*}p < 0.05$ and $^{**}p < 0.001$ *vs*. SCOP control.

Butin Effects on Acetylcholinesterase (AChE) and Choline Acetyltransferase (ChAT)

From Figure 3A-B it was evident that SCOP-injected rats increased the AChE activity of the brain to a substantial extent (p<0.001) in association with control rats. Butin treatment resulted in the decline of AChE activity in the brain to a considerable extent [F (4, 25) = 23.59, (p<0.0001)]. Butin 20 group had no significant effect on AChE levels as compared to the control group.

The activity of ChAT was reduced notably in SCOP-injected rats (p<0.001) in context to control. Treatment with butin raised the levels of ChAT to a significant extent [F (4, 25) = 11.39, (p<0.0001)]. Butin 20 group did not produce a significant effect on ChAT levels as compared to the control group.

Malonaldehyde (MDA) and Antioxidant Enzymes

Figure 4A indicates that the SCOP-injected rats had enhanced MDA levels compared to the control group. (p<0.001). The butin brought down the increased levels of MDA to a significant extent [F (4, 25) = 36.32, p<0.0001)]. It was

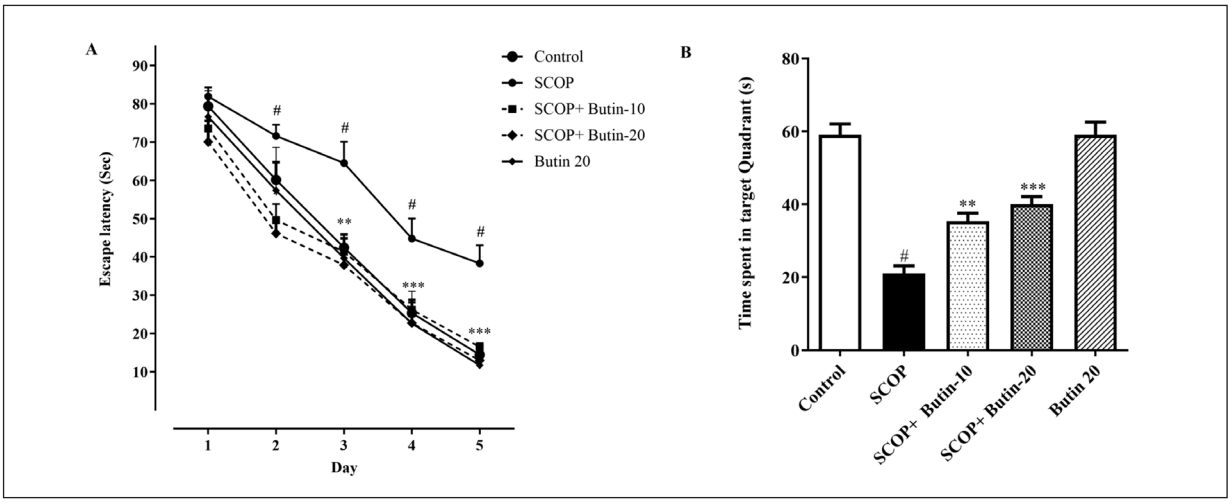

Figure 2. Shows the effect of butin on (**A**) Escape latency and (**B**) Time spent in quadrant. Mean ± S.E.M. (n = 6). Two-way ANOVA followed by Bonferroni post analytic test. $^{\#}p < 0.001$ *vs*. normal, $^{**}p < 0.001$ and $^{***}p < 0.0001$ *vs*. SCOP control.

also noted that butin 20 group lacked any effect compared to the control group.

As provided in Figure 4B-D SCOP-injected rats displayed a measurable cut-off in the functioning of SOD, GSH, and CAT (p<0.001) in association with to control group. Butin treatment elevated the amounts of these declined enzymes in SCOP-injected rats to a significant extent [F (4, 25) = 7.805, (p=0.0003)], [F (4, 25) = 11.00, (p<0.0001)], and [F (4, 25) = 9.486, (p<0.0001)]. There was no effect on antioxidant enzymes and MDA levels in butin 20 group compared to the control group.

Effect on Gamma Amino Butyric Acid (GABA)

SCOP-injected rats significantly reduced the GABA concentrations in the brain (p<0.001) in association with to control group; treatment with butin exhibited a marked rise in GABA content of the brain [F (4, 25) = 4.614, (p=0.0063)]. However, butin 20 group did not show any substantial effect on GABA levels compared to the control group (Figure 5A).

