

# Propylene Glycol induces Excessive Neurodegeneration in Combination with Amyloid Beta<sub>1-40</sub> Toxin in Male Rat Hippocampus

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Article Info	A B S T R A C T
Article type:	Introduction: Propylene glycol (PG) is frequently used as a solvent for various medications. However,
Original article	there is substantial evidence of Propylene glycol toxicity, such as depression, agitation, and seizures, particularly when used in combination with other drugs. Here, we aimed to study the effect of Propylene glycol administration in combination with amyloid $\beta_{1-40}$ injection on hippocampal neurons.
Article History:	Materials & Methods: Thirty-six male Wistar rats were randomly divided into four groups: sham,
Received: Apr. 25, 2024 Revised: May. 19, 2024 Accepted: Jun. 19, 2024	amyloid $\beta_{1-40}$ injection group, Propylene glycol group, and amyloid $\beta_{1-40}$ + Propylene glycol group. Alternation behavior, number of neurons in the hippocampus, lipid peroxidation markers, and superoxide dismutase levels were analyzed in all rats.
Published Online: Aug. 28, 2024	<b>Results:</b> When Propylene glycol was co-administered with amyloid $\beta_{1-40}$ , a notable reduction in the mean neuronal count was observed in the CA1, CA3, and DG regions compared with the amyloid $\beta_{1-40}$ only
<sup>⊠</sup> Correspondence to:	injected animals (P < 0.05). Furthermore, Propylene glycol induced an increase in lipid peroxidation markers ( $10.78 \pm 0.4$ ) and a decrease in antioxidant content ( $2.8 \pm 0.17$ ) when administered with amyloid
Maryam Bagheri Ilam University of Medical	$\beta_{1-40}$ , compared to the animals that received only amyloid $\beta_{1-40}$ (P < 0.05). A similar pattern was found in alternation behavior compared with the group with amyloid $\beta_{1-40}$ injection (P < 0.001).
Sciences, Department of Physiology, Ilam, Iran	<b>Conclusion:</b> Propylene glycol could produce excessive neurotoxicity in regions of the hippocampus when co-administered with amyloid $\beta_{1-40}$ . It likely increases lipid peroxidation and reduces superoxide dismutase in the rat brain. The use of different agents as a vehicle should be considered, especially in the
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	Keywords: Propylene Glycol, Superoxide Dismutase, Malondialdehyde, Neurodegenerative Diseases, Amyloid beta-Peptides

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#### Introduction

Nowadays, many organic compounds are widely used as solvents, vehicles, or additives. Propylene glycol (PG; 1,2-propanediol), an organic compound, is widely used in different industries, including pharmaceuticals, food production, cosmetic products, and tobacco ingredients (1, 2). However, the safety and efficacy of Propylene glycol are still debated.

Neurodegenerative diseases are characterized by the progressive loss of neurons in different parts of the brain resulting from various metabolic imbalances or toxic agents The most common (3). neurodegenerative disorders are amyloidosis, tauopathies, a-synucleinopathies and (4). Alzheimer's disease, the most common cause of dementia, is characterized by the accumulation of senile plaques and neurofibrillary tangles (5). There is substantial evidence supporting the primary role of amyloid beta  $(A\beta)$  and tau, along with glial dysfunction, in the pathology of AD (6). Dysregulation of A $\beta$  levels can lead to senile plaque accumulation (7). A $\beta$  is a protein that interacts with various receptors, and its deposition can interfere with normal neuronal function (7). Microstructural neurodegeneration has been found in different regions of the hippocampus, as well as the entorhinalhippocampal pathway, during disease progression (8). However, it is proposed that various toxins and medications might induce or accelerate the progression of neuronal loss through different mechanisms (9).

The Food and Drug Administration of the US (FDA) classified PG as a safe compound in 1970 (10), and it is commonly used as a vehicle for oral and intravenous medications. However, there is much evidence of PG side effects, including depression, agitation, seizures, hyperosmolarity, hemolysis, cardiac arrhythmia, and lactic acidosis (11, 12). Additionally, some investigations have reported PG toxicity when administered at high doses (13-15).

Previous research proposed that PG administration in combination with phenobarbital can induce apoptosis

in the developing mouse brain, resulting in neurotoxicity (16). A new study on PG neurotoxicity in zebrafish larvae indicated that high doses of PG can significantly change the eye diameter and locomotor activity of larval zebrafish (17). Furthermore, there are reports of PG toxicity in pediatric and adolescent medicine in human studies (18, 19). For instance, increased serum creatinine concentrations have been reported in patients exposed to PG with lorazepam infusion (20). Additionally, PG neurotoxicity was reported in pediatrics treated with sodium citrate for tubular acidosis (19). However, there are no scientific reports on PG hippocampal toxicity in the presence of  $\beta$ amyloid.

