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RESEARCH ARTICLE

# Detecting the neurogenesis effect of erythropoietin and galantamine in the dentate gyrus and spatial memory in Alzheimer's experimental rat model

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## ABSTRACT

**Background:** Alzheimer's disease is neurodegenerative disorder develops unreversed causing memory and behavior impairment. It is estimated that 35.5 million people are living with Alzheimer's disease worldwide. The early symptoms of Alzheimer's are short-term memory weakness and it develops gradually causing death. There is no curing medication for Alzheimer's rather than some drugs that improve memory defects for a period of time. Many studies proved that erythropoietin (EPO) and galantamine (GAL) could improve neurogenesis in hippocampus, which is promising in finding a cure for Alzheimer's. **Aims and Objectives:** In this study, we compared neurogenesis effect of GAL and EPO and its effect on spatial memory. **Materials and Methods:** We used Wistar rats to make Alzheimer's rat model by bilateral intracerebroventricular injection of streptozotocin. We used a dose of EPO 5000 IU/Kg intraperitoneal every other day for 14 days, and GAL group was treated with intraperitoneal daily dose of 5 mg/kg. Then, all groups were tested by Morris water maze test to compare spatial memory. Then, all rats were anesthetized and decapitated for immunohistochemical study by KI67 kit to investigate proliferated cells in dentate gyrus of hippocampus. **Results:** The results driven from the histological study showed that both EPO and GAL significantly increase neuronal proliferation in dentate gyrus of hippocampus, but EPO has better effect on neurogenesis, whereas GAL could improve spatial memory more than EPO in Morris water maze test. **Conclusion:** GAL has better effect on spatial memory in short period study, but EPO increases proliferation more than GAL.

**KEY WORDS:** Alzheimer's Disease; Erythropoietin; Galantamine; Streptozotocin; Neurogenesis

## INTRODUCTION

Alzheimer's disease is a neurodegenerative disorder that causes memory and cognitive defects. It is expected that the number of Alzheimer's disease patients will increase from 106.8 million worldwide by 2050.[1] The cost of health care and hospice services for people over 65 years with Alzheimer's disease and other dementias exceeded $183 billion in the United States in 2011.[2]

Alzheimer's disease initially causes memory impairment that over time gets worse. Patients can also suffer from depression, anxiety, and insomnia. As disease progresses, patients may require assistance with basic everyday activities such as dressing, bathing, and toileting. It could also develop to walking and swallowing defects and may cause aspiration pneumonia that causes death. The time from diagnosis to death varies from 3 to 10 years or more.[3]

Alzheimer's disease is associated with a disorder that leads to beta-amyloid (Aβ) aggregation in brain neural cells and disorders in hyperphosphorylated (tau) protein that leads to

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neurodegeneration in hippocampus neural cells. FDA approved treatments for Alzheimer's disease consisting cholinesterase inhibitors (donepezil, galantamine [GAL], and rivastigmine) and an N-methyl-D-aspartate receptor antagonist (memantine) just offers a limited symptomatic alleviation for Alzheimer's patients.[4,3] Finding that neurogenesis occurs in the adult brain opened a new field to cure neurodegenerative disorders such as Alzheimer's disease. Tacrine, GAL, and memantine are already used to treat AD through altering acetylcholine levels in the brain by inhibiting acetylcholine esterase enzyme. Many studies revealed that these drugs increase neurogenesis in dentate gyrus of hippocampus and this may contribute to their therapeutic effect.[5,6]

Erythropoietin (EPO) is a 30.4 KD glycoprotein that regulates red blood cell differentiation whose primary action is to increase erythroid cells survival by reducing their apoptosis. Thus, it is used extensively for the treatment of anemia.[7] Recent studies proved that EPO and its receptors are present in the central nervous system.[8,9] In addition, EPO receptor signaling is important for the development of the brain and that it has neuroprotective effects.[10] EPO protects neurons *in vitro* against oxidative stress, glutamate, and other factors that could cause neurodegenerative diseases.[11]

Many studies have revealed that EPO has neuroprotective effects in head injuries and cerebral stroke because it increases growth factors and glucose transportation.[12] EPO can also increase the expression of brain-derived neurotrophic factor, which is an important factor for maintenance and survival of neurons.[13] Previous studies have revealed that EPO protects neurons *in vitro* against oxidative stress.[14]