Impact of Butin on 5-Hydroxy Tryptamine (5-HT), Dopamine (DA), and Nor-epinephrine (NE)

As per the details in Figure 5B-D, it was noted that the SCOP-injected rats displayed a remarkable decrease in brain 5-HT, DA, and NE amounts in association with the control group. Butin treatment restored 5-HT, DA, and NE levels compared to SCOP-injected rats [F (4, 25) = 38.17, (p<0.0001)], [F (4, 25) = 23.54, (p<0.0001)], and [F (4, 25) = 32.29, (p<0.0001)]. Butin 20 group did not show any effect in brain 5-HT, DA, and NE amounts compared to the control group.

Effect of Butin on Inflammatory Parameters

The amounts of IL-1β, IL-6, and TNF-α were substantially shot up (p<0.001) in the SCOP-injected group in association with the control group. Treatment with butin declined the IL-1β, IL-6, and TNF-α amounts to a considerable extent [F (4, 25) = 66.72, (p<0.0001)], [F (4, 25) = 13.80, (p<0.0001)], and [F (4, 25) = 12.21, (p<0.0001)] (Figure 6A-C). Butin 20 group had no significant effect on IL-1β, IL-6, and TNF-α levels compared to the control group.

Nuclear Factor Kappa-B (NF-κB)

From Figure 6D it was observed that SCOP-injected rats significantly increased (p<0.001) the NF-κB levels compared to the control group. However, butin treatment at both doses showed a significant decrease in NF-κB levels compared to SCOP-injected rats [F (4, 25) = 10.14, (p<0.0001)]. The butin 20 group did not show any significant effect on NF-κB levels compared to the control group.

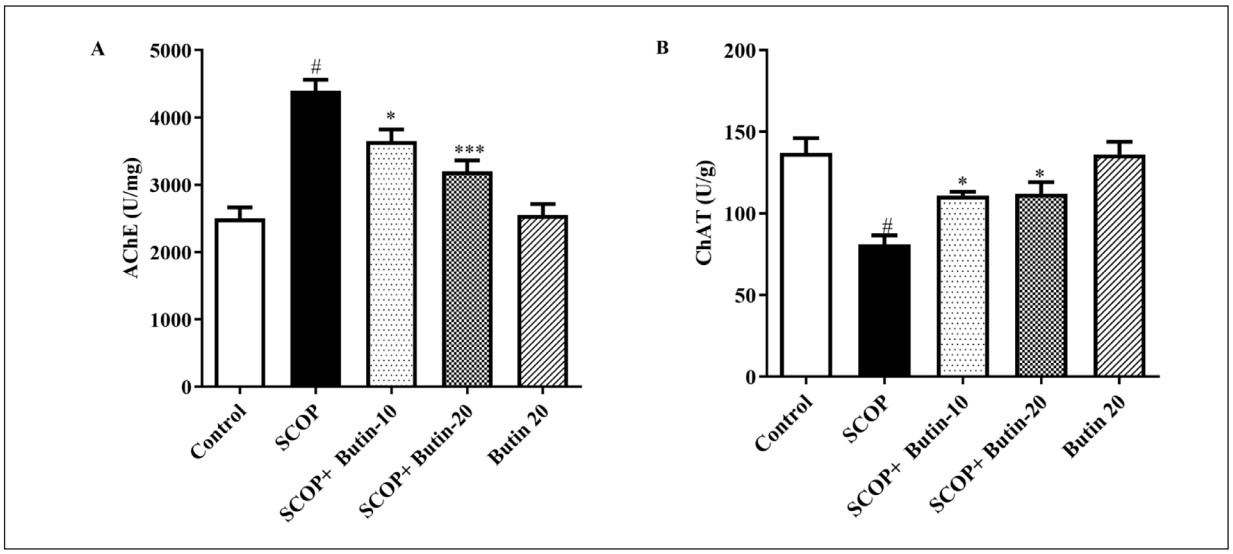

Figure 3. Shows the effect of butin on (**A**) AChE and (**B**) ChAT level. Mean ± S.E.M. (n = 6). One-way ANOVA was followed by Tukey's test. $^{\#}p < 0.001$ *vs*. normal, $^{*}p < 0.05$, and $^{***}p < 0.0001$ *vs.* SCOP control.

