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Acrylamide applied during pregnancy causes the neurotoxic effect by lowering BDNF levels in the fetal brain

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ABSTRACT

Objectives: The aim of this study is to elucidate the possible mechanism of neurotoxic effect of acrylamide (AA) applied during pregnancy on fetal brain development and to show the effect of *N*-acetylcysteine (NAC) on AA toxicity.

Materials and methods: Four groups were formed with 9 pregnant rats each as control (C), acrylamide (AA), *N*-acetylcysteine (NAC), acrylamide plus *N*-acetylcysteine (AA plus NAC) groups. Caesarian section was implemented on the 20th day of pregnancy. Malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and Brain-derived neurotrophic factor (BDNF) levels were analyzed and histopathologic examinations were performed in brain tissues of the fetuses.

Results: Our data indicated that AA caused necrotic death and hemorrhagic damages in fetal brain tissue with decreasing BNDF levels and increasing oxidative stress. *N*-acetylcysteine prevented the toxic effects of its on fetal brain ($p < 0.05$).

Conclusion: Our study indicated that acrylamide has toxic effects in the fetal brain and *N*-acetylcysteine prevents its toxic effect.

1. Introduction

Acrylamide (AA) is a water soluble monomer used in chemical manufacturing technology. It is generally used in dye, paper, mining, cosmetics industries, in polyacrylamide gel preparation for electrophoresis and treatment of polluted waters. AA is not naturally found in nutrients, but it is formed in abundant amounts during cooking food over 120 °C (FDA/CFSAN, 2003, Sharp, 2003, Tareke et al., 2000, 2002). AA could have neurotoxic and carcinogenic effects as a result of various mechanisms and is defined as “carcinogen for humans” by International Agency for Research on Cancer (1997). Although, the mechanism of the toxic effect of AA has not yet been fully elucidated, some studies demonstrated a links between AA toxicity and oxidative stress (Allam et al., 2011; Erdemli et al., 2016).

Since AA is a chemical, which is easily soluble in water, it could readily be transferred from placenta to the fetus during pregnancy and from mother's milk to the infant during nursing (Edwards, 1976; Anon, 2002). Studies demonstrated that there was a passage of acrylamide to the baby through the navel cord and mother's milk in mothers, who were nurtured on products that contain AA (Sörgel et al., 2002). Therefore, especially during intrauterine life and infancy, there is a higher possibility of AA exposure when compared to adults due to the smaller body size of fetuses and babies (Bjellaas et al., 2007; Hilbig et al., 2004). It could cause lifetime AA induced damages.

NAC, the composition of *N*-acetyl derivative's L-cysteine and a thiol molecule, is a mucolytic agent. But it also has antioxidant and neuroprotective effects and can pass easily through cellular membranes (Djukic et al., 2012; Jenkins et al., 2016; Kelly, 1998).

As these reasons, to show AA's effect on fetal brain development and to elucidate the possible mechanism of its toxicity, we administered NAC to AA applied pregnant rats. We investigated the effect of AA to fetal brain development and the protective effect of NAC against AA toxicity.

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2. Methods

2.1. Study design

This study was approved by the Ethics committee of animal experiments of İnönü University (Approval number: 2012/A-30). 36 young female Wistar albino rats weighing 200 ± 20 g and bred in İnönü University Faculty of Medicine Breeding and Research Center (INUTF-DEHUM) were used in the study. Three rats were taken into special cages at 17:00 pm so that there would be 2 females and 1 male in each cage. They were kept in the cage until 08:00 am. At the end of the period males were separated from the females. Vaginal smear was obtained from female rats and examined under the microscope and the females, whose smear was positive for sperm, were considered as half day pregnant. Females who were not pregnant as a result of smear test were excluded from the study. Pregnant rats were kept at INUTF-DEHUM for 20 days (pregnancy period) in continuously ventilated rooms under 12 h of light and 12 h of dark cycle at 21 ± 2 °C. They were fed ad libitum throughout the experiment.

Pregnant rats were separated into four groups: 1) Control (C) group ($n = 9$): Only fed ad libitum during pregnancy. 2) *N*-acetylcysteine (NAC) group ($n = 9$): NAC (250 mg/kg/day) was administered during pregnancy. 3) Acrylamide (AA) group ($n = 9$): AA was applied (25 mg/kg/day) during pregnancy. 4) Acrylamide plus *N*-acetylcysteine (AA plus NAC) group ($n = 9$): 25 mg/kg/day AA and 250 mg/kg/day NAC were administered during pregnancy.

