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Effect of acrylamide on the development of medulla oblongata in albino rat: Biochemical and morphological studies

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Acrylamide is a type-2 alkene monomer with established human neurotoxic effects. The primary source of human exposure to acrylamide is occupational; other exposure sources include food, drinking water, and smoking. The present study was performed to investigate the effects of acrylamide on the development of the medulla oblongata and oxidative stress during pre- and perinatal maternal acrylamide exposure in newborn rats. Acrylamide was orally administered to non-anesthetized pregnant females in a water solution at 10 mg/kg/day using gastric intubation. The newborn rats were divided into 3 groups: 1. Normal newborns (Group A); 2. Newborns whose mothers received acrylamide from day 7 of gestation until birth (prenatal intoxicated group; Group B); and 3. Newborns whose mothers received acrylamide from day 7 of gestation until day 28 after birth (perinatal intoxicated group; Group C). The results of this study show that acrylamide leads to significantly increased lipid peroxidation and oxidative stress disturbances in the developed medulla oblongata. This action induces various dramatic tissue changes, and these abnormalities were reflected in the external features of the newborns and their body weights. In conclusion, acrylamide and its toxic metabolites induce malformations in newborn rats if their mother is chronically exposed during the gestation and lactation periods.

Key words: Acrylamide, medulla oblongata, oxidative stress, development, newborns.

INTRODUCTION

The neurotoxicity of acrylamide in adult animals has been well studied. However, data on acrylamide-induced effects on the embryonic and postnatal development of the medulla oblongata are relatively limited. Acrylamide is neurotoxic to experimental animals and humans (Zhang et al., 2011) and has mutagenic and carcinogenic effects (Maier et al., 2012). Acrylamide can be detected in starchy foods treated at high temperatures (120°C). Individuals can be exposed to acrylamide in factories, laboratories, or daily life via food and drinking water. Recently, the toxicity of acrylamide has received increasing attention (Ma et al., 2011; Seale et al., 2012).

Recent studies in rats have suggested that axon degeneration may not be a primary effect of acrylamide (Lehning et al., 2003; Allam et al., 2011). Specifically, degeneration in the peripheral nerves (sciatic, tibial, and sural nerves) was shown to be restricted to a low-dose/long-term acrylamide intoxication paradigm (21 mg/kg/day) (Lehning et al., 2002). A recent silver stain study of rat cerebella revealed that acrylamide dose rates

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of 21 and 50 mg/kg/day produced progressive degeneration of Purkinje cell axons (Lehning et al., 2003; Allam et al., 2011). Acrylamide produces a central-peripheral neuropathy in laboratory animals, including rats, monkeys, and humans (LoPachin, 2004; Seale et al., 2012). Acrylamide neurotoxicity appears to be dose- and time-dependent, and axonal degeneration occurs over time with repeated exposure despite no apparent accumulation of acrylamide at the toxicity site (Crofton et al., 1996). Moreover, neurotoxic effects in rats have been documented in brain regions associated with higher cognitive functions (Lehning, 2003). Dysfunctions of the cerebellum and proprioceptive sensations could also contribute to abnormal performances after acrylamide intoxication (Allam et al., 2011).

Acrylamide neurotoxicity has been studied in mammalian species (that is, rats, mice, monkeys, guinea pig, dogs, and cats) at daily dosages of 0.5 to 50 mg/kg/day (LoPachin and Lehning, 1994; LoPachin et al., 2000, 2002, 2003). In well-described rodent models, acrylamide intoxication at 15 to 50 mg/kg/day produced a triad of neurological deficits, such as hind-limb foot splay, decreased fore- and hind-limb grip strength, ataxia, and skeletal muscle weakness (Burek et al., 1980; Moser et al., 1992; Shell et al., 1992; Crofton et al., 1996; LoPachin et al., 2002).

Acrylamide has been shown to induce changes in oxidative status and enzyme activities, in which the effect was pronounced at high doses (Yousef and El-Demerdash, 2006). The delicate balance between the production and catabolism of oxidants is critical for the maintenance of biological function (Allam et al., 2010). An increase in thiobarbituric acid reactive substances (TBARS; the marker of the extent of lipid peroxidation) levels in different tissues upon acrylamide exposure is con-sistent with the result of Srivastava et al. (1983), who suggested that the enhancement of lipid peroxidation was a consequence of glutathione depletion to certain critical levels. Acrylamide is oxidized to glycidamide, a reactive epoxide, and undrgoes conjugation with reduced glutathione (GSH). DNA adducts from glycidamide have been reported following acrylamide administration (Dybing and Sanner, 2003).

Acrylamide reacts with glutathione and interacts with vital cellular nucleophiles that possess –SH, $–NH_2$, or –OH and forms of glutathione S-conjugates, which is the initial step in the bio-transformation of electrophiles into mercapturic acids (Awad et al., 1998). Increased glutathione-S-transferase (GST) activity with an increase in acrylamide concentration may occur due to the increased formation of S-conjugates between acrylamide and GSH. The increase in superoxide dismutase (SOD) activity could occur to combat free radical generation during acrylamide toxicity (Sridevi et al., 1998). Thiol groups are required for the activity of many important biological proteins and are also important reducing agents and cellular antioxidants (Yousef and El-Demerdash, 2006). Glutathione is the principal thiol and redox buffer in mammalian cells, whereas serum albumin is the principal protein and thiol in the plasma fraction of blood (Tong et al., 2004). The reaction of acrylamide with thiols accounts for the majority of the elimination of acrylamide from the body (Yousef and El-Demerdash, 2006).

