

Antiamnesic activity of metformin in scopolamine-induced amnesia model in mice

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ABSTRACT The aim of this study was to screen the antiamnesic activity of metformin (MET) on scopolamine (SCOP)-induced amnesia in mice. Experiments were carried out on 20 adult albino mice, divided into 4 groups, and the experimental animals were treated for 14 days with 10 mg/kg MET orally. To induce amnesia, 3 mg/kg SCOP was administered intraperitoneally. Cognitive skills of the animals were examined after the induction of amnesia by using elevated plus maze (EPM) test, Morris water maze (MWM) test, and locomotor activity by using actophotometer. In EPM model, animals that received SCOP showed significant increase in transfer latency (TL) on day 7 and 14 when compared to that of control animals. However, animals that received piracetam and MET showed significant decrease ($p < 0.05$) in TL period when compared to that of SCOP-treated animals. In MWM test, animals that received MET showed significant decrease in escape latency (EL) period ($p < 0.05$) when compared to that of SCOP-treated animals. The results confirm that the administration of SCOP impaired learning and memory process in animals, whereas the administration of MET significantly ameliorated SCOP-induced amnesia in both EPM and MWM tests as indicated by significant reduction ($p < 0.05$) in TL ($p < 0.05$) and EL, respectively; hence, it can be concluded that MET improves cognitive functions against SCOP-treated mice.

KEYWORDS

metformin, learning and memory, elevated plus maze, Morris water maze, scopolamine, dementia

INTRODUCTION

Memory is one of the vital complex functions of brain, comprising perception, registration, consolidation, storage, and recollection, which decays over a long period of time. Any impairment in memory called amnesia affects the individual's quality of life. Amnesia is mainly characterized by loss of memory ability sufficiently and interferes with one's occupational or social activities, and it is one of the common causes that leads to a condition called dementia, which is a progressive neurodegenerative disorder associated with the loss of neurons in distinct brain areas. Moreover, dementia is associated with

various comorbid conditions such as diabetes mellitus, hypertension, dyslipidemia, and cardiovascular diseases in elderly patients.¹ Due to increase in life expectancy and lifestyle modifications, more cases of memory loss or dementia are being reported globally. As per the records, in India, around 3.7 million people are affected with dementia in 2010, while the incidence rates are expected to double by 2030 and over 100 million by 2050.^{2,3}

Only limited therapeutic interventions are available to reduce the incidence of dementia due to decline in learning and memory of individuals. Cholinesterase inhibitors, calcium channel blockers, and glutamate antagonists are few classes of pharmacological agents that are being clinically explored to reduce symptomatically the impact of cognitive dysfunction associated with vascular dementia.⁴ However, an agent that should improve both endothelial dysfunction and associated dementia still need to be explored. Very recently, the focus has been directed toward statins and other drugs such as metformin (MET).⁵

In addition to its antidiabetic potential, MET has been proved to be a therapeutically effective drug candidate in various central

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nervous system disorders such as Alzheimer's disease (AD) and Parkinson's disease⁶ and found to be neuroprotective by inhibiting apoptosis in neuronal cortical cells.⁷ It has been shown that MET promotes neurogenesis and enhances the spatial memory formation⁸ and also observed that long-term treatment with MET increases health span and lifetime.⁹ Previous studies support that MET prevented the oxidative stress-related cellular death in non-neuronal cell lines and treatment with MET prevented the appearance of pathological indices of AD.^{10,11} However, the potential of MET in amnesia remains to be explored and deserves further investigation. The aim of this study is to investigate the effects of MET on learning and memory in mice against scopolamine-induced amnesia.

MATERIALS AND METHODS

Animals and experimental conditions

Adult male albino mice (weighing 20–30 g) were kept in polypropylene cages with free access to food and water, under standardized housing conditions (natural light–dark cycle, temperature of $23 \pm 10^\circ\text{C}$, relative humidity of $55 \pm 5\%$). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to different experimental groups, and each group comprised five animals, and all tests were performed between 09:00 and 15:00 hours. The experiments on animals were conducted in accordance with the internationally accepted guidelines for laboratory animal use, and the experimental protocols were duly approved by the institution animal ethics committee (SVCP/IAEC/I-004/2015-16) of Sree Vidyanykethan College of Pharmacy, Tirupati, Andhra Pradesh, India.

Drugs, chemicals, and instruments

Scopolamine bromide and piracetam were obtained from Sigma (St. Louis, USA). MET and piracetam were suspended in 1% carboxymethyl cellulose and administered orally (po) to respective groups, whereas scopolamine was dissolved in 0.9% physiological saline and administered intraperitoneally (ip) to animals.