Applications were conducted at the same time during pregnancy for 20 days via oral gavage. Fetuses were taken out by caesarian section on the 20th day of pregnancy. One fetus per litter were randomly selected for analysis of biochemical and histopathologic parameters. The brains of fetuses were rapidly taken and right and left hemispheres were separated. The hemispheres were prepared so that histological and immunological markers could be examined in individual animals.

2.2. Histopathological analyses

Brain tissue samples were fixed with 10% formaldehyde. Later on, routine histological tissue follow up procedures were applied and they were embedded in paraffin blocks. 6 µm thick cross-sections were incised from paraffin blocks using a microtome and stained with hematoxylin-eosin (H-E). Stained cross-sections were examined under Nikon Optiphot-2 light microscope using a Nikon DS-Fi2 camera (Nikon Corporation, Tokyo, Japan) and photographs were taken.

The basophilic nuclear pyknotic and the eosinophilic acute necrosis was determined by hematoxylin and eosin staining. The number of stained cells was counted in randomly chosen 10 different sites on each slide and was received a mean of the number of these cells. The percent of necrotic cells was calculated by dividing the number of necrotic cells by the total number of cells. The degree of damage was determined as follows; if the percent of necrotic cells was < 10% the degree of necrosis was accepted as grade 1, it was between 10 and 50% the degree of necrosis was accepted as grade 2, it was between 10 and 50% the degree of necrosis was accepted as grade 3.

The grade of hemorrhage determined by the ratio of the amount of hemorrhagic areas to field. If the amount of hemorrhage was less than a third of high power field (HPF) (×40 objective), the degree of hemorrhage was accepted as grade 1. If the size hemorrhage was 1/3 to 2/3 of HPF, the degree of hemorrhage was accepted as grade 2. The hemorrhage covers more than 2/3 of HPF, it was evaluated as grade 3.

2.3. Biochemical analyses

Fetal brain tissues, taken into nitrogen tanks at the end of the study and stored at −80 °C, and were analyzed for malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), total protein and BDNF levels.

2.3.1. Preparation of tissues for biochemical analyses

Fetal brain tissues stored in the deepfreeze were taken out on the day of the study, weighed and phosphate buffer (containing protease inhibitor cocktail (Bio Shop, Canada)) was added to homogenate with 10% final concentration. These were homogenized in ice for 1–2 min at 12000 rpm (IKA, Germany). Tissue homogenates were centrifuged at 5000 rpm at 4 °C for 30 min to obtain supernatants.

2.3.1.1. Measurement of malondialdehyde (MDA) level. MDA analysis was conducted according to Mihara and Uchiyama (1978) method. MDA concentration was determined by measurement of supernatant that was extracted from n-butanol phase of the pink colored product, formed as a result of the reaction of the MDA in the supernatant with thiobarbituric acid at 95 °C, at 535 nm using the microplate reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT, USA). Results were given as nmol/g wet tissue.

2.3.1.2. Measurement of reduced glutathione (GSH) level. GSH analysis was conducted based on the description by Ellman (1959). GSH located in the analysis tube reacted with 5.5′-dithiobis 2-nitrobenzoic acid and produced a yellow-green color. Light intensity produced by this color was measured with the microplate reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT, USA) at 410 nm and GSH concentration was detected. Results were given as nmol/g wet tissue.

2.3.1.3. Measurement of Glutathione Peroxidase (GSH-Px). Determination of GSH-Px activity was conducted with Paglia and Valentine (1967) method. 50 mM potassium phosphate buffer (pH 7.0 and contain 5 mM ethylenediaminetetraacetic acid), 1 mM NADPH, 5 mM GSH, 1 mM sodium azide, glutathione reductase (1 IU), and a supernatant of tissues were included in the reaction mixture. After a 30-min incubation period at 37 °C, 1 mM hydrogen peroxide (H_2O_2) was added as the substrate and the reaction was initiated. The microplate reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT, USA) was used to measure the absorbance values (at 340 nm) and the results were recorded at the end of a 5- min period. Results were given as U/g protein.

2.3.1.4. Measurement of superoxide dismutase (SOD). The total reduction rate of nitro blue tetrazolium by the superoxide anion produced by xanthine and xanthine oxidase was utilized to measure SOD activity (Jolitha et al., 2006). The quantity of protein inhibiting the rate of NBT reduction by 50% was accepted as SOD activity unit. The results were reported as units per g protein. The method developed by Lowry et al. (1951) was used to determine the total protein content of the brain tissue homogenate samples.

2.3.1.5. Measurement of catalase (CAT). Aebi's method was used to determine CAT activity (Aebi, 1974). Rate constant k (dimension: s-1, k) of hydrogen peroxide (initial concentration 10 mM) was determined by the absorbance at 240 nm in a microplate reader (Synergy H1, Bio Tek Instruments, Inc., Winooski, VT, USA). (Casado et al., 2001). Catalase activity was reported as K (constant rate) per g protein.