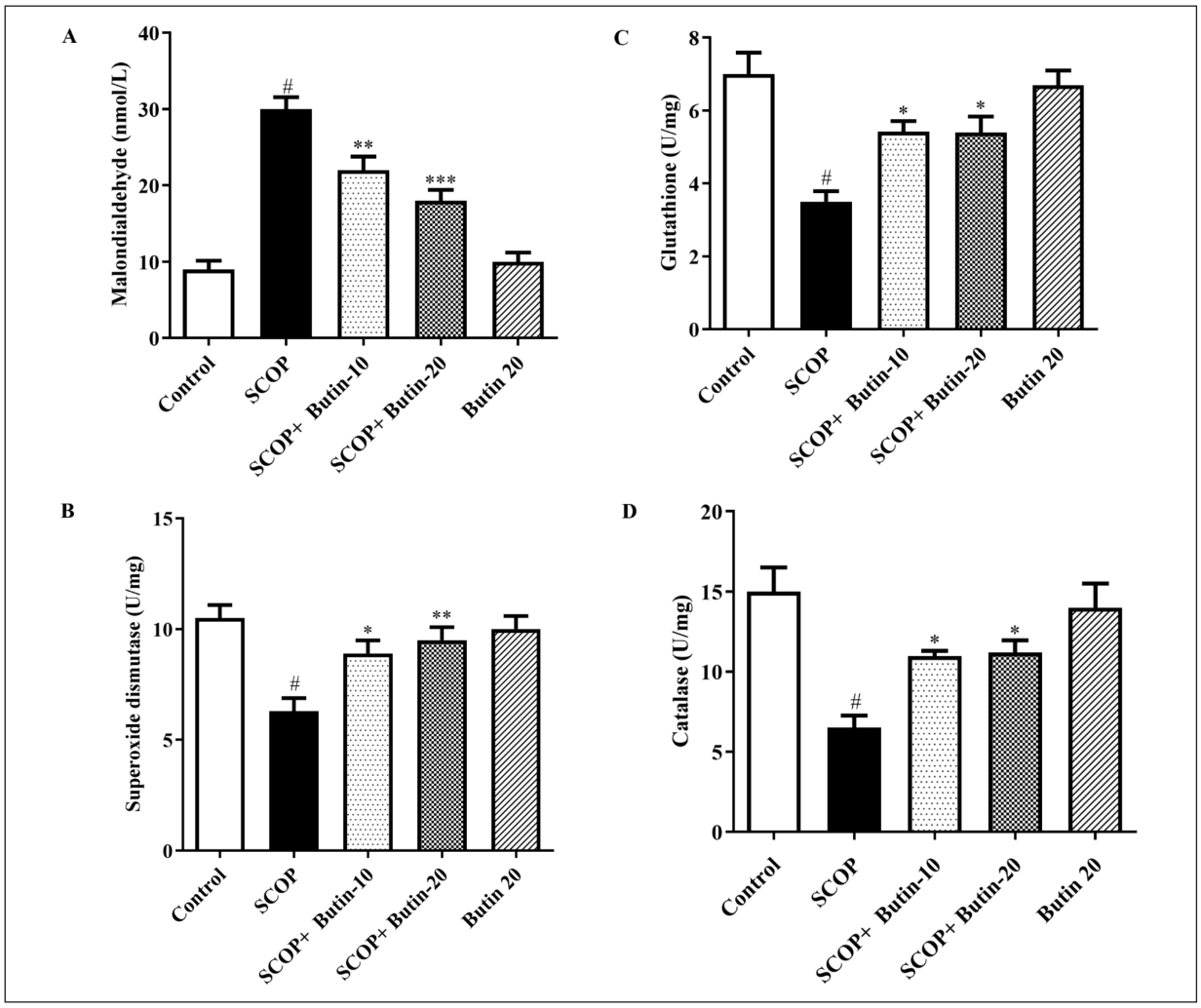

Figure 4. Shows the effect of butin on (**A**) MDA (**B**) SOD (**C**) GSH and **(D)** CAT level. Mean ± S.E.M. (n = 6). One-way ANOVA was followed by Tukey's test. $^{\#}p < 0.001$ *vs*. normal, $^{*}p < 0.05$, $^{**}p < 0.001$ and $^{***}p < 0.0001$ *vs.* SCOP control.

Nitric Oxide (NO) Levels

Figure 7 shows that SCOP-injected rats have significantly increased NO levels ($p<0.001$) compared to the control group. Moreover, butin treatment at both doses showed significantly decreased NO levels as associated with SCOP-injected rats [F (4, 25) = 15.23, ($p<0.0001$)]. Butin 20 group had no significant effect on NO levels compared to the control group.

