Peertechz

CLINICAL GROUP

ANNALS OF Alzheimer's and Dementia Care

DOI: http://dx.doi.org/10.17352/aadc

Research Article

Influence of Metformin on learning and memory in experimental Amnesia model in Mice

Jayaraman Rajangam[1]*, Shvetank Bhatt[2], Navaneetha Krishnan[1], Mounika Sammeta[1] and Lagumsani Joshna[1]

[1]Department of Pharmacology, Sree Vidyanikethan College of Pharmacy – Tirupati, 517 501. Andhra Pradesh, India

[2]Department of Pharmacology, Amity Institute of Pharmacy, Amity University, Madhya Pradesh,Gwalior– 474005, India

3Department of Pharmaceutics, Sree Vidyanikethan College of Pharmacy – Tirupati, 517 501. Andhra Pradesh, India

Received: 02 February, 2020
Accepted: 11 May, 2020
Published: 12 May, 2020

***Corresponding author:** Dr. Jayaraman Rajangam, Professor & Head, Department of Pharmacology, Sree Vidyanikethan College of Pharmacy, Tirupati, 517501. Andhra Pradesh, India, E-mail: jayaraam81@gmail.com

Keywords: Amnesia; Metformin; Cognitive Skills; Elevated plus-maze; Morris Water Maze; Scopolaminec

https://www.peertechz.com

Abstract

Background: Metformin belongs to the antidiabetic drug but it has been shown some beneficial effects towards Central Nervous System (CNS) disorders and found to be neuroprotective by inhibiting apoptosis in neuronal cortical cells in various animal models apart from its anti-diabetic potential as per the available reports.

Aim and objectives: The present study was aimed to screen the influence of Metformin (MET) against experimentally induced amnesia using scopolamine (SCOP) in mice.

Materials and methods: Twenty adult albino mice were used for the current study and all the animals were divided into 4 groups of five animals each and treated for about three weeks with MET (10 mg/kg.p.o) whereas amnesia was induced by using SCOP (3 mg/kg ip). The various cognitive skills were assessed by using conventional apparatus like an elevated plus maze (EPM), Morris water maze (MWM), motor coordination by rota rod test and neurotoxicity by chimney test.

Results: Results from EPM and MWM indicated the rise in transfer latency and escape latency respectively were due to amnesic effect by administration of SCOP when compared to control animals received normal saline. Administration of standard drug Piracetam (PTM) and test drug MET showed a significant reduction of ($p<0.05$) in the transfer latency (TL) period were noticed. Thus, the administration of MET significantly ameliorated SCOP-induced amnesia in EPM as indicated by an increase in inflection ratio and reduction in TL. In the MWM test, MET administration showed a beneficial reduction of escape latency (EL) period ($p<0.05$) when compared to SCOP induced amnesia animals. There were no significant changes in motor coordination and no neurotoxic effects were observed as per the results.

Conclusion: From the results, the administration of SCOP resulted in significant alterations in the cognitive skills of animals particularly impaired learning and memory skills whereas acute administration of MET for about three weeks, resulted in significant amelioration of scopolamine-induced amnesia. This was noticed as indicated by significant reduction ($p<0.05$) in transfer latency in the EPM test and significant reduction of ($p<0.05$) escape latency in the MWM test which reflects the beneficial effects of MET against scopolamine-induced memory and behavioral deficit in mice.

Introduction

Cognition is one of the vital complex functions of the brain of every living species including humans comprising of perception, registration, consolidation, storage, recollection throughout the life span of every individual. Any impairment in memory called amnesia affects not only the individual's quality of life and it is also considered as a most important CNS disorder due to the loss of memory due to declining of neuronal population because of the aging process, any neurodegenerative disorders, head injury, brain infections, genetic abnormalities, etc. In elderly patients, dementia is associated with various co-morbid

Citation: Rajangam J, Bhatt S, Krishnan N, Sammeta M, Joshna L (2020) Influence of Metformin on learning and memory in experimental Amnesia model in Mice. Ann Alzheimers Dement Care 4(1): 005-009. DOI: https://dx.doi.org/10.17352/aadc.000010

conditions like diabetes mellitus, hypertension, dyslipidemia, cardiovascular diseases, etc [1]. Due to an increase in life expectancy and lifestyle modifications, more incidence rates of memory loss or dementia are being reported and its burden is rising day by day globally. As per the records, in India around 3.7 million people with dementia in 2010 while the incidence rates are expected to be doubled by 2030 and over 100 million by 2050 [2].