Experimental design

Experiments were carried out on adult albino mice. A total of 20 albino mice (weight, 20–30 g) were used in this experiment. The animals were randomly divided into four groups of five animals each. Group I animals served as a control and were treated with normal saline (0.9%), whereas groups II and III animals received scopolamine (3 mg/kg ip) and piracetam + scopolamine (400 mg/kg, po + 3 mg/kg, ip), respectively. Group IV animals received MET + scopolamine (10 mg/kg po + 3 mg/kg, ip). Amnesia was induced by scopolamine injection (3 mg/kg, ip) 30 min before the behavioral experiments. All groups except the control group received scopolamine. Piracetam was used as a standard drug, and the cognitive and memory functions of the mice were assessed half an hour after the administration of scopolamine.^{12,13}

Assessment of activity

Neurotoxicity test

Neurotoxicity of MET was assessed by chimney test. This test was carried out for over the period of 45 minutes, and the animals were subjected to prior training. A Pyrex glass tube (25 cm long and 3 cm diameter) was marked at a point 20 cm from its base, and a mouse

was introduced at the end, nearest to the mark. When the animal reached the other end of the tube, the tube was moved to the vertical position and immediately the mouse tried to climb backward. The ability of mice to leave the tube within 1 minute indicates the lack of neurotoxic properties of the test drug. Screened mice were injected ip as per the experimental design and were tested after 45 minutes as described above.^{14,15}

Elevated plus maze (EPM) test

Mice were placed individually at the end of the open arm. The time for each mouse to move to the closed arm was considered as transfer latency (TL) and was noted for 90 seconds. On the first day of the study, mice were allowed to move freely to explore the apparatus for at least 10 minutes. TL recorded on 1st day was acquisition (learning), and 7th and 14th day TL reflects the retention/consolidation (memory) for learning.¹⁵

Morris water maze (MWM) test

MWM represents more specific test of spatial memory. MWM apparatus consists of a large circular pool with 150 cm in diameter and 45 cm in height, filled with water to a depth of 30 cm at $28 \pm 1^\circ\text{C}$. The water was made opaque by addition of small quantity of titanium dioxide. The tank was then divided into four equal quadrants (Q1, Q2, Q3, and Q4) by using threads. There was a hidden platform (white in color with a diameter of 10 cm) kept at the center of the 4th quadrant 1 cm below. Mice were subjected to four consecutive trials before the experiment on each day of the study (7th and 14th day). The animal was released into the water facing toward the wall of the tank and allowed to escape to the hidden platform and further allowed to remain there for 20 seconds, and the escape latency (EL) was recorded. EL is the time taken by the animal from getting dropped into the tank to escape on to the platform (cutoff period is 120 seconds).¹⁶

Rotarod test

The rotarod apparatus consisted of a rotating bar suitably machined to provide grip strength. Latency to fall animals from the bar is automatically recorded in seconds. Mice were initially selected by the ability to remain on the rotating bar rotating at a constant speed of 25 rpm for at least two consecutive 180-seconds trial before the test day. On the test day, animals were placed individually as per the experimental design in each compartment, and the fall of time of each mice was noted.^{17,18}

Statistical analysis

The statistical analysis was carried out by one-way analysis of variance. The values are represented as mean \pm standard error of the mean. Comparison of mean values of different groups treated with different dose levels of extracts and positive controls was carried out by Turkey's multiple comparison test. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In this study, the anti-amnesic effect of MET on the scopolamine-induced memory impairment was evaluated using different exteroceptive behavioral models such as MWM, EPM tests, and so on. These tasks have been extensively used to measure learning and memory in different animal models, particularly behavioral manipulations in rodents.^{19,20}

Piracetam, a nootropic agent, was used as a standard in this study, and the dose of piracetam and MET has been decided from the earlier studies.²¹ Results of this study suggest that mice treated with scopolamine showed more prolonged TL in EPM and prolonged escape latencies than the control animals. In contrast, acute administration of MET showed significant memory enhancing activity ($p < 0.05$) in the scopolamine-induced amnesia in animals.

In this study, time required to reach closed arm from open arm by each animal was considered as TL period. In EPM model, animals that received scopolamine showed significant increase in TL on both day 7 and 14 when compared to control animals. However, animals that received piracetam and MET showed significant decrease in TL period when compared to that of scopolamine-treated animals (Figure 1). The results confirm that scopolamine impaired learning and memory process in animals, whereas the MET (10 mg/kg po) treatment had significantly reduced these impairments on mice.

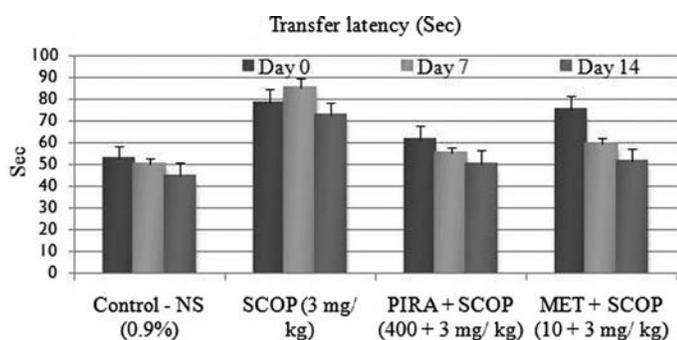


Figure 1 Effect of MET on transfer latency in elevated plus maze. MET, metformin; NS, nonsignificant; PIRA, piracetam; SCOP, scopolamine.