The medulla oblongata is the lowest region of the brain and lowest portion of the brainstem (Cormack, 1993). The medulla oblongata is connected to the midbrain by the pons and is continuous with the spinal cord at the opening (foramen magnum) at the skull base. The medulla consists of both white and gray matter (Kelly et al., 1984) and comprises two sections: an open part (near the pons) and a closed part (lower and toward the spinal cord). The cardiac center is a section of the medulla oblongata that is responsible for controlling the heart beat rate. The medullary neurons are responsible for controlling several major points and autonomic functions of the body such as respiration (via the dorsal and ventral respiratory groups), blood pressure, swallowing, vomiting, defecation, and relaying nerve signals between the brain and spinal cord (Folkerth et al., 2008).

The present study on albino rats is aimed to determine the effects of oral maternal exposure to an acrylamide monomer during pregnancy and lactation on the development of newborn rat weights, external features, medulla oblongata, and oxidative stress.

MATERIALS AND METHODS

Chemicals

Pure acrylamide (99%) and other chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA). All other chemicals were of analytical grade.

Animal dosing schedule

Sixty albino rats (*Rattus norvegicus)* were used in this study. Forty-five mature virgin females and 15 mature males weighing 140 to 150 g were purchased from the Organization for Vaccine and Biological Preparations (Helwan Laboratory Farms, Egypt). The animals were marked, housed four per cage and fed a standard rodent pellet diet manufactured by the Egyptian Company for Oil and Soap (Cairo, Egypt). Tap water was provided *ad libitum.* A daily examination of a vaginal smear from each virgin female was performed to determine her estrous cycle. The estrous females exhibited the presence of cornified cells in the vaginal smears. Mating was performed by housing two pro-estrous females with one male overnight in separate cages. The presence of sperm in the vaginal smears determined D0 of gestation. Acrylamide was dissolved in distilled water and orally administered to non-anesthetized pregnant rats at a dose of 10 mg/kg/day using gastric intubation. This chronic dose was applied because overdoses will reduce the reproductive activity of mothers and cause paralysis (Tyl et al., 2000). The newborn rats' mothers were organized into the following three groups:

1. Group A: Pregnant rats were administered saline (control).
2. Group B: Pregnant rats were administered acrylamide from Day 7 of gestation until birth (prenatal intoxication).
3. Group C: Pregnant rats were administered acrylamide from Day 7 of gestation until Day 28 after birth (perinatal intoxication).

Postnatal investigations

The experimenter investigated the newborns daily, and the following notes were recorded for each group:

1. The weights of six newborns from each group.
2. The time that fur appeared.
3. The time that the ears opened.
4. The time that the eyes opened.

Biochemical assays

The pups from each group were decapitated on Day 7, 14, 21, and 28. The medulla oblongata was dissected, and 0.25 g of tissue was homogenized in 3 ml of cold saline. The homogenate was centrifuged at 10,000 × *g* for 10 min at 4°C, and the clear supernatant was collected in a microfuge tube (0.5 ml each) and stored at -40°C.

Lipid peroxidation (TBARS)

Lipid peroxidation was determined by assaying TBARS according to the method of Preuss et al. (1998). Briefly, 1.0 ml of supernatant was precipitated with 2 ml of 7.5% trichloroacetic acid and centrifuged at 1,000 × *g* for 10 min. The clear supernatant was mixed with 1 ml of 0.70% thiobarbituric acid and incubated at 80°C; the absorbance was measured at 532 nm. Tetramethoxypropane was used as the standard.

GSH assay

The glutathione content was determined according to the procedure of Beutler et al. (1963) with some modifications. Briefly, 0.20 ml of tissue supernatant was mixed with 1.5 ml of precipitating solution containing 1.67% glacial metaphosphoric acid, 0.20% sodium ethylenediaminetetraacetic acid (Na-EDTA), and 30% NaCl. The mixture was allowed to stand for 5 min at room temperature and was centrifuged at 1,000 × *g* for 5 min. One ml of clear supernatant was mixed with 4 ml of 0.30 M Na_2HPO_4 and 0.50 ml of DTNB reagent (40 mg 5,5'dithiobis-2-nitrobenzoic acid dissolved in 1% sodium citrate). A blank was similarly prepared, in which 0.20 ml of water was used instead of the medulla oblongata supernatant. The absorbance of the color was measured at 412 nm using a spectrophotometer.

Total thiol determination

Total thiol was determined according to the method of Koster et al. (1986). Briefly, 50 µl of the supernatant and 0.75 ml of 0.1 M phosphate buffer (pH 7.4) was mixed with 0.20 ml of Ellman's reagent (2 mM 5,5'dithiobis-2-nitrobenzoic acid) and incubated for 5 min at 37°C. A blank was similarly prepared, in which 50 µl of water was used instead of the medulla oblongata supernatant. The absorbance of the color was measured at 412 nm using a spectrophotometer.

Superoxide dismutase (SOD) assay

SOD activity was assayed according to the method of Marklund and Marklund (1974). Briefly, 1.0 ml of supernatant was mixed with 0.10 ml of Tris/EDTA buffer, pH 8.0 and 0.05 ml of 10 mM pyrogallol (freshly prepared). The control was prepared by adding 1.0 ml of water instead of the medulla oblongata extract. The difference in the absorbance of the color at 430 nm was recorded immediately after adding the reagent and after 10 min, to calculate enzyme activity.