Consequently, we aimed to study the effect of PG administration in combination with  $\beta$ -amyloid injection at the levels of behavior, biochemistry, and histochemistry.

#### Materials and methods

#### Animals

Thirty-six male Wistar rats (250–300 g) were randomly divided into four groups: (A) shamoperated group, with an injection of 5 µl of normal saline in the left hippocampus (n = 9); (B)  $A\beta_{1-40}$  in the left hippocampus (n = 9); (C) Propylene glycol group, where 5 ml of propylene glycol was administered orally by gavage two times, 24 hours and 1 hour before normal saline injection (n = 9); and (D) Propylene glycol +  $A\beta_{1-40}$  group, where 5 ml of PG was administered orally by gavage two times, 24 hours and 1 hour before  $A\beta_{1-40}$  injection (n = 9).

# Surgery

Rats were anesthetized using an injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Then, the rats were placed inside the stereotaxic apparatus, and 5  $\mu$ l of normal saline or A $\beta_{1-40}$  was injected into the left hemisphere (-3.5 mm AP,  $\pm 2$  mm L, and -2.8 mm

below dura) according to the stereotaxic atlas of Paxinos and Watson (21).

## Behavioural study

We utilized the Y maze test to assess spatial recognition memory. Spontaneous alternation behavior was measured 14 days post-surgery, following previously established methods (22). In brief, rats were allowed to freely navigate the maze during an 8-minute session. An observer recorded the number of entries into different arms. Alternation behavior was defined as successive entries into all three arms in overlapping triplet sets. The alternation percentage was then calculated by dividing the actual alternations by the possible alternations and multiplying by 100.

#### **Biochemical study**

The rats were anesthetized using a high dose of ketamine (150 mg/kg) and subsequently decapitated. The left hippocampus was isolated and homogenized in a cold saline solution to prepare a 5% tissue homogenate. The homogenate was centrifuged at  $1000 \times g$ , 4°C for 10 min. The supernatant was then stored at -70°C.

#### MDA measurement

The concentration of malondialdehyde (MDA) was determined using a colorimetric assay kit (TBA method; Elabscience, E-BC-K025-S) following the manufacturer's instructions. Briefly, aliquots of the supernatant (10% rat brain tissue homogenate) were mixed with trichloroacetic acid and TBARS reagent, and then incubated at 100°C for 40 minutes. After cooling on ice, the samples were centrifuged at  $3100 \times g$  for 10 minutes, and the absorbance was measured at 532 nm. The assay demonstrated a sensitivity of 0.38 nmol/mL, with a detection range spanning from 0.38 to 133.33 nmol/mL.

#### SOD measurement

To assess superoxide dismutase (SOD) activity, the Total Superoxide Dismutase (T-SOD) Activity Assay Kit (Elabscience; E-BC-K020-M) was employed. A 10% rat brain tissue homogenate was diluted 70-fold using PBS (0.01 M, pH 7.4), and the resulting supernatant was utilized. The assay procedure involved incubating the supernatant with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37°C) for 20 minutes, followed by spectrophotometric observation of blue formazan formation at 450 nm.

The activity of SOD was quantified based on the amount of protein that inhibited NBT reduction to 50% of its maximum. One unit (U) of SOD activity was defined accordingly. The assay exhibited a sensitivity of 0.2 U/mL, with a detection range spanning from 0.2 to 14.4 U/mL.

# Histologic study

All rats were anesthetized with ketamine (100 mg/kg) for transcardial perfusion using 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Subsequently, the left hemispheres were embedded in paraffin. Coronal sections of 20 µm thickness were prepared from the left hippocampus and subjected to Nissl staining.

For neuronal quantification, sections were systematically analyzed across specific hippocampal regions: CA1 (four fields), CA3 (two fields), and the entire dentate gyrus (DG), utilizing every sixth cresyl-violet stained section in a mediolateral orientation. Neurons were identified based on the presence of a distinct membrane and discernible nucleolus. This meticulous process, conducted on five sections per specimen, facilitated the comparison of neuronal counts between experimental groups and control subjects.

#### Statistical analysis

The results were expressed as mean values accompanied by the standard error of the mean (mean  $\pm$  S.E.M.). Behavioral data were analyzed using the non-parametric Kruskal-Wallis test, followed by pairwise comparisons using the Mann-Whitney U-

test when a significant difference was detected; Bonferroni correction was applied as a post hoc test.