As per the current scenario, only limited cognitive treatment options like the use of Cholinesterase inhibitors and few other therapeutics medicaments were used to minimize dementia-related cognitive dysfunction symptomatically [3] and memory-related problems to a certain extent. Hence there are lots of potentials to develop therapeutic agents used for the management or prevention of early stages of dementia due to aging and other pathological complications and that kind of drug still need to be explored. Very recently, the focus has been directed towards statins and other drugs like metformin [4].

Apart from the anti-diabetic effect, Metformin has shown diverse therapeutic effects like neuroprotection on Central nervous system disorders like Alzheimer's and Parkinson's diseases in various animal models. Both are considered as major neurodegenerative disorders and metformin treatment found to be neuroprotective by inhibiting apoptosis in neuronal cortical cells in animals as per the reports of performed studies [5,6].

In continuation, There are many reports to support the beneficial effect of metformin on neurogenesis along with improvement in spatial memory and significant increase of health span and lifetime in various experimental animal models [7,8]. Moreover, Metformin prevented the free radical related oxidative stress which in turn prevented pathological consequences of Alzheimer's in animal models [9]. However, the potential of metformin in amnesia is remaining to be explored and deserves further investigation. Hence, we aimed to proceed to screen the influence of metformin on cognition against the experimental amnesia model in mice.

Materials and methods

Experimental animals

Adult Albino male mice of 20-25 g, aged between 3-4 months were used for the present study. After the adaptation period of about one week, all the animals were randomly assigned to different groups of 5 animals in each. All the experiments were conducted as per the CPCSEA laboratory animal guidelines and approved by the institutional animal ethics committee (SVCP/IAEC/I-004/2015-16) of Sree Vidyanikethan College of Pharmacy, Tirupati, AP, India.

Drugs and chemicals

Scopolamine was procured from Sigma Chemicals (St. Louis, USA) and dissolved in 0.9% Normal saline to inject intraperitoneally to induce amnesia to the experimental animals. Metformin and Piracetam were received as a gift sample and suspended in 1% carboxymethylcellulose (CMC) to administer orally to respective groups. Piracetam is a nootropic agent, used as a standard in the present study and the dose of Piracetam, Metformin has been taken from the earlier reported studies [10-12].

Experimental protocol

A total of twenty male albino mice (20-25gm) were randomly assigned to different groups of five animals in each.

Groups I:Control treated with Normal Saline (NS-0.9%)

Group II: Received SCOP (3mg/kg.i.p)

Group III: Received PTM (400 mg/kg,p.o.) + SCOP (3 mg/ kg, i.p)

Group IV: Received MET (10 mg/kg.p.o) + SCOP (3 mg/ kg, i.p).

The duration of the treatment was 21 days.Exactly 30 min before the screening of behavioral parameters, except control animals, all other groups were received scopolamine injection to induce amnesia whereas Piracetam was used as a standard drug and cognitive, memory deficit was assessed using standard laboratory tests [13]. Cognitive and behavioral assessment was performed every week.

Assessment of neurotoxicity by chimney test

Chimney test was used to assess the neurotoxic property of test drug MET on animals. This procedure was carried out by following the standard protocol for about 45 min subjected to sufficient trials. Briefly, mice were placed to a 25 cm long and 3cm diameter wide glass tube and made a mark at a point at 20cm from its base. As soon as the mice moved to another end of the tube, the glass tube was kept in a vertical position, and immediately the mouse will try to climb backward. The ability of animals to leave the tube and climbing backward towards moving out from the tube within 1min was taken as an endpoint to consider the lack of neurotoxic properties of Metformin [14].

Assessment of transfer latency by EPM

Mice were placed individually in the central part of the plus-maze apparatus in such a way that, head of animals facing towards the open end. The time taken for each animal to reach out to the closed platform was noted as transfer latency (TL) and observed for a cutoff period of about 90 sec. Before the beginning of the experiment adequate free trials were given and animals were allowed to move freely in the apparatus to minimize the bias [15].