Peroxidase activity

Peroxidase activity was determined according to the method of Kar and Mishra (1976). Briefly, 1.0 ml of supernatant was mixed with 3.0 ml of 0.01 M phosphate- buffered saline (pH 6.8), 315 µl of 2% pyrogallol, and 154 µl H_2O_2 and incubated for 15 min at 25°C. The reaction was halted by the addition of 0.50 ml of 5% H_2SO_4, and the absorbance was recorded at 420 nm. Peroxidase activity was expressed as the amount of purpurogallin formed per unit of absorbance.

Light microscopy

Segments of the medulla oblongata (5 mm) collected on Day 7, 14, 21, and 28 were fixed in 20% buffered formalin (pH 7.4) for 24 h. The tissue was dehydrated in ethyl alcohol followed by two changes of xylene. The tissue was impregnated and embedded in paraffin wax. The sections (4 to 5 µm) were cut, de-waxed, hydrated, and stained in Mayer's hemalum solution for 3 min. The sections were stained in eosin for one min, washed in tap water, and dehydrated in ethanol as described above. The haematoxylin and eosin stained sections were prepared according to the method of Mallory (1988). Toluidine blue was used to stain the Nissl granules and proteins according to Carleton et al. (1967). Feulgen's method was used for staining DNA (Feulgen and Rossenbeck, 1924).

Statistical analysis

The statistical package for the social sciences software (SPSS for Windows version 11.0; SPSS Inc., Chicago) was used for the statistical analyses. Comparative analyses were conducted using the general linear model procedure (SPSS, Inc.). Additionally, the data were analyzed using MANOVA followed by LSD computations to compare various groups. The results were expressed as the mean ± standard deviation (SD). The level of significance was expressed as $P < 0.05$ and was considered highly significant at $P < 0.01$ (Rao and Blane, 1995).

RESULTS

General developmental observations

The newborns in group B experienced prenatal acrylamide exposure, whereas the newborns in group C experienced perinatal acrylamide exposure. Signals of acrylamide toxicity were observed postnatally in the treated mothers and were represented by ataxia, splayed hind limbs, weakness of the hind limb muscles, and paralysis, which caused alterations in maternal behavior.

Newborns suffered from poor lactation, and consequently, malnutrition, particularly in group C. The newborns of all groups were hairless at birth. The time when fur appeared and ears and eyes opened was delayed in groups B and C (Table 1). The mean weights of the newborns of all the experimental groups varied between Day 1 and 28 (Table 2).

Table 1. External features appearance in rat newborns.

Features/Groups	A	B	C
Fur appearing	D9	D11-12	D12-13
Ear opening	D12-13	D15	D15
Eye opening	D14-15	D16-17	D16-17

D = days.

Oxidative stress

TBARS

In normal newborns, medulla lipid peroxidation was lower than in treated newborns and decreased with age. The effects of pre- and perinatal acrylamide toxicity on the medulla lipid peroxidation content are shown in Table 3. The administration of acrylamide induced a pronounced increase in the lipid peroxidation content at the investigated ages and reached a maximum value on Day 14 in group C. The general between-groups effect was highly significant ($P < 0.001$) (Table 3).

GSH content

In normal newborns, the medulla glutathione content was highest on Day 28 in all investigated organs. The intoxicated newborns indicated a marked overall decrease in the medulla glutathione content throughout the experiment compared to normal newborns, particularly for perinatal intoxicated newborns. The general between-groups effect was highly significant ($P < 0.001$) (Table 3).

Total thiol content

Table 3 indicates that the total medulla thiol markedly changed with age in the normal and treated groups. In groups B and C, the total medulla thiol content was reduced. These reductions were highly significant ($P < 0.001$) and more pronounced in group C.

Peroxidase activity

The medulla peroxidase activity in normal newborns changed slightly with age during the experiment. The acrylamide-intoxicated newborns exhibited a highly significant decrease in the medulla peroxidase activity in the two treated groups. The maximum depletion was recorded on Day 7 in group C. A one-way Multivariate analysis of variance (MANOVA) analysis of the medulla peroxidase activity showed highly significant between-group differences (Table 4).

SOD activity

In normal and treated newborns, the medulla SOD activity varied with age. In the intoxicated groups, the medulla SOD exhibited a highly significant ($P < 0.001$) decreased activity compared to normal newborns at all investigated ages (Table 4). This decrease was more pronounced in group C.

Histology of the medulla oblongata

Haematoxylin-eosin staining

On Day 7, the normal medullary neurons appeared large and varied in shape with round nuclei (Figure 1a). In group B, the majority of the medullary neurons appeared small and undifferentiated (Figure 1b). Medullary neuron chromatolysis was recorded in group C (Figure 1c). Between Day 14 and 28, the normal medullary neurons increased in size and became more differentiated (Figure 1d, g and j). Neurocyte chromatolysis and pyknosis were observed in the treated groups (Figure 1e, f, h, i and l). Group B showed improvements in the medullary neuronal state on Day 28 (Figure 1k).

Toluidine blue staining

On Day 7, the medullary neurons were well stained in group A (Figure 2a) and moderately stained in groups B and C (Figure 2b and c). Between Day 14 and 28, the cytoplasms of normal medullary neurons were deeply stained, which reflected a high amount of neuronal Nissl granules (Figure 2d, g and j). In groups B and C, the medullary neurons were faintly stained (Figure 2e, f, h, i, k and l). Additionally, improvements in the stain degree were observed in the medullary neurons of group B on Day 28.

Feulgen staining

Feulgen staining was used to demonstrate the DNA content of the medullary neurons, in which the neuronal

Table 2. Changes of body weights in rat newborns.

