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# Protective Effect of N-Acetyl Cysteine Against Formaldehyde-Induced Neuronal Damage in Cerebellum of Mice

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**ABSTRACT**

***Background:*** Formaldehyde, a colorless aldehyde with pungent odor, has negative effects on central nervous system. But, there are a little data about protective substances against neuronal damage induced by formaldehyde. Therefore, the present study was to examine histological changes in the cerebellum of formaldehyde exposed mice and possible effects of N-acetyl cysteine on these changes at histopatological level. ***Methods*:** Forty eight adult male NMRI mice were randomly divided into six groups: (I) control, (II) treated with 10 mg/kg formaldehyde, (III) treated with formaldehyde and 50 mg/kg N-acetyl cysteine, (IV) treated with formaldehyde and 100 mg/kg N-acetyl cysteine, (V) treated with formaldehyde and 200 mg/kg N-acetyl cysteine, (VI) treated with formaldehyde and 400 mg/kg N-acetyl cysteine. At the end of 14 days, the cerebellums were processed histologically and morphometric study was carried out using Image J software. Data were analyzed using SPSS software version 20.0 and ANOVA test. ***Results:*** The present study demonstrated a remarkable decrease in both the thickness and the number of the neurons of granular layer as well as the mean size and the mean number of Purkinje cells in formaldehyde-treated mice. Treatment with 50 mg/kg NAC increased the number of the neurons in molecular layer of the cerebellum as well as the thickness of the gray and white matter. Moreover, it increased the numbers of Purkinje cells (8.47±3.01 vs. 5.82±1.41). ***Conclusion*s**: Our results suggest that administration with 50 mg/kg N-acetyl cysteine prevents formaldehyde-induced neuronal damage in cerebellum of mice.

## Introduction

Formaldehyde, as a colorless substance with pungent odor, is widely used in industrial and histopathology laboratories.[1] The solid form of formaldehyde is called as paraformaldehyde, whereas the liquid form is known as formalin.[2] The exposure limit for formaldehyde in all workplaces that has recommended by The National Institute for Occupational Safety and Health is 1ppm as an 8- hour time weighted average.[3,4] Occupational safety and health administration short term exposure limit is 2 ppm during a 15 minutes period. Formaldehyde can rapidly react with DNA, RNA and protein which causes detrimental effects on human health.[5,6] In industry, formaldehyde is widely used in plastics, insulators, dyes, textiles, rubbers, cables and wood industries. In medicine, formaldehyde is used in embalming and fixation of cadavers and tissues.[2] It is also used for preserving some drugs, dental coating materials and sterilization in the hospitals. Formaldehyde is present in deodorants, toothpaste, ink, paper and cosmetic products. Therefore, everyone may be highly exposed to it.[2] Formaldehyde can affects on the skin, eyes and gonads. It also has various harmful effects on several systems such as respiratory, gastrointestinal and nervous system.[2,7-9]

It is known that formaldehyde exposure causes indigestion, headaches, malaise, memory and sleep disorders. Long-term exposure to formaldehyde can cause irreversible neurotoxicity such as brain cancer. Formaldehyde neurotoxicity causes an increase in pyknotic neuron numbers as well as a reduction in the neuron numbers in the adult rat brain. Although the exact mechanisms of formaldehyde neurotoxicity are not understood, it is postulated that formaldehyde can bond with proteins, unsaturated fatty acids and nucleic acids and it seems the neurotoxic effects of formaldehyde to be due to the molecular binding as well as formation of epoxide products.[2]

A number of studies have been reported that formaldehyde can increase the generation of reactive oxygen species (ROS) in various tissues. Many studies have shown that pretreatment with N-acetyl Cysteine remarkable protects tissues against cells from oxidative damage.[10-12] N-acetyl Cysteine (NAC), a water soluble antioxidant, can suppress the production of ROS. By facilitating glutathione synthesis, N-acetyl cysteine indicates a pro-oxidant potential. It also acts as a powerful scavenger of reactive oxygen spices and decreses inflammation. N-acetyl cysteine is known as

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a safe drug with minor side-effects.[13] Treatment of acetaminophen overdose is one of the most common clinical uses of N-acetyl cysteine. Also, it is suitable for treament of pulmonary diseases and bronchitis. Administration of NAC has a nueoprotective effect in many neurodegenerative models such as schizophrenia, Alzheimer and spinal cord injury.[14-16] Hence, this study aimed to examine the protective effects of N-acetyl Cysteine against formaldehyde-induced neuronal damage in cerebellum of mice.

## *Materials and Methods*

Forty eighth 2-3 months male mice of NMRI strain were purchased from the Razi institute (Mashhad, Iran).

