https://doi.org/10.48048/tis.2023.6656

Folic Acid Attenuates MSG-Induced Teratogenicity during A 2-Month Pregnancy by Preventing Neural Crest Cell Destruction and Malformation in Chick Embryo Models

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Received: 23 January 2023, Revised: 13 February 2023, Accepted: 15 February 2023, Published: 20 March 2023

Abstract

Monosodium glutamate (MSG), commonly used as a food enhancer, has been reported to have teratogenicity during the first 3 days of development. Furthermore, the neural crest cells (NCCs) are crucial for embryonic development during organogenesis. The present study aimed to investigate the treatment effect of folic acid (FA) on MSG-induced teratogenicity, focusing on the toxicity and teratogenic effects on somatic and neural crest cells in chick embryos as models. Six hundred and fifty fertilized eggs were divided into control, FA, MSG, and MSG with FA groups. The chemicals were administered, and the results were investigated after 3 days of incubation. The morphology and histology were studied using stereomicroscopy and hematoxylin and eosin staining, respectively. The NCC population was confirmed by the presence of HNK-1 using immunohistochemistry. The finding showed that the MSG at 2 mg/kg of egg weight induced retardation, tissue malformation, craniofacial, and heart defects, whereas the FA alleviated those adverse effects and reduced the MSG-induced NCCs destruction in the eyes, heart, stomach, and nerves. In conclusion, although MSG harms embryos, FA effectively diminished its teratogenicity in the chick embryo model. These experimental protocols are beneficial for teratogenic studies on preventing birth defects that are harmful to the embryo.

Keywords: Monosodium glutamate, Chick embryo, Birth defect, Folic acid, Neural crest cells

Introduction

Birth defects (BDs) are multifactorial diseases caused by genetics (e.g., chromosomal abnormalities as well as single gene defects) [1], environmental factors (e.g., malnutrition, drugs, toxins, infectious etiologies, teratogens, and mechanical forces), or both; therefore, these defects can occur during any stage of pregnancy, but especially in the first 3 months, which is important for embryonic development, known as organogenesis [2]. The disabilities depend on which organ or body part is involved and how much it is affected. Therefore, serious BDs can be lethal and it was estimated that the incidence rates of BDs in low-income, developing, and developed countries were 64.2, 55.7 and 47.2 per 1,000 live births, respectively [3]. The presence of these defects at birth can be a leading cause of infant mortality or the structural changes present at birth (e.g., face, head, heart, brain, and limbs), resulting in poor life quality and financial problems due to the cost of medical care for surviving patients [4].

Chick embryos are one of the most beneficial models that can serve as a substitute for developmental studies on humans, especially in the first trimester. Chick embryos were used in drug screening studies, teratogenic evaluation, and studies of the mechanisms of teratogenicity as the main characteristics of the chick embryo include its significant similarity to the human embryos at the molecular, cellular, and morphological levels [5-7]. According to the fundamentals of "Carnegie stages", which were developed by Streeter (1942) and O'Rahilly and Müller (1987), they provide a universal system for staging and comparing the embryonic development of most vertebrates. In the 8 weeks of human embryo development, which is related to a 3-day-old chick embryo, there is a critical period for organogenesis [8].

Folic acid (FA) is a water-soluble vitamin in the B complex family [9-11], which plays an important role in cell growth and development, not only in the embryonic stage but also throughout human life. Serving and maintaining these functions requires many reactions and processes to provide their activities through the folate or tetrahydrofolate (THF) cycles, a hub of pathways that stand for the 1-carbon metabolism [12]. Furthermore, the insufficiency of folate is related to neural tube defects (NTDs) [13,14], so FA has been reported to reduce the risk of birth defects [15,16]. However, the mechanical properties of FA in birth defect treatment remain unclear.

Monosodium glutamate (MSG) is a monosodium salt of glutamic acid, which is widely used as a flavor-enhancer in Asian food worldwide and provides an umami taste [17]. However, MSG has been reported to have various forms of toxicity. The evidence of MSG toxicity is proved by studies on both animals and humans. In human studies, MSG has been related to metabolic disorders and neurotoxicity [18-22]. Surprisingly, there is no evidence that maternal MSG consumption causes birth defects or any other diseases in infants. There are few reports using animal studies as models for pregnant women to study the adverse effects of MSG consumption. The growth retardation, embryonic death, and congenital malformations of several organs on chick embryo models signaled MSG-induced teratogenic effects [23,24]. However, there is insufficient evidence to support the mechanisms of MSG-induced teratogenicity during development.

Neural crest cells (NCCs) are a transient group of embryonic cells that play an important role in embryogenesis. The cells arise from the neural tube within the neural tube bordering the neural and non-neural (presumptive) ectoderm and are found in both vertebrates and invertebrates during neurulation [25-31]. Therefore, the cells have been considered a 4 germ layer as they develop to mesenchymal cells in formations known as ectomesenchyme and give rise to a variety of cell types, such as craniofacial bone and cartilage, neuronal cells (neurons and glia) of the sympathetic and parasympathetic systems, cardiac outflow tract, melanocytes of skin, etc. [32-34]. NCC abnormalities, for example, the induction, specification, migration, differentiation, or death of NCCs, cause neurocristopathies, a class of congenital diseases [35,36], and neural tube defects. Many factors that cause NCC dysfunction may be caused by a gain or loss of function, such as genetics, environmental factors, etc. [37]. The teratogen was 1 cause that affected the NCC deaths, as reported by Shi *et al.* [38].