	Groups		
Day	**A**	**B**	**C**
1	6.31±0.12	5.08±0.13***	3.88±0.11***
2	7.4±0.26	5.15±0.08***	4.47±0.50**
3	8.33±0.19	5.53±0.16***	5.27±0.38**
4	9.15±0.15	5.57±0.11***	5.83±0.53***
5	9.58±0.28	5.8±0.13***	5.48±0.29***
6	10.65±0.23	6±0.21***	6.23±0.40***
7	12.13±0.16	6.83±0.27***	6.18±0.27***
8	13.57±0.1	6.85±0.41***	6.75±0.31***
9	14.27±0.13	9.23±0.68***	7.13±0.38***
10	16.1±0.15	10.9±0.336***	7.9±0.30***
11	17.23±0.13	10.47±0.63***	9.33±0.77***
12	17.33±0.07	10.77±0.41***	9.85±0.80***
13	18.25±0.09	12.27±0.81***	9.27±0.65***
14	19.35±0.15	11.58±0.72***	9.33±0.67***
15	20.35±0.08	13.28±0.68***	10.72±0.60***
16	21.60±0.15	15.38±0.42***	10.39±0.34***
17	23.67±0.36	16.93±0.76**	12.93±0.61***
18	23.97±0.36	17.36±0.78***	11.3±0.56***
19	25.22±0.51	19.73±0.40***	11.63±0.60***
20	25.8±0.64	20.68±0.69***	12.47±0.70***
21	27.75±0.60	20.9±0.38***	14.18±0.50***
22	28.47±0.52	21.2±0.51***	16.48±0.47***
23	31.42±0.45	22.85±0.71***	20.12±0.95***
24	34.52±0.35	23.68±0.99***	20.97±0.87***
25	36.07±0.37	23.55±0.77***	21.55±1.13***
26	38.45±0.57	24.65±0.99***	22.40±1.12***
27	39.65±0.62	25.87±77***	23.25±0.64***
28	43.17±0.99	26.62±0.62***	24.21±1.11***

Data are expressed as a mean ± SE (N = 6). Values significantly compared to the control newborns; p* ≤ 0.05, p** ≤ 0.01and p*** ≤ 0.001.

Table 3. Effect of acrylamide administration on the development of medulla oblongata TBARS, GSH and total thiol contents in rat newborns.

Parameter	**Group (time)**	**D7**	**D14**	**D21**	**D28**
TBARS (nmol/100 mg)	Normal	$18.94±2.42^{gh}$	$18.70±2.28^{ghi}$	$17.46±1.04^{hi}$	$16.42±0.61^{i}$
	Group B (prenatal)	$23.01±1.55^{de}$	$25.52±4.34^{bc}$	$21.56±2.31^{ef}$	$20.62±1.66^{efg}$
	Group C (perinatally)	$27.72±0.69^{ab}$	$29.07±1.38^{a}$	$24.59±1.51^{cd}$	$19.93±1.76^{fg}$
GSH (nmol/gm)	Normal	$99.84±11.10^{abc}$	$105.43±10^{ab}$	$99.04±5.80^{abc}$	$110.22±16.32^{a}$
	Group B (prenatal)	$74.28±6.46^{efg}$	$87.06±14.02^{cde}$	$91.86±18.54^{bcd}$	$89.46±17.59^{cde}$
	Group C (perinatally)	$67.09±15.15^{fg}$	$67.89± 8.66^{fg}$	$63.90±7.21^{g}$	$82.27±14.97^{def}$
Total thiol (mol/gm)	Normal	$2.82±0.62^{ab}$	$3.12±0.17^{a}$	$2.86±0.17^{a}$	$2.52±0.31^{b}$
	Group B (prenatal)	$1.20±0.14^{de}$	$1.75±0.28^{c}$	$1.73±0.19^{c}$	$1.42±0.27^{d}$
	Group C (perinatally)	$1.00±0.11^{ef}$	$0.89±0.19^{f}$	$1.25±0.15^{de}$	$1.31±0.20^{de}$

Data are expressed as mean ± SD. (N = 6). Means which share the same superscript are not significantly different; significance level = 0.05. D = day

Table 4. Effect of acrylamide administration on the development of medulla oblongata peroxidase and SOD activities in rat newborns.

Parameter	Group (time)	D7	D14	D21	D28
Peroxidase (U/gm)	Normal	68.63±1.25^{a}	71.17±3.14^{a}	71.44±1.91^{a}	71.46±1.20^{a}
	Group B (prenatal)	56.06±3.38^{de}	59.11±1.82^{bc}	60.97±3.38^{b}	60.42±2.99^{b}
	Group C (perinatally)	51.83±1.70^{f}	53.28±3.84^{ef}	57.16±1.46^{cd}	58.95±1.84^{bcd}
SOD (U/gm)	Normal	23.01±3.22^{a}	19.37±3.33^{b}	20.78±1.88^{ab}	21.34±3.01^{ab}
	Group B (prenatal)	15.96±4.46^{c}	14.39±2.52^{cd}	12.83±1.69^{de}	14.78±2.99^{cd}
	Group C (perinatally)	10.01±2.94^{fg}	6.39±0.77^{g}	6.38±0.64^{g}	7.35±1.25^{fg}

Data are expressed as mean ± SD (N = 6). Means which share the same superscript are not significantly different; significance level = 0.05. D = day.

DNA was strongly stained from red to pink color. The nuclei of the normal medullary neurons stained well (Figure 3a, d, g and j). In group C, neurocyte chromatolysis was observed at all investigated ages (Figure 3c, f and l). Group B showed improvements in the neuronal DNA contents at late ages (Figure 3k).