Brain-Derived Neurotrophic Factor (BDNF) and Caspase-3 Activity

The SCOP-injected rats were found to have increased levels of BDNF to a substantial level ($p<0.001$) in association with the control group. Butin treatment resulted in significantly decreased BDNF levels as associated with SCOP-injected rats [F (4, 25) = 16.07, ($p<0.0001$)] (Figure 8A). The butin 20 group did not produce any significant effect on BDNF levels as compared to the control group.

From Figure 8B, it was noted that the caspase-3 function was substantially enhanced by SCOP ($p<0.001$) in association with control. Both doses of butin significantly decreased caspase-3 activity relative to a control group [F (4, 25) = 55.66, ($p<0.0001$)]. The butin 20 group had no significant effect on caspase-3 levels compared to the control group.

Discussion

AD is one of the most common disorders prevailing in developed countries affecting ma-

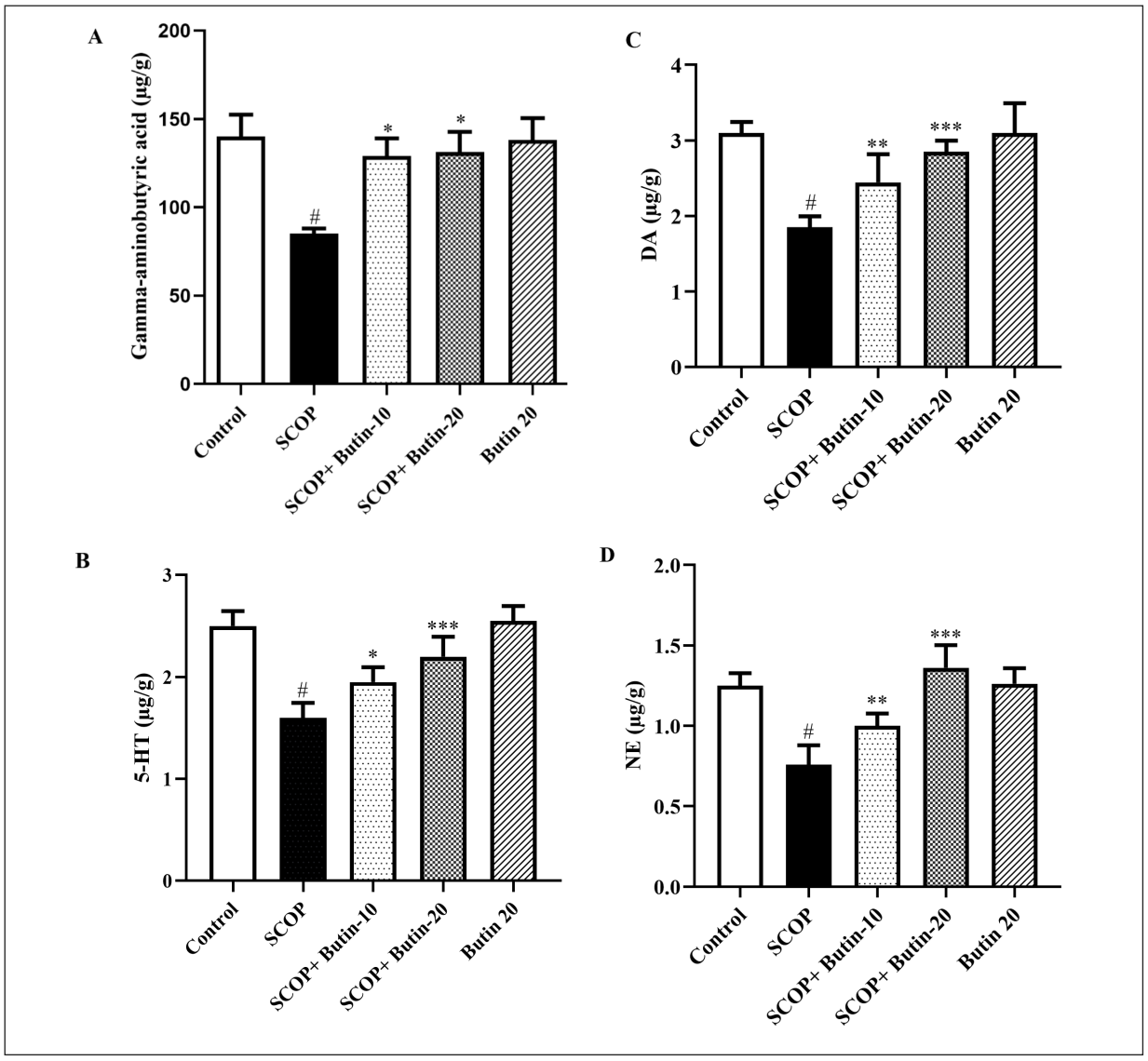

Figure 5. Shows the effect of butin on (**A**) GABA (**B**) 5-HT (**C**) DA and (**D**) NE level. Mean ± S.E.M. (n = 6). One-way ANOVA was followed by Tukey's test. $^{\#}p < 0.001$ *vs*. normal, $^{*}p < 0.05$, $^{**}p < 0.001$ and $^{***}p < 0.0001$ *vs*. SCOP control.

ny people every year. The disease is associated with the disturbance in neurological functions, causing loss of memory, thereby affecting various functions of the brain as well as neurotransmitters. Earlier studies[1-3] reported that the basic mechanism associated with AD is the formation of β-amyloid plaque, decreased activity of BDNF, and many more.

This study used a rat model injected with SCOP as a research model. The data from past research showed that the consequences of SCOP on the nervous system are directly proportional to its toxic properties. These are toxic to newborn neurons and immature granular cells, resulting in neural injury and cognitive deficits[29,30]. Antagonizing muscarinic cholinergic receptors associated with working memory, which is necessary for performing complex cognitive tasks such as reasoning, comprehension, and learning, was a classical use of SCOP. Moreover, working memory deficits are associated with AD[18,21].

In the present study, the effect of butin was investigated against SCOP-injected memory deficits in rats at both doses. Butin was effective in restoring memory loss and associated parameters to maintain up to normal levels. The probable