### Experimental Model

One of the relevant animal rat models of Alzheimer's disease could be induced by injection of streptozotocin (STZ) in the lateral cerebroventricular (intracerebroventricular [ICV]-STZ). STZ is a chemical that causes prolonged impairment of memory and brain metabolism similar to that caused by Alzheimer's diseases.[15]

## MATERIALS AND METHODS

Male Wistar rats 180–230 g weigh housed one per cage in 23 ± 1°C on a 12 h light-dark cycle were used for the study. Rats were used after approval from the institutional animal ethics committee. The rats were divided into four groups: The first group (negative control group) had lateral cerebroventricular injection with saline and received treatment with intraperitoneal injection of saline. The second group (positive control group) had lateral cerebroventricular injection with STZ and received treatment with intraperitoneal injection of saline every other day for 2 weeks. The third group (EPO group) had lateral cerebroventricular injection with STZ and received treatment with intraperitoneal injection of EPO 5000 IU/kg every other day for 2 weeks. The fourth group (GAL group) had lateral cerebroventricular injection with STZ and received treatment with intraperitoneal injection of GAL 0.005 mg/kg once a day for 2 weeks.

After 5 days of the last dose, the rats were tested by Morris water maze, then decapitated for immunohistochemically study.

### Surgical Procedure

Rats were anesthetized with intraperitoneal injection of chloral hydrate (dose of 350 mg/kg in saline). A dose of 3 mg/kg in 4 μL saline of STZ in injection site was injected in the rat brain lateral ventricles using Hamilton syringe and stereotaxic device to inject in the obtained coordinates from the pilot study (AP = −0.9 mm, L = ±1.6 mm, and DV = −4.1 mm). The same procedure was performed to the negative control group but using an equal volume of saline instead of STZ.

The animals were anesthetized then their heads were shaved, and the area of incision was disinfected using povidone iodine. Then, the animals were fixed into the brain surgery stereotaxic device. Then, an incision on the posterior part of the head was made using a surgical blade, and two holes were made using a driller after determining the stereotaxic coordinates for brain lateral ventricles. STZ was injected into each ventricle by a Hamilton syringe during 5 min. After surgery, rats were put in their cages and had access to food and water without limitation for 10 days to recover from the surgery [Figure 1].

### Morris Water Maze Test

Spatial learning memory was tested by the Morris water maze test. The water maze apparatus consists of a circular pool (150 cm diameter and 60 cm high) divided into four equally spaced quadrants. A translucent platform was hidden 2 cm below the surface of the water in the center of quadrant 4. Each rat was allowed to swim in the pool for 120 s to find the platform. Then, the rat was left on the platform for 10 s. If a rat did not find the platform, it was carried and put on it for 10 s. This task was performed for 5 consecutive days. Moreover, the time to find the platform in the 5$^{th}$ day was recorded. We analyzed data using analysis of variance (ANOVA) Bonferroni test using international business machines (IBM) Statistical Package for the Social Sciences (SPSS) statistics 24.

### Histological Study

Rats were anesthetized then beheaded and the brains were removed, fixed in 10% buffered formalin for 24 h, then embedded in paraffin. After embedding, coronal cuts were made through the targeted area and tested using immunohistochemical staining by Ki67 Kit (based on kit instructions). Then, the tissue slides were studied under an optic microscope for proliferated neurons counting in dentate gyrus [Figures 2 and 3]. We analyzed data using ANOVA Bonferroni test using IBM SPSS statistics 24.

## RESULTS

Our study revealed a very important difference ($P < 0.001$) in the time needed to find translucent platform in the pool between the positive control group (mean of time in the fifth trial = 59.75 s) and the EPO group (mean of time in the fifth trial = 31.83 s), difference $P < 0.001$ between the GAL group (mean of time in the fifth trial = 23.67 s) and the positive control group, and difference $P < 0.01$ between EPO group and GAL group.

Mean of time in the fifth trail for the negative control group rats to find translucent platform was 6.33 s witch has very important deference ($P < 0.001$) from positive control group and EPO group and It is significantly better than GAL group $P < 0.01$ [Figure 4].