DISCUSSION

This study was designed to examine the effect of acrylamide on the appearance of external features, body weight, and the development of the medulla oblongata in newborn rats under different conditions of maternal acrylamide exposure. The effects of the low-dose acrylamide were recorded in several sections of the medulla oblongata at different ages.

Acrylamide and its metabolites, such as glycidamide, readily pass through the placenta because of their solubility in water (Sorgel et al., 2002) and are distributed in many fetus tissues during gestation (Marlowe et al. 1986 and Sumner et al., 2001). Acrylamide passes through the mother's milk to her newborns during lactation (Sorgel et al., 2002). Acrylamide results in poor lactation, which results from poor maternal behaviors and consequently leads to postnatal malnutrition (Frieda and William, 1999; Shaheed et al., 2006). Therefore, the newborns of groups B and C suffered from exposure to acrylamide and malnutrition.

In group B, the newborns suffered from prenatal acrylamide exposure. In addition, postnatal malnutrition related to maternal acrylamide exposure during the gestation period led to an alteration of maternal behaviors (Shaheed et al., 2006). In group C, the newborns suffered from acrylamide exposure during gestation and lactation and were consequently exposed to malnutrition. The fur on normal newborns appeared on Day 9. The appearance of fur was delayed in the treated groups. Gold et al. (2000) reported that acrylamide causes growth retardation, which results from growth and protein deficiencies due to malnutrition during development (Allam et al., 2010). In normal newborns, ear opening was detected on Day 12 to 13. Smart et al. (1971) detected similar results in newborn rats. In the treated groups, ear opening was delayed (Day 15). This retardation demonstrated that acrylamide exposure impaired organogenesis, as noted by Marlowe et al. (1986). These results are consistent with those reported by Garey et al. (2005). Eye opening occurred on Day 14 to 15 in group A, as observed by Bolles and Woods (1964), whereas it was detected in groups B and C on Day 16 to 17. The retardation in the treated groups was consistent with a report by Sumner et al. (2001), who observed that acrylamide causes developmental alterations.

The newborns of the treated dams suffered weight loss. Prenatal weight reductions mainly resulted from intrauterine acrylamide exposures that led to growth deficiencies of the developing fetus (Tyl et al., 2000). Newborn body weights are the most sensitive indicator of developmental toxicity (Wise et al., 1995). In the treated groups, acrylamide affected the function of mammary glands because acrylamide led to a prolactin reduction in animals, thus impairing lactation (Uphouse et al., 1982). Therefore, there were nutritional deprivations, and consequently, newborn body weight loss was observed. Frieda and William (1999) showed postnatal weight reduction in treated newborns because they suffered from alterations in maternal behaviors caused by acrylamide and a decrease in the lactation index.

The maternal acrylamide exposure during the gestation and lactation periods in the present study produced a pronounced increase in oxidative stress and marked suppression in the antioxidant defense system in the medulla oblongata of newborn rats. The lipid peroxidation level was markedly elevated, whereas the GSH and total thiol content were greatly depleted. Moreover, the antioxidant enzyme activities (SOD and peroxidase) were also depressed in the treated groups. The most pronounced decrease of these variables relative to the controls was observed in group C due to perinatal acrylamide intoxication.