Biochemical and histological findings underwent parametric one-way ANOVA. Statistical significance was considered for p-values less than 0.05.

#### Results

### Alternation Behavior in Y Maze Task

Spatial recognition memory was assessed using the Y-maze task (Figure 1). The alternation score was markedly reduced in the A $\beta$  group (53%) compared to the sham group (82%, P < 0.0001). Similarly, the score was significantly lower in the A $\beta$ +PG group (43%) compared to both sham (P < 0.0001) and NS+PG groups (P < 0.0001). Notably, pretreatment of A $\beta$ -injected animals with PG (A $\beta$ +PG group) significantly attenuated the alternation behavior in this task compared to the A $\beta$  group (p < 0.001).



**Figure 1. Alternation behavior in Y Maze Task.** Significant lower alternation behavior in A $\beta$  group vs. sham group (P<0.0001). Significant lower score in A $\beta$ +PG group vs. sham (P<0.0001) and vs. PG groups (P<0.0001). Significant lower score in A $\beta$ +PG group vs. A $\beta$  group (p<0.001). A $\beta$ : Amyloid beta1-40, PG: propylene glycol. \*\*\* indicates p<0.0001, ¥ ¥ indicates p<0.001, ¥ ¥ indicates p<0.0001

# Neuronal Counting

#### Mean number of neurons in CA1 region

The mean number of neurons in CA1 was  $154.1 \pm 4.7$ in the sham group,  $147.9 \pm 5.2$  in the PG group, 112.7  $\pm$  8.2 in the A $\beta$ -injected group, and 84.43  $\pm$  7.3 in the PG + A $\beta$  group. This value was significantly decreased in animals receiving both PG and amyloid beta (P = 0.02) (Figure 2A).



**Figure 2. The mean number of neurons in CA1, CA3 and DG. A.** Significant reduced number of neurons was found in CA1 region in A $\beta_{1-40}$  injected animals vs. sham group (P<0.001), and this was significantly reduced in PG administration and A $\beta_{1-40}$  injected rats vs. A $\beta_{1-40}$  group (P =0.02). **B.** Significant reduced number of neurons was found in CA3 region in A $\beta_{1-40}$  injected animals vs. sham group (P<0.0001). In addition, it was significantly reduced in PG administration and A $\beta_{1-40}$  group (P =0.04). **C.** Significant reduced number of neurons was found in DG region in A $\beta_{1-40}$  injected animals vs. sham group (P<0.0001), and was significantly reduced in PG administration and A $\beta_{1-40}$  injected number of neurons was found in DG region in A $\beta_{1-40}$  injected animals vs. sham group (P<0.0001), and was significantly reduced in PG administration and A $\beta_{1-40}$  injected rats vs. A $\beta_{1-40}$  group (P =0.03). PG: propylene glycol. \*\*\* indicates p<0.0001, ¥ indicates p<0.05, ¥ ¥ indicates p<0.01, ¥ ¥ indicates p<0.0001.

## Mean number of neurons in CA3 region

The mean number of neurons in the CA3 region of the hippocampus was  $111.3 \pm 5$  in the sham group,  $104.6 \pm 5.9$  in the PG group,  $71.29 \pm 4.4$  in the Aβ-injected group, and  $62.71 \pm 4.7$  in the animals co-

administered with PG + A $\beta$ . This parameter was significantly decreased in the A $\beta$ -injected group compared to the sham group (P < 0.0001). Moreover, there was a substantial reduction in the mean number

of neurons in the hippocampus of rats receiving both PG and amyloid beta (P = 0.04) (Figure 2B).

## Mean number of neurons in DGlb region

The mean number of neurons in DGlb is depicted in Figure 2C. This value was  $302.1 \pm 15.32$  in the sham group,  $281.1 \pm 13.97$  in the PG group,  $77.86 \pm 11.73$  in the A $\beta$ -injected group, and  $27.71 \pm 8.7$  in the animals co-administered with PG + A $\beta$ . Similar to CA3, this parameter showed a significant reduction in the A $\beta$ -injected group compared to the sham group (P < 0.0001). Furthermore, there was a notable decrease in the group receiving both PG and amyloid beta (P = 0.03).

# Measurement of Lipid Peroxidation (nmol/mg protein)

Figure 3 illustrates the levels of MDA in hippocampal tissue homogenates across different groups. A $\beta$  injection led to an increase in MDA levels (8.87 ± 0.47) compared to the sham group (6.35 ± 0.2; P < 0.001). Interestingly, the MDA level was significantly higher in the A $\beta$ +PG group (10.87 ± 0.4), not only compared to the sham (P < 0.0001), but also in comparison with rats that received only the A $\beta$  injection (P = 0.04).



