

# Histopathological and Stereological Study of the Effects of Gallic Acid Administration on Hippocampal Neuronal Density after Trimethyltin Toxication

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**Received:** 5 February 2018

**Accepted:** 8 May 2018

**Published online:** 1 June 2018

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**Competing interests:** The authors declare that no competing interests exist.

**Citation:** Aghababa H, Ghezelbash M, Edalatmanesh MA, Dehghan Sh. Histopathological and stereological study of the effects of gallic acid administration on hippocampal neuronal density after trimethyltin toxication. Rep Health Care. 2018; 4 (2): 22- 27.

## Abstract

**Introduction:** Trimethyltin is a methylated organotin, which induces selective damage and neuronal death in human and rodents' CNS. On the other hand, the neuroprotective effects of Gallic acid can prevent toxicity of trimethyltin. In the current study, the repairing effect of Gallic acid in cell injury caused by trimethyltin in rats was evaluated. Also, this study assessed the effects of Gallic acid on neuronal density of the different hippocampal areas of intoxicated rats with trimethyltin.

**Methods:** 30 rats were divided in three groups: the no treatment control group, the control group, and the experimental group with intraperitoneal injection of trimethyltin (8 mg/kg) and Gallic acid solvent for 14 days. In the experimental group, after the administration of trimethyltin, 50 mg/kg of Gallic acid was injected. It was continued for 14 days. Finally, the rats were killed with transcardial perfusion. Histopathological and stereological analysis was performed on the rats. In this research, Kolmogorov-Smirnov test, one-way analysis of variance, and Tukey's post-hoc test were used.

**Results:** The results of this study showed that neuronal density of different hippocampal areas of the no treatment control group was significantly increased compared to the rats of the control and experimental groups ( $P < 0.05$ ), while chronic administration of Gallic acid could prevent apoptosis and protect hippocampal cells.

**Conclusions:** According to the results, it is suggested that chronic administration of Gallic acid decreases the effects of trimethyltin and therefore, it prevents the reduction of hippocampal cells.

**Keywords:** Hippocampus, Gallic Acid, Trimethyltin, Rat

## Introduction

Gallic acid (3, 4, 5- trihydroxybenzoic acid) is one of the most important polyphenolic compounds in plants, and a natural product of hydrolysis of tannins. Gallic acid shows anti-diabetic, anti-carcinogenic and anti-darkening effects. It prevents myocardial infarction and oxidative stress of liver and kidneys, also it protects the neurons from apoptosis induced by  $\beta$ -amyloid peptide ( $A\beta$ ) (1, 2). Gallic acid is found in foods, pharmaceutical and cosmetic products as an antioxidant. Strawberry, pineapple, banana, lemon, red and white grapes, sumac, elm, tea leaves, oak bark, and apple skin are some of the natural products

enriched with Gallic acid. Gallic acid through inhibition of tyrosine activity, induces antibacterial, antiviral, anti-inflammatory and antioxidant activities (3, 4). Relationship between damage to the hippocampus region and cognitive impairment (memory intervention and learning disorder) has been identified, which leads to the infertility of trimethyltin as an effective factor in the study of Alzheimer's disease (5). Trimethyltin affects several processes in sensitive cells. Various theories have been presented to explain the mechanism of neurotoxic trimethyltin function, which includes glutamate manifestations, increased

intracellular calcium, and ion transfer impairment. Oxidative stress and thermal shock protein activity are also followed by trimethyltin therapy (6). Laboratory studies have shown that impairment of memory and learning process is sought after the trimethyltin-induced death and selective death of neurons in the limbic system, and in particular the hippocampus (7, 8). Trimethyltin is known for using in laboratory animals to induce severe selective neuronal death, which is associated with microglial and astroglial activities in selective limbic areas, especially in hippocampus. Therefore, it presents a valuable method for studying hippocampal function during neurodegenerative disorders (9). Trimethyltin-induced neuronal damage in various species of rodents, is depending on different parameters such as race, age, dosage and type of administration, which leads to various consequences in metabolism and pathological toxic effects. Also it has been reported that trimethyltin can cause Alzheimer's disease (10). Laboratory findings have shown that oxidative stress could play an important role in Alzheimer's pathogenesis. Therefore, the risk of Alzheimer's disease could be reduced by consumption of the antioxidants which neutralize the adverse effects of oxidative stress (11).