2.3.1.6. Measurement of BDNF. Fresh brain tissues were rapidly weighed and transferred to liquid nitrogen tank and kept at −80 °C deepfreeze until the day of the study. At that time, PBS (pH 7.4) buffer was prepared with Rat Boster (Boster Biological Tech. Co., Ltd., CA, USA, Catalogno. EK0308) as per manufacturer's directions. After the tissues thawed, brain tissues were diluted 5 times with the PBS buffer and supernatants were obtained by homogenizing the samples in ice at 12000 rpm (IKA, Germany) and centrifuging for 10 min at 10,000 xg. At the end of the study, samples were measured automatically by ELISA analyzer (Basic Radim Immunoassay Operator, BRIO; Pomezia, Italy) at 450 nm according to the kit procedures and BDNF levels were determined.

2.4. Statistical analyses

Statistical analyses were conducted with SPSS 21.0 for Windows software package. Normal distribution of data was determined using Shapiro-Wilk test. Since the data did not demonstrate normal distribution, they were summarized using median (min-max). Since one fetus from one litter has randomly chosen, there was no dependency among fetuses and no need to eliminate the litter effect and the probability of a Type I error. Kruskal-Wallis test was used to compare the groups. Pairwise comparisons were conducted with Conover method, following Kruskal-Wallis test. Level of significance was considered as 0.05 in all tests ($p \leq 0.05$). Superscript letters (a,b.c) show the significance after all possible pairwise comparisons. The difference between the groups with different superscript letter are found to be statistically significant. So if two groups have different symbols they are statistically different, otherwise there is no difference between the groups.

3. Results

3.1. Biochemical findings

MDA, GSH, GSH-Px, SOD, CAT and BDNF results are presented in Fig. 1. The median levels of brain MDA in C, AA, NAC and NAC + AA groups were 201 (171–251), 320 (281–397), 184 (151–221) and 178 (168–295) nmol/gwt respectively. The median levels of brain GSH in C, AA, NAC and NAC + AA groups were 699 (526–1051), 769 (654–885), 994 (940–1033) and 808 (705–936) nmol/gwt respectively. The median levels of brain GSH-Px in C, AA, NAC and NAC + AA groups were 283 (222–366), 167(106–253), 266(207–419) and 218(183–300) U/g protein respectively. The median levels of brain SOD in C, AA, NAC and NAC + AA groups were 56(36–87), 107(98–135), 58(45–71) and 61(32–75) U/g protein respectively. The median levels of brain CAT in C, AA, NAC and NAC + AA groups were 3.99 (1.53–4.78), 1.39 (1.02–1.80), 4.91 (3.72–5.50) and 3.05(1.38–3.9) K/g protein respectively. The median levels of brain BDNF in C, AA, NAC and NAC + AA groups were 3036 (2500–4643), 1333(1005–2000), 3750 (2600–4500) and 2589 (2000–5000) pg/mL respectively.

Comparison of groups demonstrated that fetal brain tissue MDA and SOD levels in AA group were statistically significantly higher that levels in the C group ($p < 0.05$). NAC application decreased those levels up to those of control group. MDA and SOD levels in NAC and NAC + AA groups were statistically significantly lesser than that levels in AA group ($p < 0.05$), while there was not any difference in those levels between NAC and NAC + AA groups and, between control groups and each of these groups.

Comparison of GSH-Px and CAT levels on fetal brain tissue demonstrated that the levels of GSH-Px and CAT were significantly lower in the AA group compared to C group ($p < 0.05$). NAC application increased that levels approximately up to those of control group. GSH-Px and CAT levels in NAC and NAC + AA groups were statistically significantly higher than that levels in AA group ($p < 0.05$).

The fetal brain tissue GSH levels were significantly higher in the NAC group compared to other groups ($p < 0.05$). Although the fetal brain tissue GSH levels were higher in the NAC + AA group compared to C and AA groups, there was not any statistically significant difference in those levels between C, AA and NAC + AA groups.

It was also found that fetal brain tissue BDNF levels were statistically significantly lower in AA group compared to the control group ($p < 0.05$), while NAC application statistically significantly increased BDNF levels ($p < 0.05$) up to control group levels. Histopathologic findings of the present study were consistent with biochemical findings.

Histopathologic findings.

Histopathologic appearances of C, NAC, AA, NAC + AA groups were shown in Figs. 2, 3, 4, and 5 respectively. In control group cross-sections, cerebral cortex was observed as surrounded externally by piamater. Lamina molecular layer of the cerebral cortex was observed to have lower cellular intensity on the outermost areas. Nuclei of pyramidal neurons and other large neurons of the cerebral cortex were observed typically in euchromatic structure with significant nucleus. (Fig 2A B, C).