The mice were maintained according to the guidelines of Institutional Animal Ethics Committee. Mice were housed under a standard condition (12 light/dark cycles, 24˚C) with free access water and standard chow diet.

After two week of adaptation, mice were randomly divided into six groups. The mice in Group I were used as a control, whereas the animals in group II were injected intraperitoneally every day with 10 mg/kg formaldehyde for 2 weeks. The mice in group III-VI received intraperitoneally NAC at doses of 50, 100, 200 and 400 mg/kg daily while exposed to formaldehyde. We calculated the dose of N-acetyl cysteine and formaldehye by pilot study. After 2 weeks, the mice pefused with paraformaldehayde 4% and then the brain fixed with 10% formalin. Samples were embedded in paraffin and 5 µ sections were carried out and stained with haematoxylin–eosin (H&E). All sections were obtained by an Olympus BH2 light microscope by an observer blind to animal experiment. We were prepared one block from each mouse. Then, first section was randomly selected and after that, it was selected 1 section from each 5 sections.

The number and thickness of cerebellum cells was counted by total magnification ×400 using Image J software version 1.48. For counting the cells, the single color images were used. To begin counting, click on the image. Then, click on Adjust and threshold. After that, we can see a Table. It was selected selection form options in the below of the Table. We finished counting by clicking on analyze particles. In this part you can see a Table consist of count, total area, and average size of each neuron.[17]

The image J analyzer was used to investigate these parameters as follows: estimating the thickness of the white and gray matter, counting the Purkinje, granular and molecular cells nuclei, comparison of the thickness of the gray matter to the white matter in the cerebellum.

**Table 1.** Mean values of the thickness and the number of the cerebellum layers in all groups

| | Group I | Group II | Group III | Group IV | Group V | Group VI |
|---|---|---|---|---|---|---|
| Molecular layer thickness (µ) | 528.59±111.45 | 554.21±104.62 | 597.72±215.10 | 522.73±153.74 | 534.71±141.33 | 592.51±236.19 |
| Granular layer thickness (µ) | 452.71±127.35 | 400.2±78.39 | 451.21±176.8 | 299.01±73.12 | 440.94±127.57 | 523.46±213.45 |
| Gray matter thickness (µ) | 1072.77±119.4 | 1014.48±91.50* | 1134.66±195.9* | 1052.33±113.4* | 1060.78±134.45* | 1211.43±224.82* |
| White matter thickness (µ) | 69.39±26.52 | 80.64±29.09 | 181.08±120.54* | 147.51±82.90 | 67.24±11.59 | 113.2±106.63 |
| Gray to white matter ratio | 14.81±1.31 | 12.58±0.40* | 6.26±2.95* | 7.13±1.2* | 15.77±1.5* | 10.69±1.60* |
| Number of molecular cell(n/mm2) | 12.86±5.03 | 11.83±1.16 | 15.92±4.87* | 10.4±3.37 | 10.0±2.95* | 13.53±3.79 |
| Number of granular cell(n/mm2) | 99.6±22.52 | 43.77±6.41* | 100±32.42 | 49.71±8.67* | 63.44±22.48 | 141.45±70.74* |
| Number of purkinje cell(n/mm2) | 5.82±1.41 | 3.14±0.36* | 8.47±3.01* | 6.13±1.84 | 6.15±1.72 | 6.57±1.80 |
| Height of purkinje cell (µ) | 91.47±16.45 | 60.07±8.07* | 85.73±12.13 | 83.43±12.18* | 85.13±11.38 | 95.46±23.73 |

Values are presented as Mean± SD. Group I: Control, Group II: formaldehyde group (FA), Group III: 50mg/kg NAC+FA, Group IV: 100mg/kg NAC+FA, Group V: 200mg/kg NAC+FA, Group VI: 400mg/kg NAC+FA.
* P <0.05 compared to the control group

### *Statistical analysis*

All statistical analyses were carried out using the SPSS statistical package, version 20.00 (SPSS, Chicago, IL, USA). Data were analyzed using one-way ANOVA followed by a tuckey post-hoc test. P value less than 0.05 were considered to be statistically significant.