## Methods

In this experimental study, animals included 30 Sprague Dawley rats, which were selected randomly as the sample. The animals were obtained from the laboratory animal reproduction and breeding center in Shiraz, and they were kept and tested in the zoology research laboratory of Islamic Azad university of Shiraz. For absorption of urine and feces, sterile wood chips were used. The optimal temperature was 20 to 24 °C and relative humidity was about 55 to 65 percent. A twelve-hour light-dark cycle was controlled accurately by salon's electronic light regulator. In this research, there was no food restriction for the animals. Rats in the study were divided

into 3 groups: 1. The no treatment control group: the animals of this group did not receive any treatment. Histopathological analysis of different hippocampal areas was simultaneously performed to be compared with the other groups. 2. The control group: The animals of this group were injected with 8 mg/kg intraperitoneal trimethyltin and normal saline for 14 days. Histopathological analysis was performed to evaluate degenerative effects of this substance on hippocampal neurons during stereological analysis. And 3. The experimental group: They received 50 mg/kg of Gallic acid and 8 mg/kg of trimethyltin. After administration of trimethyltin and induction of hippocampal degeneration, the animals of this group were injected with multiple intraperitoneal Gallic acid shots during 14 days. To conduct histopathological study in the end of treatment, the animals were anaesthetized with the combination of ketamine hydrochloride (100 mg/kg) and xylazine (50 mg/kg). Then, the transcardial perfusion was performed. After the perfusion, the rats' brains were extracted from their skulls and were immediately weighed with an accurate scale. Then, cerebellum tissue was prepared for slicing, and the obtained slices were stained using hematoxylin and eosin, and the microscopic imaging was conducted using a light microscope. To determine the location of different areas of hippocampus, the obtained images of the slices were matched with the atlas pictures. After assurance of location of the different hippocampal areas, the total number of questions in different hippocampal areas were estimated using hematoxylin and eosin staining. Both left and right sides of hippocampus were observed, then the mean of the obtained numbers from the different areas of hippocampus was evaluated. In this step, random sampling was used and to count DG/CA<sub>3</sub>/CA<sub>2</sub>/CA<sub>1</sub> the dissector counting technique was utilized. In this method, cells are counted in a counting frame. The gathered data from the measured variables were described using dispersion

index and central tendency. In this research, Kolmogorov-Smirnov test, one-way analysis of variance, and Tukey's post-hoc test were used. The significance level was considered as  $P \leq 0.05$  for all of the calculations.