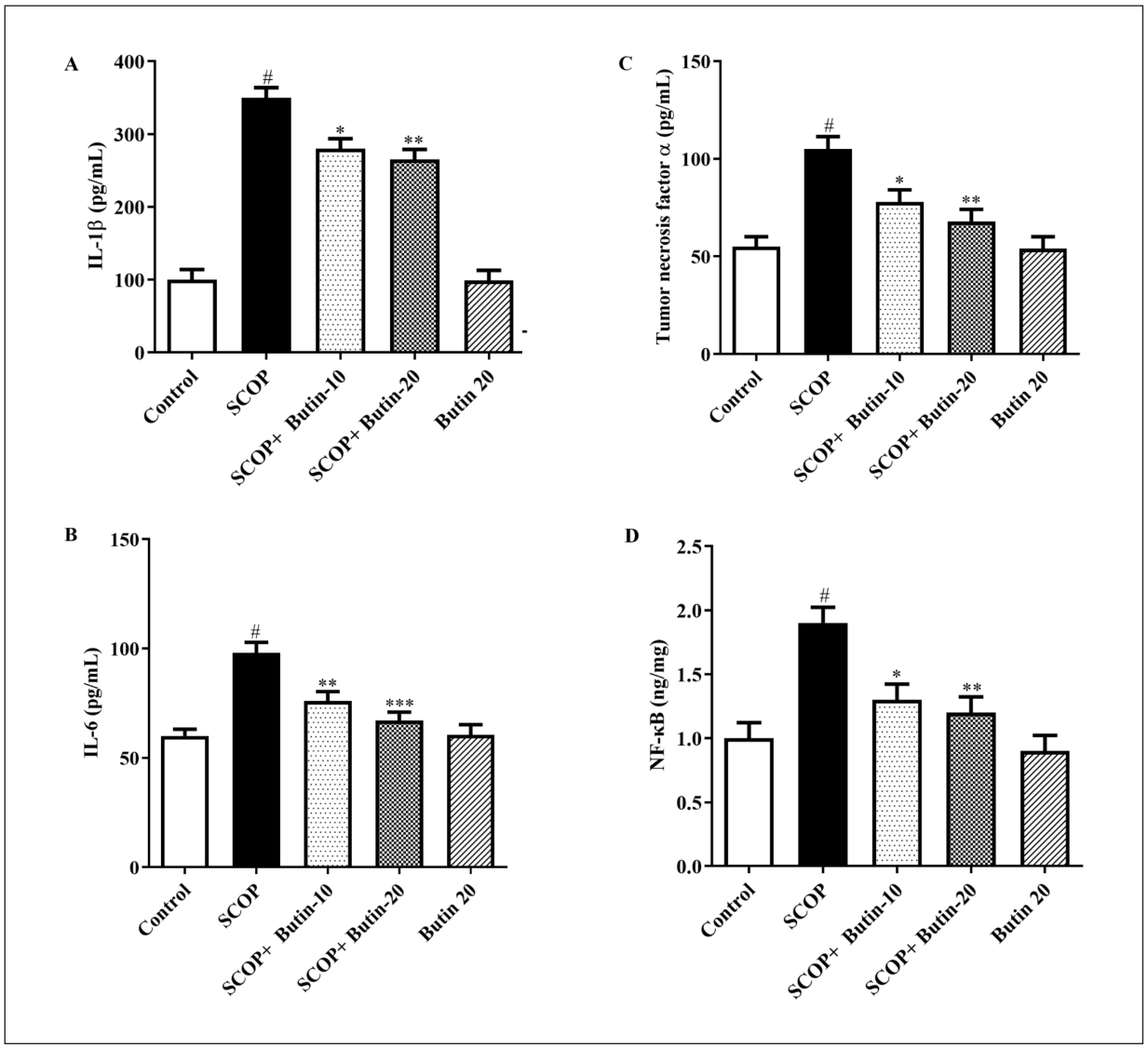

Figure 6. Shows the effect of butin on (**A**) IL-1β, (**B**) IL-6 (**C**) TNF-α and (**D**) NF-κB level. Mean ± S.E.M. (n = 6). One-way ANOVA was followed by Tukey's test. $^{\#}p < 0.001$ *vs*. normal, $^{*}p < 0.05$, $^{**}p < 0.001$ and $^{***}p < 0.0001$ *vs.* SCOP control.

mechanism of action of butin was found to be the inhibitor of cholinesterase prevention and degradation of free radicals and to maintain the normal levels of neurochemicals and neuroinflammatory cytokines[31-33].

In this study, the behavioral parameters were chiefly estimated with the help of the Y-maze and MWMT. The rodents were trained well in these apparatuses to incorporate the learning memory before treatment. These trained rats were injected with SCOP followed by treatment with butin[34,35]. It was observed that in the Y-maze test, the SCOP-injected group exhibited a decline in SAP as well as total arm entries, which indicated that the rodents were suffering from loss of memory. It was further explored that the treatment with butin brought up the SAP and total arm entries exhibiting the enhancement in the behavioral study of rodents at both the doses[15,36,37].