Assessment of escape latency by MWM

A large circular pool (Diameter of 150 cm and height of 45 cm) filled with water to a depth of 30 cm at 28±1°C was used. To this pinch of titanium dioxide powder was added to make opaque of the circular pool. The whole pool was then equally divided into four equal quadrants and named as Q1, Q2, Q3, and Q4 and the hidden platform was placed at the center of the pool. Animals were allowed to move freely and sufficient trial was allowed before the experiments to avoid bias. Time is taken by

Citation: Rajangam J, Bhatt S, Krishnan N, Sammeta M, Joshna L (2020) Influence of Metformin on learning and memory in experimental Amnesia model in Mice. Ann Alzheimers Dement Care 4(1): 005-009. DOI: https://dx.doi.org/10.17352/aadc.000010

each animal to identify the hidden platform was noted as escape latency (EL) and continued to a Cut off period of about 120sec [16].

Assessment of muscle grip strength by rotarod Test

Rotarod is considered an important experimental tool used to record the muscle coordination and relaxant property of the given drug. In the present study in the rotarod test, latency to fall animals is automatically recorded. Animals were subjected to prior exposure to the rotating bar and selected upon the suitability and ability to hold the rotating bar in a specified time limit [17].

Statistical analysis

The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons using GraphPad Prism 5.00® software (GraphPad Software, Inc., San Diego, CA). The values are represented as mean ±SEM. $p < 0.05$ was considered significant.

Results and Discussion

Morris water maze (MWM), elevated plus maze tests were commonly used to measure learning and memory parameters in different animal models and particularly behavioral manipulations in rodents models among the different models available [18]. In the present study, Transfer latency was considered as one of the important parameters to assess the learning and memory process in the experimental animals used. Time required by every animal to reach the closed platform from the open arm as noted as transfer latency. The results of the present study indicate that the administration of scopolamine was altered the learning and memory patterns of the experimental animals. As per the results, scopolamine received animals showed rising of transfer latency period whereas acute administration standard drug PTM and test drug MET significantly altered the learning and memory process which was ultimately reflected as a significant reduction of transfer latency when compared to scopolamine treated animals (Figure 1). Results of the present study suggest that animals treated with SCOP showed significantly prolonged TL in a plus-maze test and prolonged EL in water maze models than that of control animals. In contrast, acute administration of MET showed significant memory enhancing activity ($p<0.05$) in the scopolamine-induced amnesia in animals. Hence, the results of the study confirm that scopolamine administration impaired learning and memory processes in animals, whereas the MET (10 mg/kg p.o) treatment had significantly reduced these impairments on mice.

The influence of MET on EL was depicted in Table 1. As indicated in different earlier studies, EL was significantly raised by SCOP administration. The same trend was noticed in the present study also. The results of the MWM test indicated that acute administration of SCOP significantly increased the EL period when compared to control groups received normal saline. But, the groups treated with standard drug PTM had significantly reduced the EL period on days 14 and 21 ($p<0.05$) as shown in Figure 2 From the results, acute administration of MET reduced the TL period when compared to SCOP treated animals ($p<0.05$. The results confirm that SCOP impaired learning and memory processes in animals, whereas administration of MET significantly ameliorated scopolamine-induced amnesia as indicated by significant reduction ($p<0.05$) in EL against scopolamine-induced in mice.

Chimney test is one of the most widely employed screening models for the assessment of the neurotoxic profile of the compounds. In the present study, results from the chimney test suggest that experimental animals received MET was able to climb backward in a specified period of about 1 min which indicates, there were no neurotoxic effects were observed at the investigated dose level of MET (10mg/kg i.p.).Also, the results indicate that MET does not cause any impact on motor