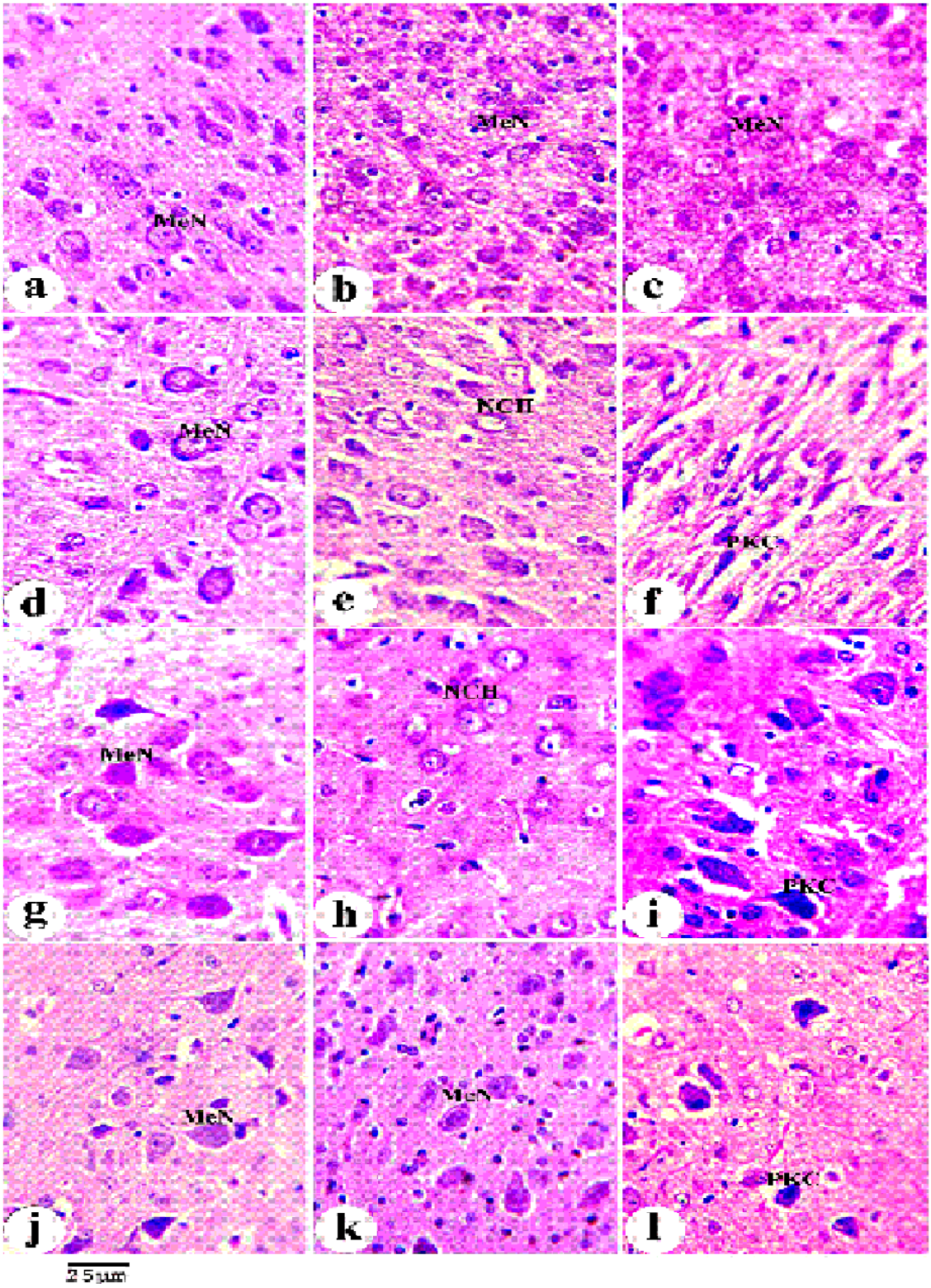

Figure 1. Sagittal sections in the medulla oblongata showing the medulla neurons (MeN), neurocyte chromatolysis (NCH) and pyknosis (PKC). (a) Normal group at Day 7, (b) group B at Day 7, (c) group C at Day 7, (d) normal group at Day 14, (e) group B at Day 14, (f) group C at Day 14, (g) normal group at Day 21, (h) group B at Day 21, (i) group C at Day 21, (j) normal group at Day 28, (k) group B at Day 28, (l) group C at Day 28. Scale bar = 25 µm (H and E).

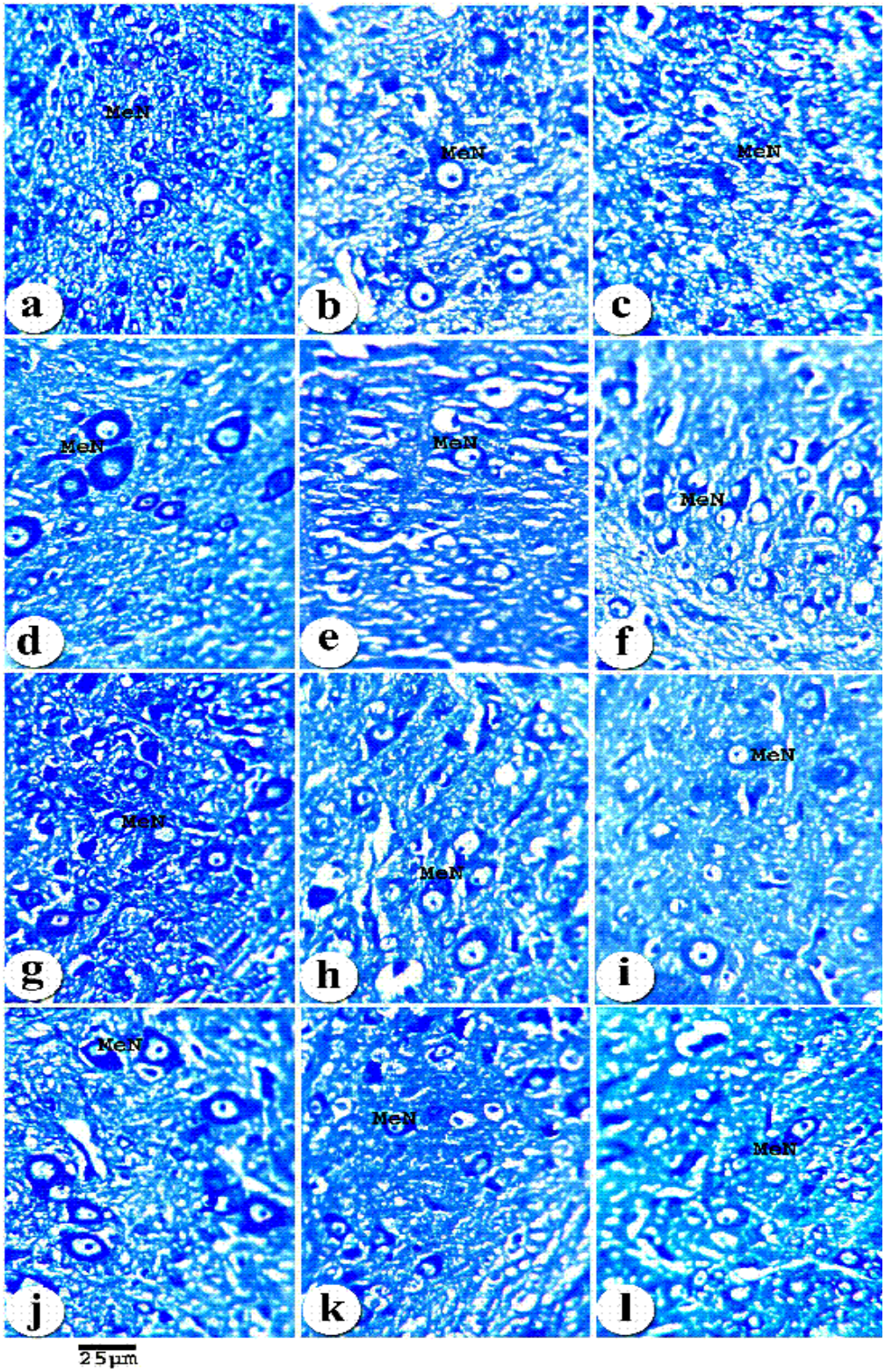

Figure 2. Sagittal sections in the medulla oblongata showing the distribution of Nissl granules in medulla neurons (MeN). (a) Normal group at Day 7, (b) group B at Day 7, (c) group C at Day 7, (d) normal group at Day 14, (e) group B at Day 14, (f) group C at Day 14, (g) normal group at Day 21, (h) group B at Day 21, (i) group C at Day 21, (j) normal group at Day 28, (k) group B at Day 28, (l) group C at Day 28. Scale bar = 25 µm (Toludine-blue stain).