Histological structure of NAC group cerebral cortex, outlook of neurons and glia cells were similar to control group findings (Fig. 3A B, C).

AA group cross-sections demonstrated irregular neuron groups with pycnotic nuclei and neurofibrillary intensity in cerebral cortex fields. Furthermore, degenerated neurons and glia cells were observed (Fig. 4A). Advanced pycnosis (Fig. 4B, C) and eosinophilic stained nucleus structure (Fig. 4C) were significant in neurons and glia cells. Local hemorrhage areas were identified in cerebral cortex (Fig. 4A, B).

In NAC plus AA group, majority of the neuron and glial cells in cerebral cortex cross-sections was considered as normal histomorphological structure similar to control and NAC groups (Fig. 5).

The histopathological findings of groups presented in Table.1. The grade of basophilic nuclear pyknotic necrosis on brain in C, AA, NAC and NAC + AA groups were 0, 2, 0 and 0 respectively. The grade of acute eosinophilic necrosis on brain in C, AA, NAC and NAC + AA groups were 0, 1, 0 and 0 respectively. The grade of acute hemorrhage on brain in C, AA, NAC and NAC + AA groups were 0, 1, 0 and 0 respectively. There was grade 2 basophilic nuclear pyknotic and grade 1 eosinophilic necrotic and, grade 1 hemorrhagic changes in AA group, while there were not observed any histopathological changes in the other groups.

4. Discussion

It was indicated that AA can be dissolved in water, can pass through placenta and can easily reach fetal tissues (Edwards, 1976). In experimental studies, therefore, investigated the effects of AA toxicity on development of fetuses or off springs of pregnant rats (Allam et al., 2011; El-Sayyad et al., 2011; Erdemli et al., 2016; Ogawa et al., 2012). Although Erdemli et al. (2016) previously investigated the effects of AA on brain development of fetuses, to our knowledge, our study was first study that investigated AA toxicity in fetuses of pregnant rats with considerably higher AA doses (25 mg/kg body weight).

In the present study, histopathological analyses of AA group revealed important degrees of necrotic and hemorrhagic changes showing toxic effect of AA on fetal brain, which in accordance the study of Erdemli et al. (2016) previously investigated the effects of AA on brain development of fetuses at lower dose (5 mg/kg body weight) AA. However, the occurrence of hemorrhagic areas due to AA application, there was not in study of Erdemli et al. (2016). This difference between our study and former study may be due to our higher dose AA application.

In our study, it was determined that AA application significantly increased MDA and SOD levels and significantly reduced BDNF, GSH-Px and CAT levels together with its toxic effect in fetal brain. The elevation of MDA as the marker for oxidative stress confirms previously reported studies that demonstrated the links between AA toxicity and oxidative stress (Allam et al., 2011; Erdemli et al., 2016).

Under physiological conditions, free radicals are produced continuously in the body, concomitantly these harmful radicals are detoxified by protective antioxidant mechanisms. These antioxidant mechanisms include enzymatic antioxidants (SOD, CAT, GST, GSH-Px) and nonenzymatic antioxidants such as vitamins (Vitamin A, E and C), NAC, GSH, melatonin and selenium. There is an equilibrium between antioxidants and oxidants and when this balance is lost in favor of the oxidants, oxidative stress occurs, causing the development of damaging events. Oxidative stress can also result from increased production of free radicals from exposure to toxins like AA. SOD represents the first line of intracellular defense mechanisms against oxidative stress. SOD exerts the antioxidant effect by converting the highly toxic superoxide