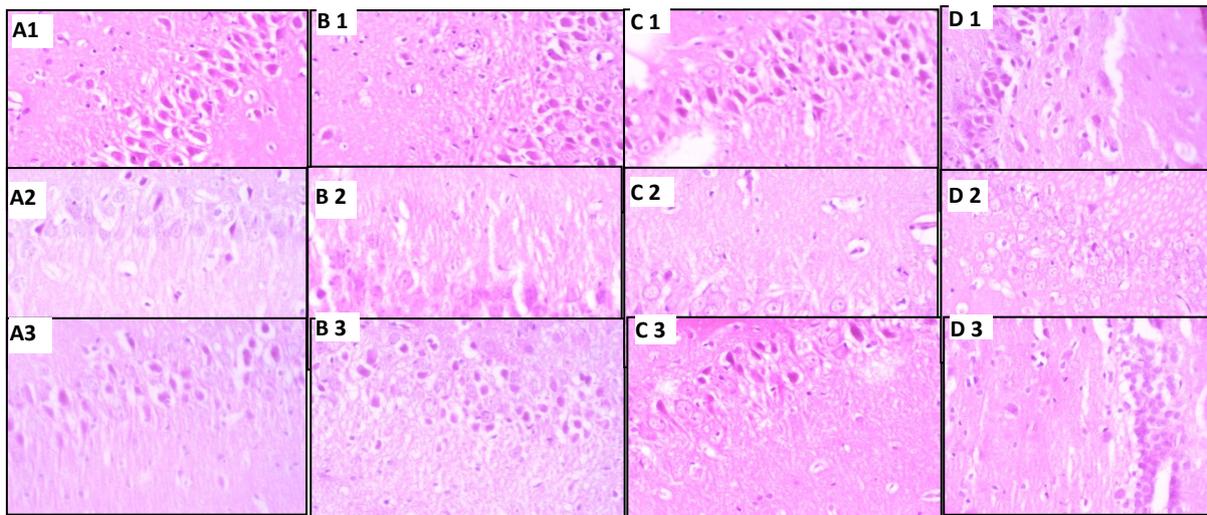
## Results

The qualitative evaluation of different hippocampal areas including CA<sub>1</sub>/CA<sub>2</sub>/CA<sub>3</sub>/DG showed that after administration of trimethyltin, the CA<sub>1</sub> neurons and cell volume were decreased, pyknosis and karyorrhexis were seen in the hippocampus. With chronic administration of Gallic acid for 14 days, these changes were less than the control group. After trimethyltin administration, the number of the neurons and cell volume were decreased and the cell wall was destructed, which indicates apoptosis. In addition to reducing the number of apoptotic neurons, Gallic acid has neuroprotective effects, therefore in the experimental group, the number and morphology of cells were closer to normal conditions. After administration of trimethyltin in CA<sub>3</sub>, the number of neurons and cell volume were decreased, while chronic treatment with Gallic acid could preserve normal morphology of cells in this area. In DG of hippocampus, after administration of trimethyltin, a large number of control group's neurons were injured, although the injury severity was significantly lower. Treating with Gallic acid reduced the number of the injured neurons of dentate gyrus. Also, the morphology of many cells of the experimental group was similar to the control group (figure 1). The stereological results showed a significant decrease in the number and density of neurons in different hippocampal areas of the rats treated with trimethyltin. The neuronal density of the treated group with Gallic acid (the experimental group) was increased compared to the control group. The results show that neuronal density in right and left CA<sub>1</sub> in both control and experimental groups were

significantly lower than the control group without any treatment, also, it is significantly lower in the control group compared to the experimental group ( $P < 0.001$ ). Moreover, there is no significant difference in neuronal density of right and left CA<sub>2</sub> area between the groups. The results show that neuronal density of right and left CA<sub>3</sub> area in the control group is significantly lower than the no treatment control group, also it is significantly lower in the control group compared to the experimental group ( $P < 0.001$ ). The results show that there is a significant difference in neuronal density of left and right DG between the groups. The neuronal density of left and right DG in the control group was significantly lower than the no treatment control group, and it is significantly lower in the control group compared to the experimental group ( $P < 0.001$ ). (Table 1).

## Discussion

In the current study, apoptosis, neuronal necrosis, neuronal transmutation and nuclear fragmentation were seen after intoxication with Trimethyltin. In contrast, trimethyltin led to neurogenesis and neuronal regeneration. In this practical study, the dual effect of trimethyltin in different hippocampal areas was shown. Trimethyltin is an organotin compound, its neurotoxic effects occur in cerebellum, limbic system and specially in hippocampus. These effects were shown experimentally in human and animal models. Trimethyltin is used widely in hippocampal destruction models of the cognitive deficits and temporal lobe epilepsy. However, the molecular mechanisms of selective neuronal death are not known clearly (10). Trimethyltin causes selective injury of the dentate gyrus of hippocampus. Neuronal/microglial interactions play the main role in neuronal inflammation signal and trimethyltin- induced programmed neuronal death (11).



**Figure 1.** A photomicrograph of hippocampal layers of the assessed groups.  $\times 400$  magnification, hematoxylin and eosin staining. A, B, C, D respectively represents: the control group without any treatment (A1), the control group (A2), and the experimental group (A3) in CA<sub>1</sub> area. The control group without any treatment (B1), the control group (B2), and the experimental group (B3) in CA<sub>2</sub>. The control group without any treatment (C1), the control group (C2), and the experimental group (C3) in CA<sub>3</sub>. The control group without any treatment (D1), the control group (D2), and the experimental group (D3) in DG.