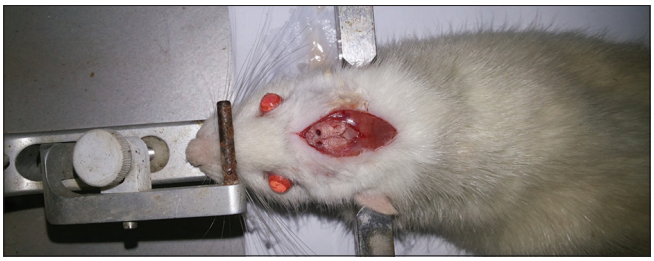

**Figure 1:** Rat fixed to stereotaxic, incision was made using a surgical blade, and two holes were made using a driller after determining the stereotaxic coordinates for brain lateral ventricles

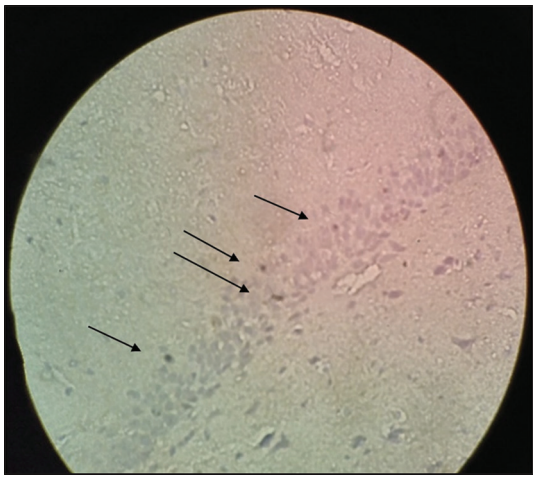

**Figure 2:** New proliferated cells, positive to KI67 staining, in dentate gyrus of hippocampus. Optic microscope, 10*40

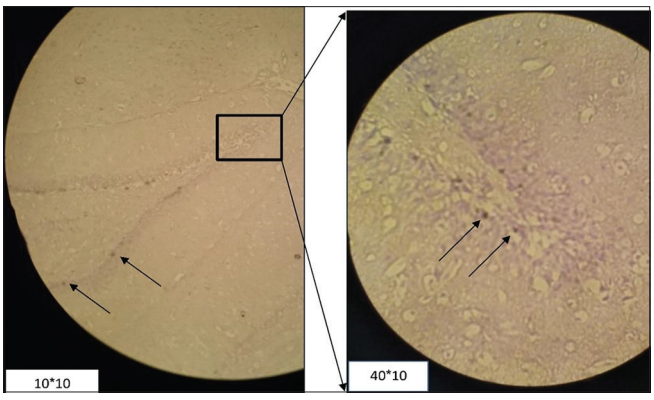


**Figure 3:** New proliferated cells, positive to KI67 staining, in dentate gyrus of hippocampus. Optic microscope, 10*10, 10*40

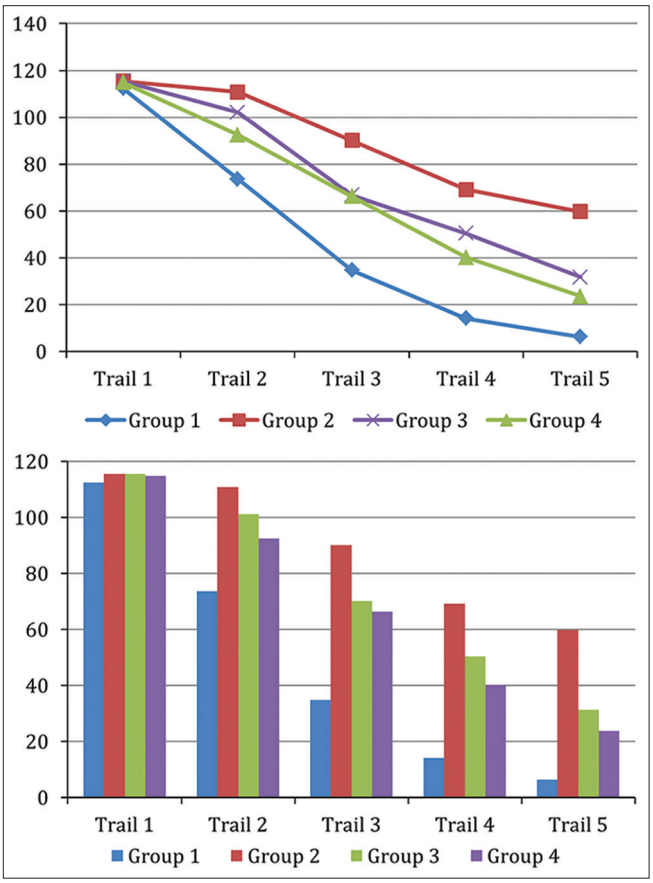


**Figure 4:** Comparison between the time needed to find the translucent platform escape latency time in the pool for the Group 1 (negative control group), Group 2 (positive control group (intracerebroventricular [ICV] with streptozotocin [STZ]), Group 3 (ICV with STZ treated with erythropoietin [EPO]), and Group 4 (ICV with STZ treated with galantamine) in five trials performed for 5 consecutive days after the last dose