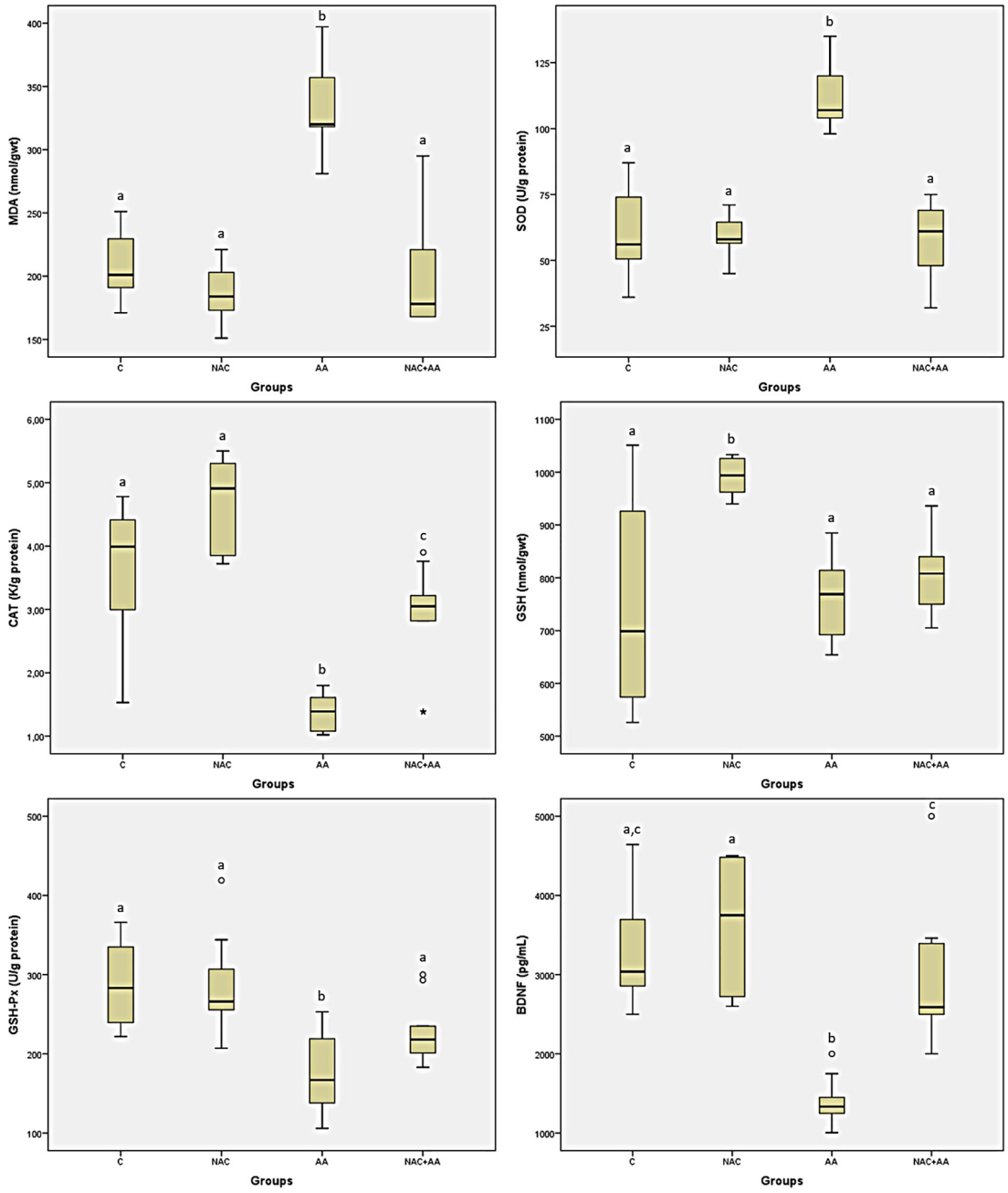

Fig. 1. Fetal brain tissue oxidant–antioxidant parameters and BDNF levels of groups. *C: Control,* AA: acrylamide, NAC: *N*-acetylcysteine, AA plus NAC: acrylamide plus *N*-acetylcysteine *group.* The difference between the groups with different superscript letter is found to be statistically significant "o" represents a outlier value; "*" represents a extreme value.

radicals to less toxic hydrogen peroxide. So, we think that the increase in SOD levels is a compensation mechanism against oxidative stress. Our result is consistent with the finding of previous reports that indicated the increased activity of SOD after exposure to AA (Yousef and El-Demerdash, 2006; Zhu et al., 2008).

NAC is a nonenzymatic antioxidant. Biologic activity of NAC is derived from sulfhydryl group. Acetyl group bound to the amino group provides stability to the molecule against oxidation. Due to the sulfhydryl group in its structure, it increases glutathione-S-transferase (GST) activity and GSH synthesis, affecting free oxygen radicals. NAC is not only a GSH precursor, but also removes free oxygen radicals. Pharmacological effects of NAC are realized by replacing reduced GSH depots in the cells and hence, increasing antioxidant potential of the cells. Accumulating evidence indicated that *N*-acetylcysteine is neuroprotective for human and animals (Falluel-Morel et al., 2012; Flanagan and Meredith, 1991; Kelly, 1998; Jenkins et al., 2016; Nazıroğlu et al., 2014). Allam et al. (2011) conducted a study where they applied 10 mg/kg/day AA orally to pregnant rats between the 7th day of pregnancy and 28th perinatal day and they reported a significant increase in MDA levels and a significant decrease in GSH and GSH-Px levels in pup brain tissues compared to the control group. Krishna and Muralidhara (2015) reported that 200 ppm AA application increased fetal brain tissue MDA level and decreased CAT and GSH-Px levels significantly compared to the control group based on the dose. Lebda et al. (2015) added 0.05% AA into the drinking water of male rats for 21 days and identified a significant increase in brain tissue MDA levels and a significant decrease in GSH and GSH-Px levels in the AA applied group when compared to the control group. In another study, Baykara