The SCOP-injected rodents exerted a marked decrease in escape latency, which showed an impairment in cognitive function, which was later on brought to normal by butin, indicated by an increase in escape latency. The second parameter in the MWMT was the time spent in the target quadrant by the rodents. The SCOP-injected rats

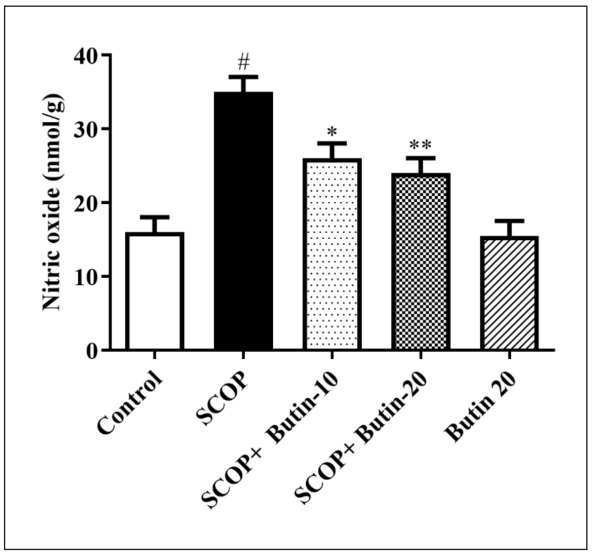

Figure 7. Shows the effect of butin on NO level. Mean ± S.E.M. (n = 6). One-way ANOVA was followed by Tukey's test. $^{\#}p < 0.001$ *vs*. normal, $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* SCOP control.

showed the least time spent in the target quadrant, indicating an impairment in spatial learning and memory. Butin treatment raised the time spent in the target quadrant exhibiting the enhancement in cognitive functions[6,23,38].

The prior research claimed that ACh has a crucial character in memory enhancement as well as function. The reduced levels lead to loss of memory. The Ach is synthesized by the enzyme ChAT and degraded by AChE[39,40]. SCOP-injected rodents were found with decreased levels of ChAT and high amounts of AChE, which indirectly resulted in lowering the levels of Ach, resulting in memory deficits. Butin was proved to be effective in bringing back the levels of these enzymes, butin reduced the enhanced amounts of AChE and elevated the declined amounts of ChAT, maintaining the normal concentration of ACh in the brain, thereby imparting a boosting effect in the recovery of impaired memory[41-43].