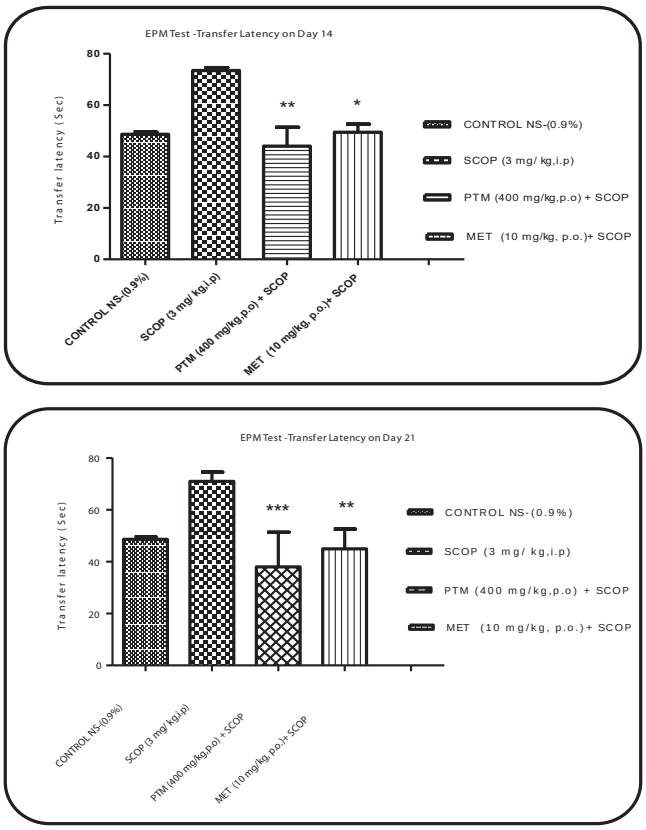

Figure 1: Effect of Metformin on transfer latency in Elevated plus maze on Day 14 & 21.

Table 1: Effect of MET on the rotarod test against scopolamine-induced amnesia in mice.

Groupsa	Fall of Time (Sec)			
	Day 0	Day 7	Day 14	Day 21
Control - NS (0.9%)	104.54±3.36	124.54±2.87	122.81±1.57	119.72±1.29
SCOP (3 mg/ kg, i.p.)	119.69±5.85^{ns}	121.47±1.61^{ns}	121.65±3.61^{ns}	121.55±2.31^{ns}
PTM (400 mg/kg, p.o) + SCOP (3 mg/kg, i.p)	118.36±4.65^{ns}	125.27±3.36^{ns}	121.38±1.57 cns	118.38±2.49^{ns}
MET (10 mg/k g, p.o.)+ SCOP (3 mg/ kg, i.p)	115.57±4.54^{ns}	119.62±5.64^{ns}	125.64±2.34^{ns}	122.64±3.19^{ns}

Number of animals (n= 5): Values are mean ± SEM: *p<0.05,** p<0.01 & *** p<0.001 When compared to control and scopolamine treated groups;

Citation: Rajangam J, Bhatt S, Krishnan N, Sammeta M, Joshna L (2020) Influence of Metformin on learning and memory in experimental Amnesia model in Mice. Ann Alzheimers Dement Care 4(1): 005-009. DOI: https://dx.doi.org/10.17352/aadc.000010

coordination. The same trend was observed in the rotarod test also. In the rotarod test, between the control and experimental group on throughout experiment, there was no significant observables difference were noticed in the fall off time among the animals (Table 1) which suggest that MET does not cause any deviation in the muscle coordination process of animals.

Chimney test is one of the most widely employed screening models for the assessment of the neurotoxic profile of the compounds. In the present study, results from the chimney test suggest that experimental animals received MET was able to climb backward in a specified period of about 1 min which indicates, there were no neurotoxic effects were observed at the investigated dose level of MET (10mg/kg i.p.).Also, the results indicate that MET does not cause any impact on motor coordination. The same trend was observed in the rotarod test also. In the rotarod test, between the control and experimental group on throughout experiment, there was no significant observables difference were noticed in the fall off time among the animals (Table 1) which suggest that MET does not cause any deviation in the muscle coordination process of animals.

In the present study, the protective effect of MET on experimental amnesia model using SCOP was screened using different exteroceptive behavioral models like MWM, EPM tests and rotarod test, etc. Among many behavioral screening models, both EPM and MWM models were used extensively to screen learning and memory impairments in different animal models particularly, behavioral manipulations in rodents [19-20]. On the other hand, PTM is a nootropic agent, used as a standard in the present study and the dose of PTM has been taken from the earlier studies [21].

As per the results of the present investigation, SCOP administration to experimental animals reflected significant prolonged TL in the EPM task and prolonged EL in MWM task than the control animals received normal saline. In contrast, acute administration of MET showed significant protection of these parameters, and the diminishment of both TL and EL were noticed.

The key finding of this present investigation is that MET attenuated the SCOP-induced learning and memory impairments to a significant extent were reflected in EPM and MWM models in mice and this may be due to protective effect by MET treatment, probably MET attenuated SCOP-induced neuronal damage in the brain. Because there were no observable significant changes that were seen in motor activity and or motor coordination in the rotarod test. Hence, results show that MET treatment improves spatial learning and memory in SCOP received animals, and they were able to find the hidden platform as soon as quickly and thereby reduced escape latency was noticed.