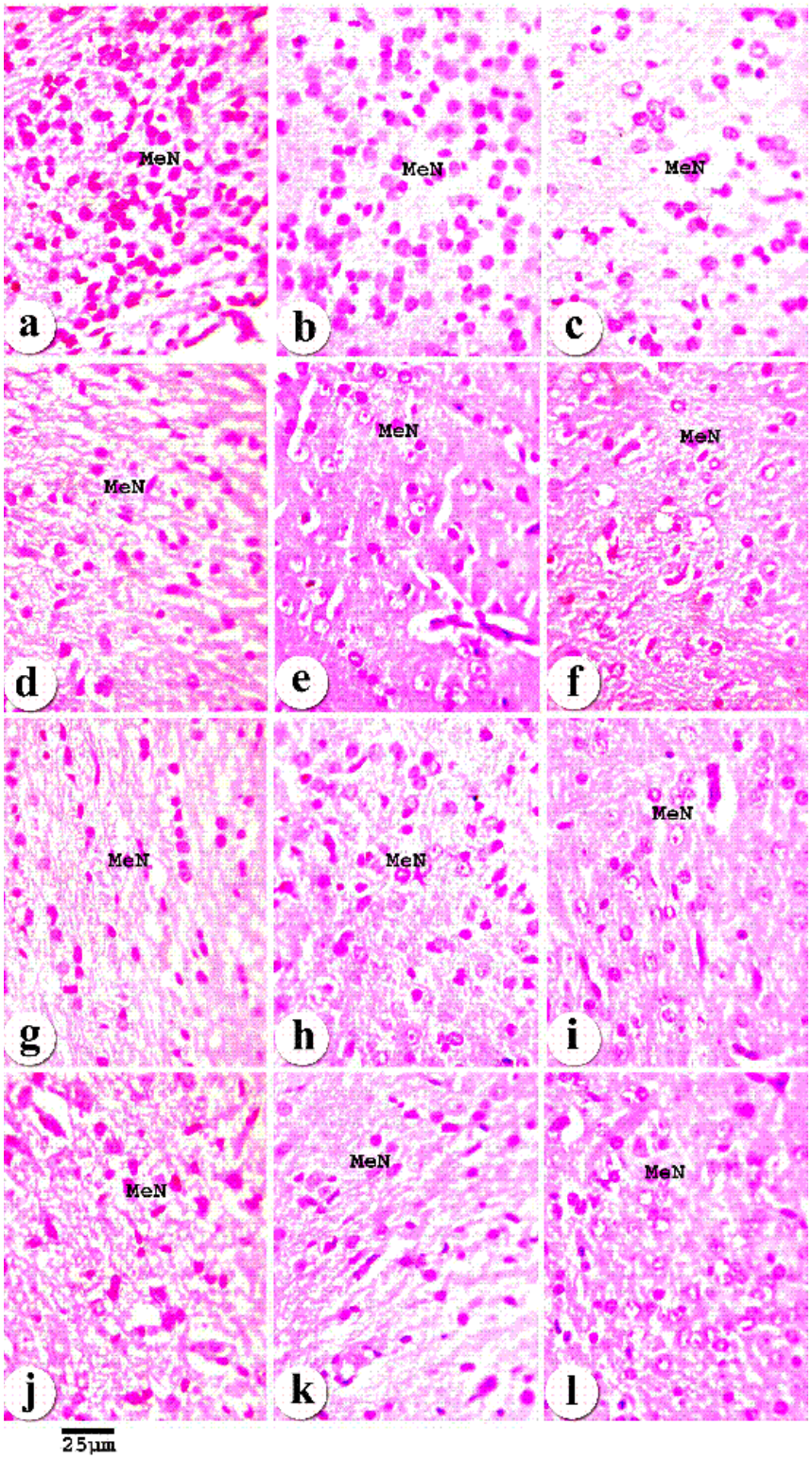

Figure 3. Sagittal sections in the medulla oblongata showing DNA in the nuclei of medulla neurons (MeN). (a) Normal group at Day 7, (b) group B at Day 7, (c) group C at Day 7, (d) normal group at Day 14, (e) group B at Day 14, (f) group C at Day 14, (g) normal group at Day 21, (h) group B at Day 21, (i) group C at Day 21, (j) normal group at Day 28, (k) group B at Day 28, (l) group C at Day 28. Scale bar = 25 µm (Feulgen staining technique).

In normal newborns, the biochemical parameters and oxidative stress changed with age because of maturation and tissue differentiation.

Yousef and El-Demerdash (2006) indicated that rats that received oral acrylamide exhibited markedly elevated levels of lipid peroxidation. Bhadauria et al. (2002) and Uličná et al. (2003) observed that acrylamide toxicity induced an increase in the hepatic malondialdehyde level and a reduction in the GSH level. Srivastava et al. (1983) suggested that an enhancement of lipid peroxidation (TBARS) occurs due to glutathione depletion to certain critical levels.