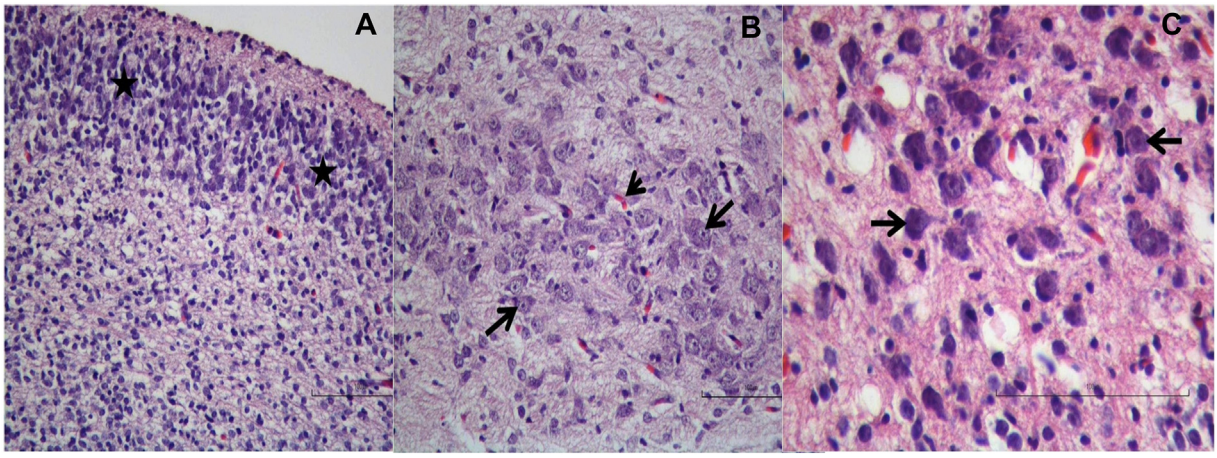

Fig. 2. Histopathologic appearances of control group. Scale bars = 100 μm.
A. The structure of cerebral cortex, neurons, glial cells (asterisk), and were normal in appearance (H-E; ×10).
B. Cerebral cortex, neurons (arrow), capillary (arrowhead) (H-E; ×20).
C. Cerebral cortex, neurons (arrow (H-E; ×40).

et al. (2015) created a nephropathy model on male rats and applied 300 mg/kg i.p. NAC as a protective, they determined that NAC application caused a decrease in MDA levels and an increase in GSH-Px and CAT levels in nephropathy induced group compared to the control group. Bueche et al. (2013) reported that GSH levels increased in male rat brains, which were applied Spontaneously Hypersensitive Stroke-Prone Rats (SHRSP) model and 3–30 weeks of 12 mg/kg/bw NAC as a protective.

In present study, NAC administration restored necrotic and hemorrhagic changes in AA group towards normal histological appearances. This indicated the protective role of NAC against AA-induced fetal brain toxicity. NAC administration decreased MDA levels up to control levels so that there were not any significant differences between the MDA levels of C, NAC and NAC + AA. There was similar approximation between the same groups of SOD and GSH-Px levels. Specifically, MDA and SOD levels of NAC and NAC + AA groups appear to have similar levels. At first glance, although, it may seem to be an inconsistency about these situations, there were reasonable explanations each of them. Firstly, MDA is marker of free radical-mediated lipid peroxidation and the reduced MDA levels in NAC + AA group up to the levels NAC and C levels is due to the strong antioxidant and free radical scavenger effects of NAC. Secondly, the reduction of SOD and GSH-Px levels in NAC + AA group could be due to restoration of them towards normal level, resulting from increased GSH synthesis or free radical scavenging properties of NAC (Falluel-Morel et al., 2012; Flanagan and Meredith, 1991; Kelly, 1998; Jenkins et al., 2016; Nazıroğlu et al., 2014).