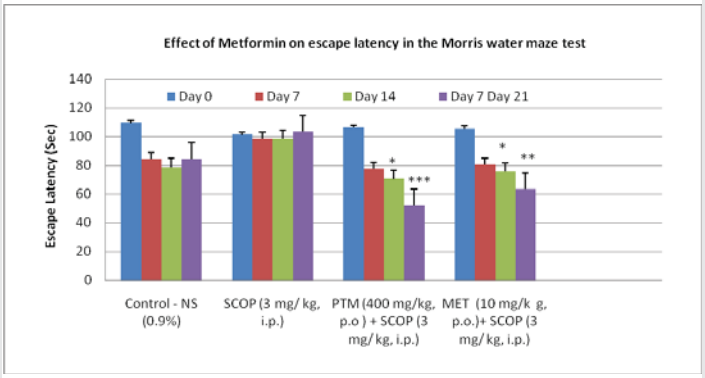

Figure 2: Effect of Metformin on escape latency in the Morris water maze test. Number of animals (n=5): Values are mean ± SEM: *p<0.05,** p<0.01 & *** ᶜp<0.001 When compared to control and scopolamine treated groups;

Results from chimney test reveal that all the mice were able to leave the tube within 1 min which indicates that there were no neurotoxic effects were observed with MET (10 mg/kg p.o) and it has no impact on motor coordination, muscle grip strength in the rotarod test against SCOP-induced amnesia model.

Also, it has been shown that in earlier studies, MET can act as a neuroprotectant against apoptotic cell death [22] and it can attenuate tau phosphorylation, A generation, and increases antioxidant protection, can improve cognitive function [23]. Following that, our result also demonstrates that MET can improve learning and memory function of mice against scopolamine-induced amnesia.

Conclusion

In conclusion, the administration of SCOP resulted in significant alterations in the cognitive skills of animals particularly impaired learning and memory skills whereas acute administration of MET attenuated the scopolamine-induced learning and memory impairments to a significant extent were reflected in EPM and MWM models in mice. Moreover, there were no neurotoxic effect and no significant changes in motor coordination were observed in the chimney and the rotarod test indicated the protective effect of MET, probably it attenuated scopolamine-induced neuronal damage in the brain. Hence it can be concluded that MET offers protection and improves cognitive functions against scopolamine-induced amnesia in mice.

Acknowledgments

The authors are grateful to the management of Sree Vidyanikethan Educational Trust for providing the necessary facilities to carry out the research work. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Dunning J, During MJ (2003) Molecular mechanisms of learning and memory. Expert Rev Mol Med 25: 1-11. Link: https://bit.ly/2Wx3f6D

2. Shaji KS, Jotheeswaran AT, Girish N, Srikala B, Amit D, Meera P, Mathew V (2010) Dementia India report 2010.Link: https://bit.ly/2zFy6oF

3. Korczyn AD, Kalvach P, Bornstein ND (2005) Vascular dementia. J Neurol Sci 2003:1-2.

4. Parle M, Singh N (2007) Reversal of memory deficits by atorvastatin and simvastatin in rats. Yakugaku Zasshi 127: 1125-1137. Link: https://bit.ly/2Z5BAeH

5. Ashabi G, Khodagholi F, Khalaj L, Goudarzvand M, Nasiri M (2014)

Citation: Rajangam J, Bhatt S, Krishnan N, Sammeta M, Joshna L (2020) Influence of Metformin on learning and memory in experimental Amnesia model in Mice. Ann Alzheimers Dement Care 4(1): 005-009. DOI: https://dx.doi.org/10.17352/aadc.000010

Activation of AMPactivated protein kinase by Metformin protects against global cerebral ischemia in male rats: interference of AMPK/PGC-1a pathway. Metab Brain Dis 29: 47–58. Link: https://bit.ly/2WRy7xq

6. El-Mir MY, Detaille DGRV, Delgado-Esteban M, Guigas B, Attia SF, et al. (2008) Neuroprotective role of the antidiabetic drug Metformin against apoptotic cell death in primary cortical neurons. J Mol Neurosci 34: 77-87. Link: https://bit.ly/3dTOZuF

7. Wang J, Gallagher D, De Vito LM, Cancino GI, Tsui D, et al. (2012) Metformin activates an atypical PKC-CBP pathway to promote neurogenesis and enhance spatial memory formation. Cell Stem Cell 11: 23–35. Link: https://bit.ly/3bw4nM9