**Table 1.** Comparison of Mean  $\pm$  SE of neuronal density and one-way ANOVA analysis P value between CA1, CA2, CA3 and DG (right and left) areas in the control, sham and experimental groups.

Groups (N=8)	DG Right (n/mm <sup>3</sup> )	DG Left (n/mm <sup>3</sup> )	CA3 Right (n/mm <sup>3</sup> )	CA3 Left (n/mm <sup>3</sup> )	CA2 Right (n/mm <sup>3</sup> )	CA2 Left (n/mm <sup>3</sup> )	CA1 Right (n/mm <sup>3</sup> )	CA1 Left (n/mm <sup>3</sup> )
Control	$\pm 2.02$ 26.30	$\pm 6.31$ 26.51	$\pm 2.45$ 27.74	$\pm 6.91$ 26.21	$\pm 2.63$ 23.67	$\pm 6.17$ 24.50	$\pm 2.21$ 23.44	$\pm 4.76$ 24.35
Sham	$\pm 2.80$ 13.69	$\pm 3.24$ 13.69	$\pm 4.80$ 18.03	$\pm 4.85$ 18.17	$\pm 2.98$ 23.77	$\pm 3.67$ 24.36	$\pm 3.06$ 15.44	$\pm 3.86$ 15.89
Experimental	$\pm 2.10$ 19.43	$\pm 4.81$ 22.50	$\pm 4.25$ 25.08	$\pm 3.04$ 25.26	$\pm 5.13$ 23.97	$\pm 6.44$ 23.74	$\pm 1.78$ 19.96	$\pm 3.38$ 19.09
P Value	0.001	0.001	0.001	0.001	0.97	0.90	0.001	0.001

Administration of trimethyltin was assessed in at least two animal models under laboratory conditions. trimethyltin-induced neuronal death was assessed from two different aspects. In one model of rats, acute apoptosis of dentate granule cells was observed in 48 hours after the administration, and in the other model, progressive death of pyramidal cells in CA1/CA2 during 3 weeks were seen (12).

Neuronal death happens at the end of the attack (2 days after treatment), it progresses exponentially, and it will develop after 3 weeks, which is probably caused by strong adhesion of rat's hemoglobin to trimethyltin. Different studies have proved that mitochondrial dysfunction and lack of Ca<sup>2+</sup> homeostasis in the initial location of trimethyltin induction would lead to neuronal

death (10). In 2011 Funk *et al.* reported that two days after treating with trimethyltin, plasma levels of corticosterone were doubled, and adrenalectomy caused by trimethyltin would lead to more susceptibility to the injury (13). Mansouri *et al.* (2013) evaluated the protective effect of oral Gallic acid on memory disorders and brain oxidative stress in the rats with Parkinson's disease. The results of this study shows that Gallic acid has deep neuroprotective effects, which is probably explained by its significant antioxidant feature (14). The Gallic acid derivatives include 4-5-methyl gallic acid, which is reported as the main metabolite of this substance in humans and rats (15). The study of Farbood *et al.* (2013) has shown that gallic acid has helpful effects on behavioral disorders caused by brain injuries. The results of this study showed that gallic acid treatment will improve brain ischemia / reperfusion injuries, and it will improve antioxidant defense of the rats, therefore it will protect the brain (4). In this study, by injecting trimethyltin in animals and the stereological analysis of hippocampal neurons and comparing it with the control group, we showed that this organotin is a neurodegenerative combination. In another study, the effects of nerve protection of gallic acid as a protecting agent and antitrimethyltin in the test group were evaluated. As expected, this compound could significantly reduce the effects of trimethyltin cell cytotoxicity. A quick look into these findings once again confirmed the potential potential for nerve protection of gallic acid and the role of trimethyltin in apoptosis, cell necrosis.

### Conclusions

According to the results of present study it appears that chronic administration of gallic acid decreases the effects of trimethyltin and therefore, it prevents the reduction of hippocampal cells.

### Ethical issues

Not applicable.

### Authors' contributions

All authors equally contributed to the writing and revision of this paper.

### Acknowledgments

The authors would like to thank Dr. Seyyed Ali Hosseini for contributing to the statistical analysis of this research.

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