## Results

The results of morphometric study for all groups are summarized in Table 1. The cerebellum sections of the control group showed a normal morphology (Figure. 1A). The present study demonstrated a significant difference between the formaldehyde and the control group ($P<0.05$). A remarkable decrease in both the thickness and the number of the neurons of granular layer of cerebellum was observed in formaldehyde-treated mice. Besides, the mean size and mean number of Purkinje cells in the formaldehyde group significantly decreased (60.07±8.07 and 3.14±0.36, respectively) compared to those (91.47±16.45 and 5.82±1.41, respectively) of the control group ($P<0.05$). Treatment with 50 mg/kg NAC increased the cell numbers in molecular and purkinje layers of the cerebellum. Moreover, it increased the thickness of gray matter (1134.66±195.9vs. 1072.77±119.4) as well as the thickness of white matter (181.08±120.54 vs. 69.39±26.52), compared to the control ones ($P<0.05$). The lowest thickness of molecular layer was observed in group IV whereas; it was in the highest level in group III. Thickness and the numbers of neurons in granular layer was the lowest in group IV and the most level was found in group VI. The most height of purkinje cells was observed in group VI whereas; the most numbers of purkinje cells was observed in group III. Gray to white matter ration and the numbers of neurons in molecular layer was the lowest in group VI and the highest in group V (Fig. 1,2).

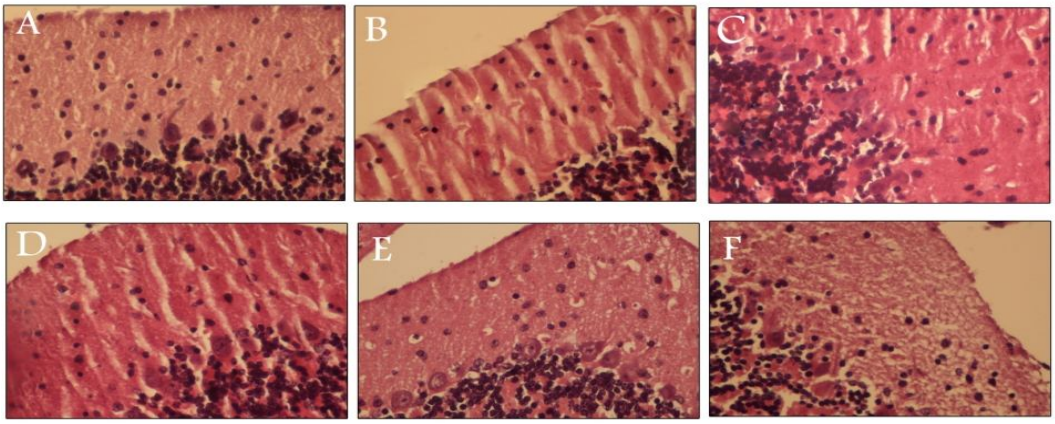


**Figure 1.** A-F are H&E stained sections of mice cerebellum of all groups. A: control group shows normal appearance of three layers of cerebellum namely molecular layer, purkinje layer and granular cell layer. B: formaldehyde group indicates loss of purkinje cells and dissociation of Purkinje cells from the granular layer. C: formaldehyde+50mg/kg NAC group shows an improvement in the number of the purkinje cells as well as the molecular cell. D: formaldehyde+100mg/kg NAC group. E: formaldehyde+200mg/kg NAC group. F: formaldehyde+400mg/kg NAC group (40X).

## Discussion

Our histological findings showed that 50 mg/kg N-acetyl cysteine protects against neurotoxicity caused by formaldehyde in the cerebellum of mice. Formaldehyde affects on different systems especially the central nervous system. The reports show indigestion, headache, mental, memory and sleep disorders after formaldehyde exposure. Besides, it was found sensory-emotional and balance disorders in the industries and hospitals employees.[18]. It also has been shown that formaldehyde is substrate for cytochrome P-450 monooxygenase system 2E1 isozyme that can activate enzymes such as peroxidase, aldehyde oxidase and xanthine oxidase and the result of this activation is increasing the formation of reactive oxygen species. The production of ROS leads to oxidative stress in the brain. Antioxidants play a critical role in scavenging free oxygen radicals. Many studies have reported that antioxidant treatment can prevent from oxidative stress in the tissues. For example, Zararsiz et al. reported that treatment of rats with 400 mg/kg/day Ш-3 fatty acids decreased cellular damage in the hippocampus and prefrontal tissues. Besides, Ш-3 fatty acids administration increased superoxide dismutase whereas it reduced malondealdehyde levels. Their histological findings also showed apoptotic cells with fragmented nuclei in the prefrontal of formaldehyde-administered rats. Besides, losing of nuclei and apoptotic bodies were observed in shrinking pycnotic cells. Treatment with omega-3 caused a decrease in the cellular damage due to FA administration compared to the control ones.[18]

On the other hands, the numbers of the studies have been shown low level of formaldehyde can cause the stimulation in the neurons while higher level of formaldehyde leads to a depressant on the nervous system.[18] However, based on our search we did not find any histopatological study in the literature about effects of formaldehyde on the cerebellum tissue.