BDNF is an important neurotrophic brain development factor that maintains neuron growth during brain development and continuity of synaptic function and neural plasticity. The most significant functions of BDNF are protection of neurons from toxic effects and survival of neurons. Pardo et al. (2016) induced sleep restriction rat model during pregnancy. At the end of pregnancy, they examined the rat offspring brain tissues and found that BDNF levels were lower in the rat offspring when compared to the control group. Madhyastha et al. (2013) created stress model during the first 10 days of pregnancy in rats and applied 10 mg/kg/day resveratrol orally during pregnancy as a protective agent. When they examined the effects of the stress model and resveratrol application on fetal hippocampus tissue BDNF levels during the first 10 days of pregnancy, they found that the stress decreased BDNF levels significantly, however resveratrol application increased BDNF levels. In a study in which a hypothyroidism model was developed by applying propylthiouracil (PTU) to female rats during pregnancy, it was found that BDNF levels decreased in rat fetus hippocampus (Chakraborty et al., 2012). Erdemli et al. (2016) demonstrated that AA-induced toxicity has oxidative tissue damage on fetal brain tissues with reduced fetal BDNF levels. Erdemli et al. (2016) also indicated the preventive effect of an antioxidant, Vitamin E on AA toxicity on fetal brain with normalized BDNF levels. The results of the current study have also shown that acrylamide administration to pregnant rats

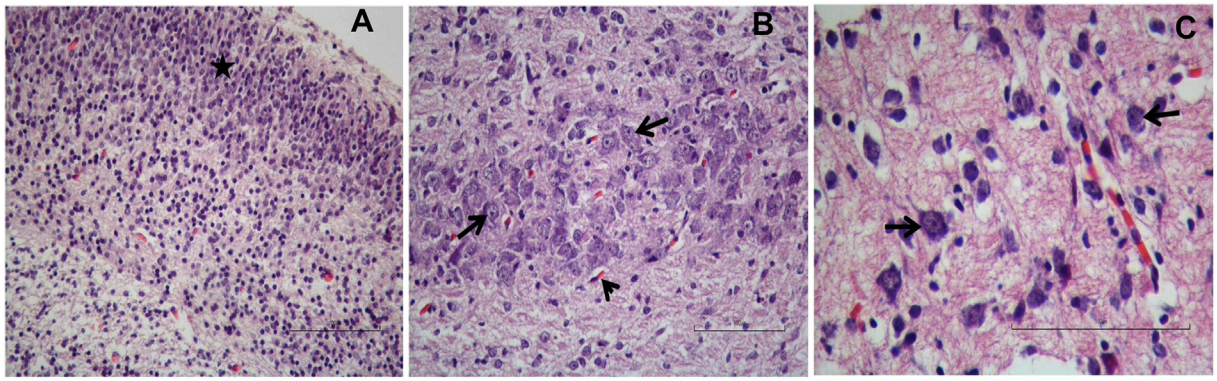

Fig. 3. Histopathologic appearances of NAC group. Scale bars = 100 μm.
A. The structure of cerebral cortex, neurons, and glial cells (asterisk) were normal in appearance (H-E; ×10).
B. Cerebral cortex, neurons (arrow), capillary (arrowhead) (H-E; ×20).
C. Cerebral cortex, neurons (arrow) (H-E; ×40).

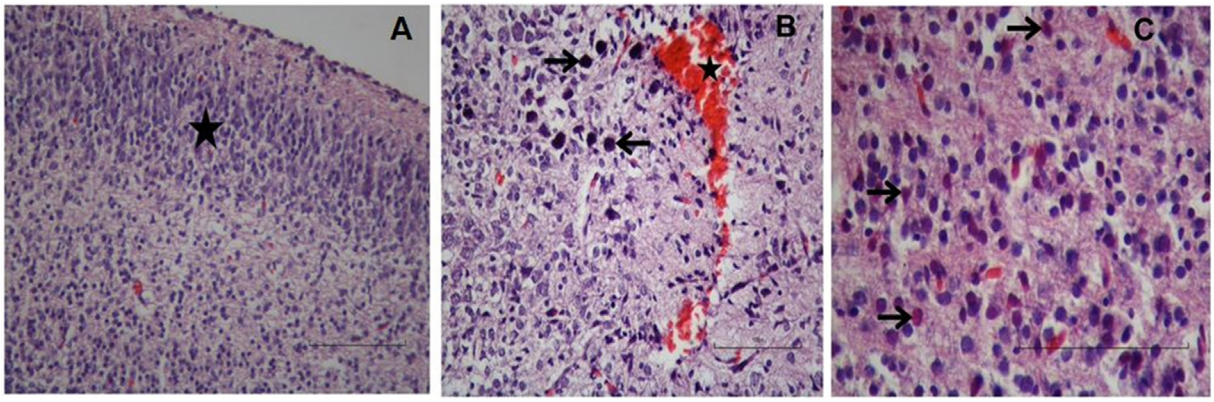

Fig. 4. Histopathologic appearances of AA group. Scale bars = 100 μm.
A. Irregular neurons with pyknotic nuclei and neurofibrillary density (asterisk) in cerebral cortex (H-E; ×10).
B. Heterochromatic-pyknotic neuron nuclei (arrow), hemorrhage (asterisk) in cerebral cortex (H-E; ×20).
C. Eosinophilic stained and pyknotic nuclei in neurons (arrow) (H-E; ×40).

significantly reduces fetal brain BDNF levels. We used another antioxidant, NAC to prevent AA toxicity on fetal brain. In our study, concomitant NAC administration prevented fully the AA toxicity damages on fetal brain and brought the fetal brain BDNF levels up close to the control group values. However, additional studies are needed to understand whether the reduction of BDNF contribute to the mechanism of acrylamide-mediated neurotoxicity.