8. Klinkenberg I, Blokland A (2010) The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. Neurosci Biobehav Rev 34: 1307-1350. Link: https://bit.ly/363CSZ9

9. Labuzek K, Liber S, Gabryel, Okopien B (2010) Metformin has adenosine-mono phosphate activated protein kinase (AMPK)-independent effects on LPS-stimulated rat primary microglial cultures. Pharmacol Rep 62: 827–848. Link: https://bit.ly/2ZcYPDs

10. Georgieva-Kotetarova MT, Kostadinova II (2013) Effect of atorvastatin and Metformin on learning and memory in rats with diazepam-induced amnesia. Folia Med 55: 58-65. Link: https://bit.ly/2Lz21Bi

11. Gouliaev AH, Senning A (1994) Piracetam and other structurally related nootropics. Brain Res Rev 19: 180-222. Link: https://bit.ly/3fPWvs4

12. Lenegre A, Chermat R, Avril I, Steru L, Porsolt RD (1988) The specificity of piracetam's anti-amnesic activity in three models of amnesia in the mouse. Pharmacol Biochem Behav 29: 625-629. Link: https://bit.ly/2yYE9EJ

13. Gouliaev AH, Senning A (1994) Piracetam and other structurally related nootropics. Brain Res Rev 19: 180-222. Link: https://bit.ly/3fPWvs4

14. Mandhane SN, Aavula K, Rajamannar T (2007) Timed pentylenetetrazole infusion test: A comparative analysis with s.c. PTZ and MES models of anticonvulsant screening in mice. Seizure 16: 636-644. Link: https://bit.ly/2y5q1sX

15. Joshi H, Parle M (2006) Zingiber Officinale: evaluation of its nootropic effect in mice. Afr J Trad CAM 3: 64-74. Link: https://bit.ly/3dMVhMl

16. Lavinsky D, Arteni NS, Netto CA (2003) Agmatine induces anxiolysis in the elevated plus-maze task in adult mice. Behav Brain Res 141: 19-24. Link: https://bit.ly/3dNgcij

17. Jayaraman R, Lavanya O (2018) Effect of Rosuvastatin on learning and memory in scopolamine-induced amnesia in Mice. Trends Med 18: 1-4. Link: https://bit.ly/2LuWoUP

18. Oh JH, Choi BJ, Chang MS, Park SK (2009) Nelumbo nucifera semen extract improves memory in rats with scopolamine-induced amnesia through the induction of choline acetyltransferase expression. Neurosci Lett 461: 41-44. Link: https://bit.ly/2y8bdty

19. Oh JH, Choi BJ, Chang MS, Park SK (2009) Nelumbo nucifera semen extract improves memory in rats with scopolamine-induced amnesia through the induction of choline acetyltransferase expression. Neurosci Lett 461: 41-44. Link: https://bit.ly/2y8bdty

20. Terry AV (2000) Spatial navigation (water maze) tasks. In: JJ. Buccafusco (Eds), Methods of Behavior Analysis in Neuroscience. (2nd edition). Boca Raton (FL): CRC Press.

21. Georgieva-Kotetarova MT, Kostadinova II (2013) Effect of atorvastatin and rosuvastatin on learning and memory in rats with diazepam-induced amnesia. Folia med 55: 58-65. Link: https://bit.ly/2Lz21Bi

22. El-Mir MY, Detaille DGRV, Delgado-Esteban M, Guigas B, Attia SF, et al. (2008) Neuroprotective role of the antidiabetic drug Metformin against apoptotic cell death in primary cortical neurons. Journal Mol Neurosci 34: 77-87. Link: https://bit.ly/3dTOZuF

23. Ashabi G, Khodagholi F, Khalaj L, Goudarzvand M, Nasiri M (2014) Activation of AMPactivated protein kinase by Metformin protects against global cerebral ischemia in male rats: interference of AMPK/PGC-1a pathway. Metab Brain Dis 29: 47-58. Link: https://bit.ly/2WRy7xq

Citation: Rajangam J, Bhatt S, Krishnan N, Sammeta M, Joshna L (2020) Influence of Metformin on learning and memory in experimental Amnesia model in Mice. Ann Alzheimers Dement Care 4(1): 005-009. DOI: https://dx.doi.org/10.17352/aadc.000010