MDA is the marker of oxidative stress, resulting in free radical generation and causing the worsening condition in the body. Oxidative stress further contributes to memory loss. SCOP-injected rats showed an increase in MDA levels, while butin declined these elevated levels substantially. The formation of free radicals and stress from oxidation leading to oxidation is prevented with the help of antioxidant enzymes. The SCOP-injected rats were decreased amounts of antioxidant enzymes SOD, GSH, and CAT. The butin boosted these reduced concentrations of SOD, GSH, and CAT to normal[9,33,44].

Rats injected with SCOP had lower levels of GABA in the brain, which played a key role in memory deficit. It has also been reported that lower levels of GABA damage nerve cells and cause aging[43,45,46]. Treatment with butin raised the amounts of GABA in the brain to a substantial extent. The study found that rats injected with SCOP had lower levels of 5-HT, DA, and NE.

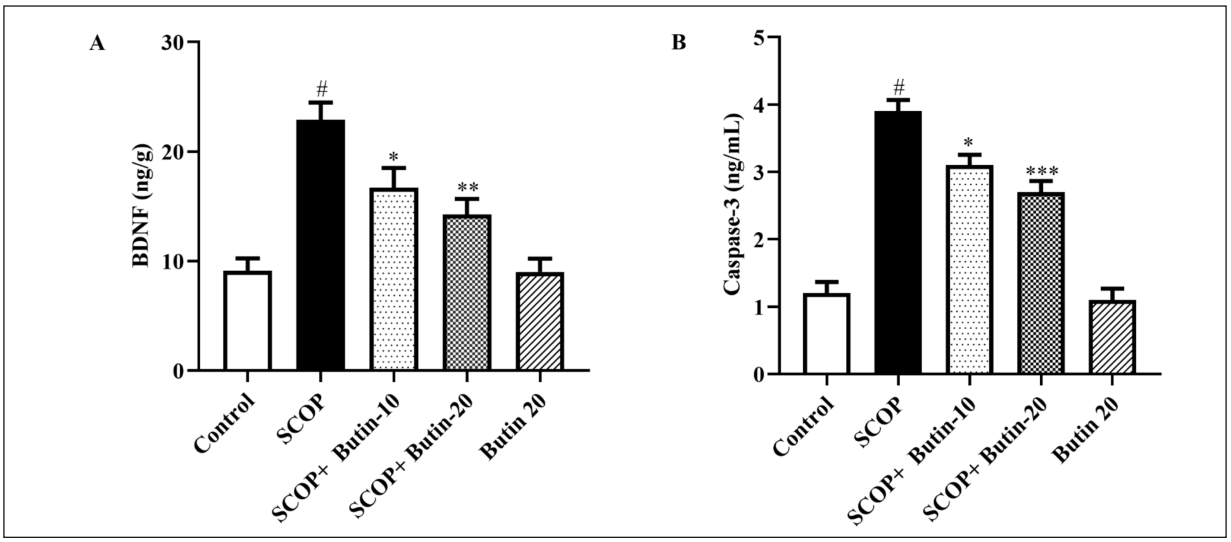

Figure 8. Shows the effect of butin on (**A**) BNDF and (**B**) Caspase-3 level. Mean ± S.E.M. (n = 6). One-way ANOVA was followed by Tukey's test. $^{\#}p < 0.001$ *vs*. normal, $^{*}p < 0.05$, $^{**}p < 0.001$ and $^{***}p < 0.0001$ *vs.* SCOP control.

These neurotransmitters are involved in memory function, and their decrease could contribute to the observed memory impairment. The butin remarkably restored 5-HT, DA, and NE levels[36,37].

The levels of neuro-inflammatory cytokines were markedly increased in SCOP-injected rats resulting in inflammation of brain cells and promoting memory impairment. Butin lowered these raised concentrations of IL-1β, IL-6, and TNF-α to normal. NF-κB was also responsible for generating an inflammatory response and SCOP-injected rats had increased levels of NF-κB in the present study. Butin significantly declined the levels of NF-κB to a significant extent[47,48].

SCOP significantly increases NO levels in the brain, whereas butin treatment decreases NO levels and enhances learning memory[19,26,37].