The increase in TBARS observed in the present study paralleled the decrease in the GSH concentration in the medulla oblongata of acrylamide-treated newborns. GSH is a powerful reducing agent that can interrupt the radical chain production of lipid peroxidation. Lipid peroxidation of polyunsaturated lipids has been implicated in a variety of diseased states (Ghosh et al., 1994). The delicate balance between the production and catabolism of oxidants is critical for the maintenance of biological functions (Allam et al., 2010).

Glutathione is one of the essential compounds for maintaining cell integrity because of its reducing properties and participation in cell metabolism (Conklin, 2000). Alterations in the ratio of GSH and oxidized glutathione have been used as indicators of oxidative stress and/or diseases in humans and laboratory animals (Gohil et al., 1988). In states of oxidative stress, GSH is converted into its oxidized form, glutathione disulfide (GSSG), and is thus depleted, leading to lipid peroxidation. Therefore, GSH is an important marker for the evaluation of oxidative stress (Recknagel et al., 1991). To prevent lipid peroxidation, it is important to maintain the GSH level. GSSG is reduced to GSH by glutathione reductase (GR), which is nicotinamide adenine dinucleotide phosphate (NADPH)-dependent. Therefore, the reduction of GR from the interaction of free radicals and lipid peroxides formed by acrylamide with the sulfhydryl (SH) group present at the active site of the enzyme, which in turn prevents the enzyme from participating in the reaction, results in decreased GSH levels (Recknagel et al., 1991; Yousef and El-Demerdash, 2006). Furthermore, GST catalyzes the conjugation of the glutathione thiol functional groups to electrophylic xenobiotics and results in increased solubility. The xenobiotic-GSH conjugate is then either eliminated or converted to mercapturic acid (Rao et al., 2006).

Acrylamide reacts with glutathione to interact with vital cellular nucleophiles possessing –SH, -NH_2 or –OH and forms of glutathione S-conjugates, which is the initial step in the biotransformation of electrophiles into mercapturic acid (Awad et al., 1998). A decreased GSH content in the tissues with an increase in acrylamide concentration could occur because of the increased formation of S-conjugates between acrylamide and GSH. Thiol groups are required for the activity of many important biological proteins and are important reducing agents and cellular antioxidants (Yousef and El-Demerdash, 2006). Meng et al. (2001) observed that acrylamide concentrations of 0 to 1 M markedly deplete the thiol groups of protein, similar to the results of the present study.

The antioxidant enzymes SOD, catalase, and peroxidase comprise a mutually supportive defensive consortium against reactive oxygen species (Tabatabaie and Floyd, 1994; Bandhopadhay et al., 1999). The decrease in the activity of SOD and peroxidase in acrylamide-treated newborns may have occurred due to the increased lipid peroxidation or inactivation of enzymes by cross-linking with malondialdehyde. This cross-linking will cause an increased accumulation of both superoxide and hydrogen peroxide radicals, which could further stimulate lipid peroxidation (Rister and Bachner, 1976; Rajesh and Latha, 2004). The peroxidases assayed in this study using pyrogallol as a substrate may be myeloperoxidase and eosinophil peroxidase (heme peroxidase) rather than glutathione peroxidase, which is highly specific for glutathione and cannot oxidize other substrates (Shigeoka et al., 1991).

Based on the results presented herein, it is notable that the enhanced lipid peroxidation and deterioration of the antioxidant defense system that resulted from acrylamide exposure may play a significant role in the pathogenesis and deleterious histological effects on the medulla oblongata of newborns.

The normal medullary neurons appeared large in size, varied in shape, and had round nuclei. Similar results were observed by Folkerth et al. (2008) who reported that medullary neurons have vital respiratory functions. The present results demonstrated that the normal medullary neurons increased in size and became more differentiated with age. The most striking features of acrylamide toxicity in the medulla oblongata in the treated groups were pyknosis and neurocyte chromatolysis, which were detected at all investigated ages. The severity of neurocyte chromatolysis increased with age in group C. The above pathological cases reflect central nervous system (CNS) neuropathy, which was caused by acrylamide as was stated by He et al. (1989), Deng et al. (1993), and LoPachin (2004). This damage may have resulted from the metabolic and biochemical alterations caused by acrylamide and its metabolites (Seale et al., 2012). Group B showed some improvement on Day 28 because of the absence of postnatal maternal acrylamide exposure.

The intensity of Nissl granules in the neurons corresponds to the high metabolic activity of the neurons (Stevens and Lowe, 1997). In group A, the intensity of Nissl granules in the medulla neurons was high at the investigated ages, which revealed a high metabolic rate in the neurons. Stevens and Lowe (1997) observed that Nissl granules were present in the cell body and dendrites.

Feulgen staining was used to investigate the levels of DNA in the medulla neurons. The stain color intensity in the normal neurons was high. The nuclei of groups B and C showed a marked decrease in the intensity of the color specific for DNA, thus indicating a marked loss of DNA. This color reduction decreased with age in group B but increased in group C. Central chromatolysis was observed for most ages in the treated groups due to DNA damage induced by acrylamide (Sega et al., 1989; Tyl et al., 2000).

Conclusion

Acrylamide affects the medulla oblongata of developed newborn rats if their mothers are exposed to acrylamide during gestation and lactation. These effects, which appeared as histopathological changes within the medulla oblongata, resulted from perturbations of oxidative stress. These disturbances in newborns led to healthy abnormalities. Therefore, acrylamide causes malformations in newborn rats.

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