**Figure 3. lipid peroxidation marker measurement (MDA level).** A $\beta$  injection elevated the MDA level vs. sham group (P <0.001). MDA level was significantly higher in A $\beta$ +PG group vs. sham (P <0.0001) and vs. A $\beta_{140}$  injection group (P =0.04). A $\beta$ : Amyloid beta<sub>1-40</sub>, PG: propylene glycol. \*\* indicates p<0.01, \*\*\* indicates p<0.0001, ¥ indicates p<0.05, ¥ ¥ indicates p<0.0001.

# Measurement of Hippocampal SOD Activity (unit/mg protein)

Figure 4 displays the SOD levels measured in all animals to assess antioxidant capacity in the rat brain. SOD levels were significantly lower in A $\beta$ -injected rats (3.6 ± 0.17) compared to the sham group (7.08 ±

0.2; p < 0.0001). Furthermore, SOD levels were significantly decreased in the A $\beta$ +PG group (2.8 ± 0.17) compared to the sham group (p < 0.0001). Interestingly, pretreatment with PG in A $\beta$ -injected animals led to a further reduction in SOD content compared to the A $\beta$  group (p = 0.04).



**Figure 4. Super oxide dismutase measurement (SOD).** SOD level was significantly lower in the A $\beta$  injected rats vs. sham group (p<0.0001). Significant lower SOD in A $\beta$ +PG vs. sham group (p<0.0001) and vs. PG group (p<0.0001). SOD activity was significantly decreased in A $\beta$ +PG vs. A $\beta$ 1-40 group (p=0.04). \*\*\* indicates p<0.0001, ¥ indicates p<0.05, ¥ ¥ indicates p<0.0001.

# Discussion

This research aimed to evaluate the impact of propylene glycol (PG) on hippocampal neurons in male rats exposed to A $\beta$ 1-40. Co-administration of PG and A $\beta$  exacerbated neurodegeneration, leading to heightened neurotoxicity in the CA1 and DG regions. This effect was mediated by increased lipid peroxidation and reduced antioxidant capacity. Furthermore, the combination of PG with A $\beta$  resulted in a more pronounced decline in spatial recognition memory scores. Importantly, when administered alone, PG did not exhibit neurotoxic effects.

Various compounds, including sesame oil, olive oil, DMSO, Cremophor EL, ethanol, and others, are commonly used as vehicles (23). Propylene glycol (1,2-propanediol), also known as propane-1,2-diol, is an organic compound with the chemical formula C3H8O2. It is widely utilized as a solvent and moisturizer in medicines, cosmetics, and tobacco products. PG is a viscous, colorless liquid that is odorless and has a slightly sweet taste. In 1982, the US FDA classified PG as "generally regarded as safe"

(GRAS). However, recent reports have highlighted potential toxicity associated with PG (24).

Oral propylene glycol (PG) is rapidly absorbed through the gastrointestinal system and has an elimination half-life of approximately  $2.3 \pm 0.7$  hours in adults. About 45% of absorbed PG is excreted in urine, while the remainder is metabolized by the liver to produce lactate, acetate, and pyruvate (25).

There is evidence suggesting that certain medications and vehicle substances can impact various brain regions (22, 26). For instance, gray matter volume in regions such as the medial temporal cortex, temporal pole, para-hippocampal gyrus, insula, orbitofrontal cortex, and substantia nigra pars compacta can be affected in a dose-dependent manner, influenced by the age at which medications are used (26). Additionally, co-administration of specific drugs may lead to brain toxicity, necessitating careful monitoring of drug interactions. Consistent with our findings, Cremophor EL has also been implicated in exacerbating the toxicity of A $\beta$ 1-40 on hippocampal neurons (23). Gaunt and colleagues have found that long-term oral administration of rats with PG (2-5 g/kg/day) had no effect on mortality, body-weight gain, food consumption, hematology, or urinary cell excretion (27). Similarly, Tackaberry in 2010 reported no toxic effects in rats with oral PG administration up to 1000 mg/kg/day (28). Consistent with these findings, in the present study 1.5 g/kg/day of PG administered without inducing significant side effects in shamoperated animals, and no evidence of neurotoxicity was observed in the hippocampus of rats, aligning with previous research.

Studies indicate that toxicity is more likely to occur when serum PG concentrations exceed 25 mg/dL. Histopathological findings such as interlobular liver hypertrophy have been reported in F344 rats following propylene glycol acetal administration at doses of 1000 mg/kg/body weight (29, 30). Furthermore, there is evidence supporting adverse effects of PG when administered concurrently with other medications. For instance, PG has been implicated in neurotoxicity when combined with sodium citrate (19) and has been shown to induce CNS apoptosis when administered in combination with phenobarbital (16).