Finally, our results indicated that AA-induced toxicity in pregnant rats caused necrotic death and hemorrhagic damages in fetal brain tissue with increased oxidative stress and reduced fetal brain tissue BDNF levels. Our results also show that an antioxidant NAC administration prevents these damages with reducing oxidative stress and increasing fetal brain BDNF levels.

5. Conclusion

AA has toxic effect on fetal brain development and *N*-acetylcysteine administration prevents its toxicity, reducing oxidative stress and elevating BDNF levels. However, further studies are necessary to understand the contribution of oxidative stress and BDNF levels on AA-induced toxic damages and the mechanism of NAC's preventive effect on AA toxicity.

Table 1
Histopathologic analysis of groups.

Groups	The grade of basophilic nuclear pyknotic necrosis Median (min.–max.)	The grade of acute eosinophilic necrosis Median (min.–max.)	The grade of hemorrhage Median (min.–max.)
C	0 (0–0)[a]	0 (0–0)[a]	0 (0–0)[a]
NAC	0 (0–0)[a]	0 (0–0)[a]	0 (0–0)[a]
AA	2 (1–3)[b]	1 (0–2)[b]	1 (0–2)[b]
NAC + AA	0 (0–0)[a]	0 (0–0)[a]	0 (0–0)[a]
p Value	0,0005	0,004	0,004

The difference between the groups with different superscript letter is found to be statistically significant.
C: Control, AA: acrylamide, NAC: *N*-acetylcysteine, AA plus NAC: acrylamide plus *N*-acetylcysteine *group.*

Author contributions

M.E. Erdemli and M.A. Aladag worked in the phases of research design, tissue extraction and writing the article, M.E. Erdemli, E. Altinoz and S. Demirtas worked in studying and calculating biochemical parameters, B. Yigitcan worked for histopathological preparations and analyzing histopathologic parameters, H.G. Bag worked in statistical calculation of the data, Y. Turkoz worked in critical editing of the article.

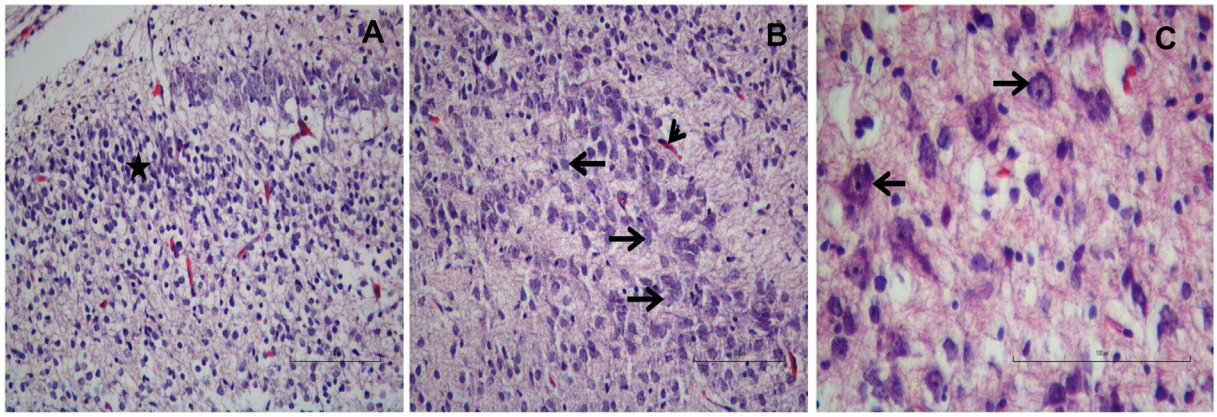

Fig. 5. Histopathologic appearances of NAC + AA group. Scale bars = 100 μm.
A. Neuron and glia cell (asterisk) in cerebral cortex (H-E; ×10).
B. Normal neurons (arrow), capillary (arrowhead) in cerebral cortex (H-E; ×20).
C. Normal neurons (arrow) in cerebral cortex (H-E; ×40).

Conflict of interest

The authors declare that they have no personal financial or institutional interests in any of the drugs, materials, or devices described in this article.

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Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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