Results from chimney test suggest that there were no neurotoxic effects after administration of MET (10 mg/kg ip), and it has no impact on motor coordination in mice at the investigated dose, and animals were able to leave the tube within 1 minute. In rotarod test, between control and experimental group on day 7 and 14, there was no significant observable difference in the fall off time among different experimental groups (Table 1).

In MWM test, results show that animals that received scopolamine showed significant increase in EL (103.56 seconds on

day 7 and 101.51 seconds on day 14) when compared to control groups. However, animals that received piracetam and MET showed significant decrease in the latency period on day 7 and 14 (Table 2). Acute administration of MET reduced the TL period when compared to that of scopolamine-treated animals ($p < 0.05$) on day 7 and 14. The results confirm that scopolamine impaired learning and memory process in animals, whereas the administration of MET significantly ameliorated scopolamine-induced amnesia in MWM test as indicated by significant reduction ($p < 0.05$) in EL against scopolamine-treated mice.

The main finding of this study is that MET treatment is capable to attenuate scopolamine-induced impairment in learning and memory consolidation. Because there was no significant difference in the swimming speed among experimental groups including scopolamine and scopolamine + MET groups, the effect of MET treatment was not due to the improvement in motor activity of rats. Therefore, MET treatment probably attenuated scopolamine-induced neuronal damage of the brain. Our results show that MET treatment improves spatial learning and memory in scopolamine-received animals; hence, MET groups found hidden platform as soon as possible, and a decrease in EL was noticed.

Also it has been shown that in earlier studies, MET can act as a neuroprotectant against apoptotic cell death,⁷ and it can attenuate tau phosphorylation and A β generation, can increase antioxidant protection, and can improve cognitive function.^{6,22} In accordance with that, our result also demonstrated that MET can improve learning and memory function of mice against scopolamine-induced amnesia.

Results from chimney test revealed that all mice were able to leave the tube within 1 minute, which indicates that there were no neurotoxic effects after administration of MET (10 mg/kg po), and it has no impact on motor coordination, muscle grip strength in the rotarod test against scopolamine-induced amnesia model.

CONCLUSION

In conclusion, results from the present investigation show that scopolamine impaired learning and memory process in mice, whereas administration of MET significantly ameliorated scopolamine-induced amnesia in both EPM and MWM test as indicated by significant reduction in TL and EL, respectively; hence, it can be concluded that MET improves cognitive functions against scopolamine-treated mice. However, the beneficial effects and mechanism responsible for improved learning and memory showed by MET remain unclear and deserves further investigations.

Table 1 Effect of MET on fall of time in rotarod against scopolamine-induced amnesia in mice

Groups	Fall of time (seconds)		
	Day 0	Day 7	Day 14
Control—NS (0.9%)	104.54 \pm 3.36	124.54 \pm 2.87	122.81 \pm 1.57
Scopolamine (3 mg/kg, ip)	119.69 \pm 5.85 (NS)	121.47 \pm 1.61 (NS)	121.65 \pm 3.61 (NS)
Piracetam + scopolamine (400 mg/kg, po + 3 mg/kg, ip)	118.36 \pm 4.65 (NS)	125.27 \pm 3.36 (NS)	121.38 \pm 1.57 (NS)
Metformin + scopolamine (10 mg/kg, po + 3 mg/kg, ip)	115.57 \pm 4.54 (NS)	119.62 \pm 5.64 (NS)	125.64 \pm 2.34 (NS)

Number of animals (n) = 5. Data are represented as mean \pm standard error of the mean: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ when compared to control and scopolamine-treated groups. ip, intraperitoneal; MET, metformin; NS, nonsignificant; po, orally.

Table 2 Effect of MET on escape latency in Morris water maze test

Groups	Escape latency (seconds)		
	Day 0	Day 7	Day 14
Control—NS (0.9%)	109.58 ± 2.74	84.43 ± 3.49	78.63 ± 1.57
SCOP (3 mg/kg, ip)	101.29 ± 1.53 (NS)	98.56 ± 1.40*	98.51 ± 1.49*
PIRA + SCOP (400 mg/kg, po + 3 mg/kg, ip)	106.29 ± 2.53 (NS)	77.64 ± 2.61*	70.52 ± 2.65*
MET + SCOP (10 mg/kg, po + 3 mg/kg, ip)	105.49 ± 2.58 (NS)	80.56 ± 2.56*	75.62 ± 2.48*

Number of animals (n) = 5. Values are represented as mean ± standard error of the mean: **p* < 0.05, ***p* < 0.01, and ****p* < 0.001 when compared to control and scopolamine-treated groups. ip, intraperitoneal; MET, metformin; NS, nonsignificant; PIRA, piracetam; po, orally; SCOP, scopolamine.

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