Zararsiz and colleagues in their study found that melatonin administration, a well known antioxidant, reverses negative effects in the rats that were exposed

to formaldehyde.[19] Moreover, Gurelet al. reported degenerative damage and dark neurons were observed in the mice that exposed with formaldehyde and treatment with 300 mg/kg vitamin E significantly prevents the neuronal damage due to exposure with formaldehyde.[20] There damages were significantly less in the vitamin-treated group than those in the formaldehyde group.

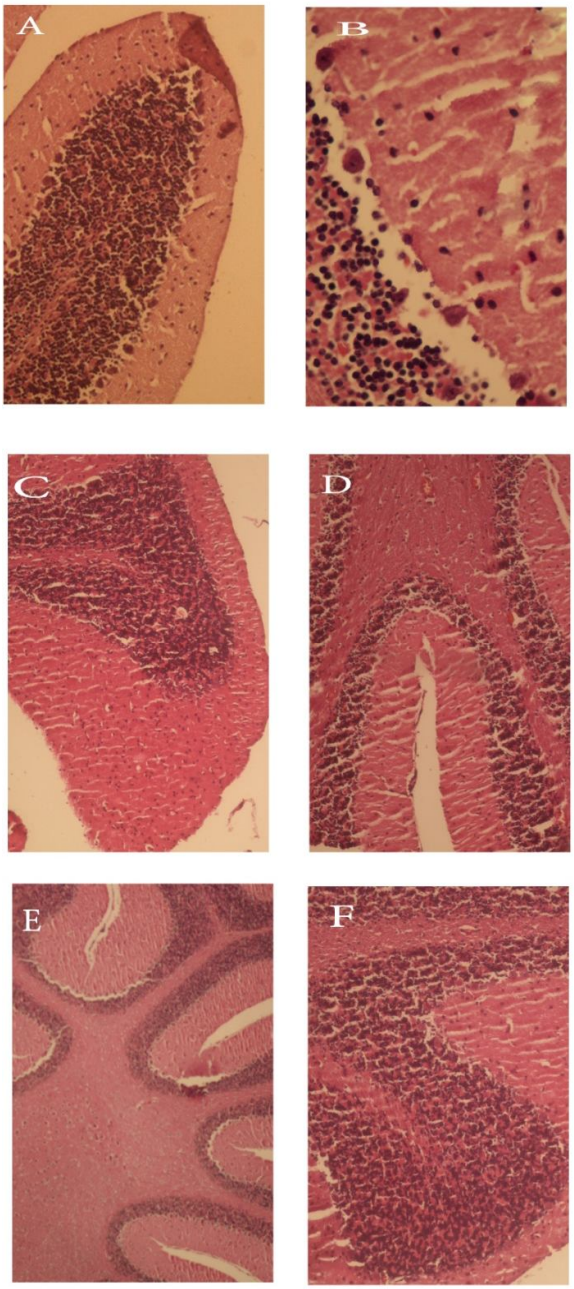


**Figure 2**. A-F are H&E stained sections of mice cerebellum of all groups.
A: control group.
B: formaldehyde group.
C: formaldehyde+50mg/kg NAC group.
D: formaldehyde+100mg/kg NAC group.
E: formaldehyde+200mg/kg NAC group.
F: formaldehyde+400mg/kg NAC group.

In mice exposed to formaldehyde was observed dark picnotic nuclei as well as reduction in the neuron counts. Administration with vitamin E caused decrease in the severity of degenerative changes.[20] Besides, the number of the neurons was higher in the vitamin-treated group than those of the formaldehyde group.
Similarly in our study, the formaldehyde-induced changes in the neurons of the cerebellum were reversed by N-acetyl cysteine treatment. Treatment with 50 mg/kg NAC caused a remarkable increase in thickness of gray and white matter as well as the number of cells in the granular layer and purkinje cells.
Khalaf et al showed that lead caused an increase of vacuoles in the cerebellum and administration of 5 gram/liter green-tea alleviated these adverse effects. Also, Musa et al reported that lead exposure induced a remarkable neuronal damage in the cerebellum of rats especially in purkinje cells and vitamin C prevents from degeneration of purkinje and granular cells in the cerebellum of rat exposed with lead acetate. However, the protective effect of vitamin C was dose dependent. Similarly, in our study antioxidant treatment with optimum dose caused a preventive effect against formaldehyde-induced damage in cerebellum of mice.[21,22] Treatment with unsuitable dose especially 100 mg/kg NAC caused a reverse effect on neural damage in the cerebellum.
Limitations: Due to of financial support, there was not biochemical finding parallel with histopathological evaluations.

## Conclusion

In conclusion, our results show that formaldehyde causes neurotoxicity in the mice's cerebellum which can be prevented by administration with 50 mg/kg NAC.

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