In our current study, we observed neurotoxic effects of PG at safe doses (1.5 mg/kg) when administered in combination with A\beta1-40. However, PG was found to be safe when administered independently, consistent with previous research findings. Various mechanisms may contribute to its neurotoxicity. Given the observed increase in lipid peroxidation markers and decrease in SOD levels, it is plausible that an imbalance between oxidants and antioxidants played a significant role in PG-induced neurotoxic effects. Additionally, PG-induced neurotoxicity manifested not only at the biochemical level but also altered working memory in animals at the behavioral level. Moreover, the combination of A<sub>β1</sub>-40 and PG may induce apoptosis, as evidenced by severe neurodegeneration observed in the CA1 and DG regions. These findings align with a study by Shen et al. in 2022, which similarly demonstrated the neurotoxic potential of PG in zebrafish (17).

Various isoforms of amyloid beta, including 25-35, 1-40, and 1-42, have been implicated in animal models of Alzheimer's disease (22, 31). Key features of the disease include hippocampal neurodegeneration, the presence of beta-amyloid plaques, and deterioration in learning and memory (22). Amyloid beta protein is predominantly found in AD-associated plaques and is produced through cleavage of the type I transmembrane amyloid precursor protein (APP) by proteases (32).

APP has been extensively studied, yet its primary physiological functions remain debated. The extracellular domain of APP facilitates synaptic connections through cell adhesion and may function as a G protein-coupled receptor involved in neuronal signaling neurotransmitter and release (33). Specifically, APP regulates hippocampal GABAergic inhibition by stabilizing the K+-Clcotransporter (KCC2) on cell membranes; deficiency in APP leads to KCC2 degradation and impaired GABAergic inhibition.

Soluble cleavage products of APP, particularly sAPP $\alpha$ , play critical roles in neuronal plasticity, survival, and protection against A $\beta$  toxicity. They are also essential for neuronal stem cell proliferation and central nervous system (CNS) development. However, dysregulated processing of APP may contribute to AD pathogenesis by increasing A $\beta$  production (34).

Furthermore, microglial-activated neuroinflammation through reactive astrogliosis is another mechanism implicated in the neuropathology of Alzheimer's disease (35). On the other hand, mitochondria play a crucial role in cellular ATP production via oxidative phosphorylation. Any impairment in oxidative phosphorylation can lead to the generation of reactive oxygen species (ROS) and mitochondrial dysfunction. Consequently, neurons with high lipid content become susceptible to lipid peroxidation and oxidative stress. Biochemical changes following oxidative stress have been implicated in many neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (36). In our study, we observed exacerbated neurodegeneration in various regions of the hippocampus after co-administration of PG and amyloid beta, which may be attributed to these mechanisms.

A study by Yixi Zhou reported decreased levels of superoxide dismutase, catalase, and glutathione peroxidase, alongside increased levels of malondialdehyde (MDA),  $\text{TNF}\alpha$ , IL-1 $\beta$ , and IL-6 in mice treated with 1 and 5 g/kg/day of PG for 28 days (37). These findings support our observations regarding the oxidant/antioxidant imbalance in the co-administration of PG and beta-amyloid.

Adverse effects of PG include depression, agitation, seizures, hyperosmolarity, hemolysis, cardiac arrhythmia, and lactic acidosis (12).

# Conclusion

In conclusion, administration of PG in combination with A $\beta$ 1-40 exacerbates neurodegeneration and neurotoxicity in the CA1 and DG regions of the hippocampus. This effect is likely mediated through increased lipid peroxidation and reduced SOD content, indicating an imbalance between oxidants and antioxidants in rat brains. The combined administration of PG and A $\beta$ 1-40 may contribute to establishing a more relevant animal model of Alzheimer's disease (AD), which warrants further detailed investigation in future studies.

Furthermore, these findings underscore the potential for vehicle chemicals to induce varying toxicological and biological effects that could potentially influence study outcomes. This consideration is particularly critical in vulnerable populations such as children and seniors, where sensitivity to such compounds may be heightened. Therefore, careful attention to vehicle selection and its potential effects on experimental outcomes is essential in neuroscientific research.

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### **Conflict of interest**

The authors declare no conflict of interest.

# **Authors' contributions**

Study conception: SS, Material preparations: MB, Data collection: SS & MB, Data analysis: SS, Primary draft preparation: MB, Final draft preparation: SS & MB.

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