BDNF is the factor responsible for memory enhancement by increasing the storage capacity in the brain. The SCOP-injected rats showed reduced levels of BDNF that were reversed by treatment with butin, which increased the levels of BDNF to normal[18,49].

Caspase-3 causes damage to brain cells resulting in cell death. The SCOP-injected rats considerably increased the caspase-3 activity. Administration of butin in both dosages was associated with reduced caspase-3 expression and prevented brain cell loss[47,50]. From the study's results butin, as a natural compound, may be helpful in conditions such as Alzheimer's by preventing or treating memory deficits *via* restoring AChE, ChAT, GABA, NO, neurotransmitters, lipid peroxidation, antioxidants and neuroinflammatory markers, NF-κB, BDNF, and caspase-3 levels (Figure 9). Butin's ability to affect cognitive function requires further research, as well as its potential therapeutic activities. Conducting additional studies using cell-based assays, Western blot analyses, and gene expression analyses can be used to understand further how butin impacts cellular processes and molecular mechanisms. This study had the limitation of being conducted over a short period and only using a small number of animals.

Conclusions

Recent research on the effects of butin on scopolamine-induced memory impairment in rodents suggests that it may be an effective treatment for memory deficit, AD, and related disorders. Butin may work by controlling various neurochemicals, such as AChE and ChAT, neuroinflammatory cytokines, and oxidation potential. Further studies are needed to confirm these findings. However, butin appears to be a safe and effective natural source of treatment for memory deficit and related disorders.

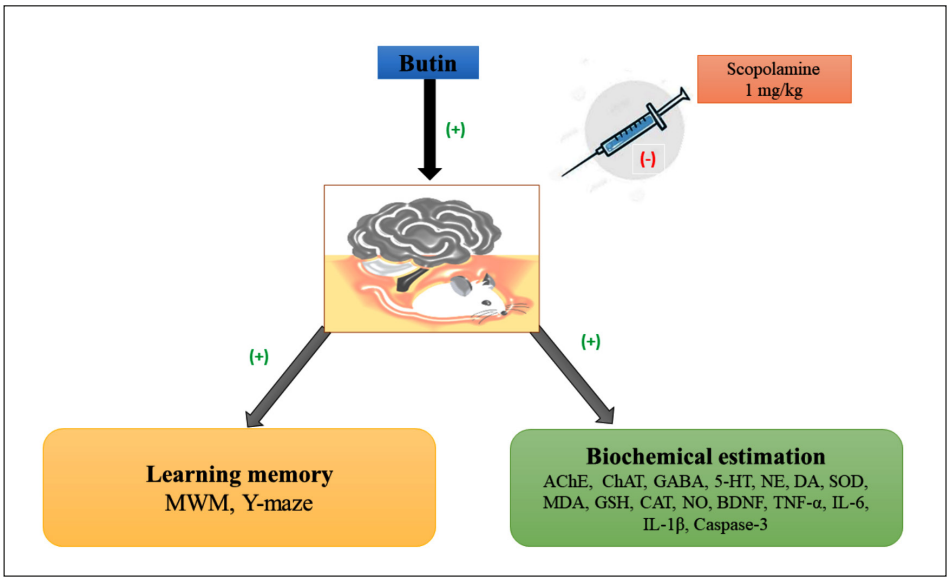

Figure 9. Probable mechanism of butin. 5-HT: 5-hydroxytryptamine; AChE: Acetylcholinesterase; BDNF: Brain-derived neurotrophic factor; CAT: Catalase; ChAT: Choline acetyltransferase; DA: Dopamine; GABA: Gamma-aminobutyric acid; GSH: Glutathione; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; MDA: Malondialdehyde; MWM: Morris water maze; NO: Nitric oxide; SOD: Superoxide dismutase; TNF-α: Tumor necrosis factor-alpha.

Conflict of Interest
The authors declare that they have no conflict of interests.

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Informed Consent
Not applicable.

Ethics Approval
Institutional Animal Ethics Committee of Transgenica Research Services, M.S., India (IAEC/TRS/PT/023/028), approved the research protocol, and research was conducted per the criteria mentioned in ARRIVE guidelines.

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Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' Contribution
Imran Kazmi: Conceptualization; methodology and the first draft of the manuscript: Nadeem Sayyed; Fund acquisition: Imran Kazmi; Critical Revision of manuscript: Mustafa Zeyadi, Fahad A. Al-Abbasi, Muhammad Afzal, Azizah Salim Bawadood, Ryan A. Sheikh, Sami I. Alzarea. All authors read and approved the manuscript.

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