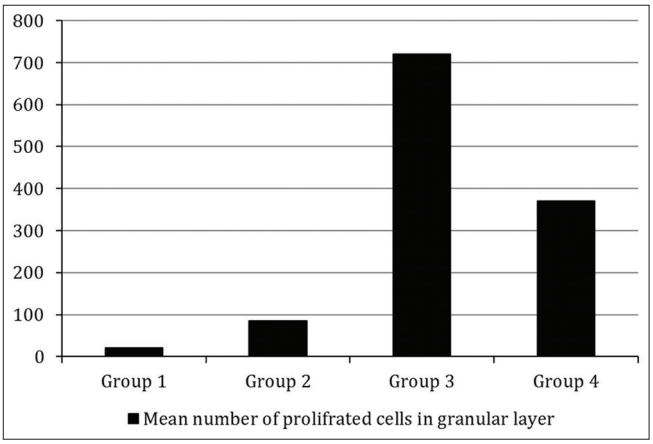


**Figure 5:** Comparison between the number of new proliferated cells in section for the Group 1 (negative control group), Group 2 (positive control group (intracerebroventricular [ICV] with STZ), Group 3 (ICV with STZ treated with EPO), and Group 4

Immunohistochemical staining revealed significant increase in number of proliferated cells in granular layer of dentate gyrus in the EPO group comparing with the positive control group ($P < 0.001$) which showed only a small number of new proliferated cells in section, whereas there were no significant proliferated cells in the sections from negative control group.

GAL group revealed significant increase in neurogenesis in the granular layer of dentate gyrus comparing with the positive control group and negative control group ($P < 0.001$), but it was less than the EPO group ($P < 0.01$) [Figure 5].

## DISCUSSION

According to Morris water maze test results, we found that negative control group had the best results in spatial memory test and it was important difference from other groups. The GAL-treated group had better results from EPO and it could be due to altering acetylcholine levels in brain which has positive effect on memory and cognition, even though EPO could stimulate neurogenesis better than GAL did.

Our results are compatible with previous studies that revealed that ICV injection of STZ causes severe learning and memory disorders.[16] Our results showed that EPO has a significant effect on neurogenesis in the granular zone of dentate gyrus in Alzheimer's rats. The factors that induce neurogenesis in the dentate gyrus can improve learning and memory[17] and that were clear in Morris water maze test results where we found a significant difference between the time needed to find the platform in the pool between the rats treated with EPO (Group 3) and the untreated ones (Group 2). Whereas the negative control group rats (Group 1) needed the least time to find the platform among all groups. Immunohistochemical staining revealed significant increase in the neurogenesis in the granular layer of dentate gyrus in ICV-STZ rats treated with EPO, whereas the untreated ICV-STZ rats showed only a small number of new proliferated cells in dentate gyrus which shows the neurogenesis effect of EPO, whereas there were no significant proliferated cells in the dentate gyrus of negative control group, which is compatible with many other studies.[18,19] Immunohistochemical staining revealed significant increase in the number of proliferated cells in the granular layer of dentate gyrus in ICV-STZ rats treated with GAL, which is compatible with many studies.[6,20]

In this study, we used EPO and GAL as short-term treatment to detect its effect on spatial memory tested by Morris water maze and its effect on proliferation in dentate gyrus of hippocampus. Acetylcholine altering stimulated by GAL is effective to improve spatial memory for just a period of time.[4] However, neurogenesis effect of GAL and EPO could be a new line in curing Alzheimer's disease and other neurodegenerative disorders.

Our study revealed that EPO that regulates red blood cell differentiation had an important effect on dentate gyrus of hippocampus by improving proliferation of stem cells in granular layer of dentate gyrus. Moreover, this effect is better than GAL, which is used previously for Alzheimer's patients and offers a limited symptomatic alleviation.

## CONCLUSION

Our results showed that EPO could increase neurogenesis in the granular zone of dentate gyrus in Alzheimer's rat model more than GAL does, but GAL proved more efficacy to improve spatial learning memory in Alzheimer's experimental rat model within a short period.[4]

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