In the present study, we investigated the effect of high MSG consumption during the prenatal period on the impairment of embryonic development and the treatment role of FA in the birth defects caused by MSG-induced toxicity and teratogenicity during the first 3 days of development. We focused on the toxicity and their teratogenic effects on somatic and neural crest cells, using chick embryos as models.

Materials and methods

Chemicals

Monosodium glutamate (MSG) and folic acid (FA) were purchased from Sigma-Aldrich (USA). MSG was prepared to 4 mg MSG by dissolving 0.0479 g of MSG powder into 10 mL of warm, sterile, normal saline. The folic acid (FA) solution was prepared to 0.6 mg in normal saline by dissolving 0.00719 g of FA powder into 10 µL of 0.1M NaOH after the FA dissolved, then adding the sterile, normal saline to a final volume of 10 mL. The anti-HNK-1 and goat anti-mouse IgG conjugated to the enzyme HRP were also purchased from Sigma-Aldrich (USA).

Chicken embryo experiments

The freshly laid, fertilized, white leghorn hen eggs (*Gallus gallus domesticus*) were obtained from Luang Suwan Vajokkasikij farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Thailand. The 650 fertilized eggs were cleaned with sterile water, then weighed, and incubated at 37 ± 0.5 °C with 70 - 80 % humidity in a humidified incubator and automatically turned 120 degrees 3 times per day. The experiments used a single injection of 50-µL soluble agent to deliver the soluble agent into the fertilized egg after 21-h incubation using the *ovo*-injection method [23]. Two hundred and 50 fertilized eggs, whose average weight was 0.62 ± 0.37 kg, were divided into 3 experiments; (1) determining 50 % lethal concentrations (LC50); (2) a morphological study of folic acid's effects on MSG-induced embryonic mortality, retardation, and malformation; (3) the histopathological study of folic acid's effect on the MSG-induced tissues' teratogenicity, as shown in **Figure 1**.

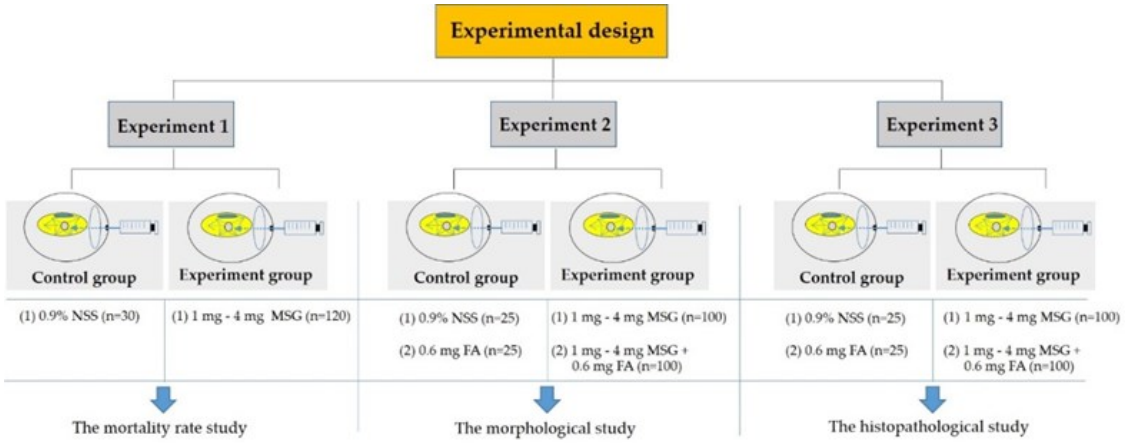

Figure 1 The schismatic diagram of experimental design. Experiment 1 represents the study of the mortality rate of MSG-induced embryonic death by observing the absence of a heartbeat. Experiment 2 represents the morphological study of folic acid on MSG-induced embryos. Experiment 3 represents the histopathological study of the treatment effect of folic acid on MSG-induced embryos. All of the experiments were obtained after 3 days of administration by opened and chick embryos were examined and recorded. The surviving embryos in experiment 1 and experiment 2 were sacrificed, removed from the extra-embryonic membranes, and fixed overnight in 4 % PFA in PBS at 4 °C.

The morphological study

For the morphological study, the formation and development of the brain, craniofacial components, brachial arches, neural tubes, somites, heart, and limb buds were investigated on 3-day-old embryos. The whole body of embryos in each group was observed under a stereomicroscope (Leica EZ4 W, German) and photographed. HH 18 stage was used as a criterion for morphological study, as follows: the embryo showed the formation of the craniofacial structures, auditory vesicle (AV), eye (E) and nasal placode (NP), 5-brain vesicles, 4 pairs of branchial arches (BA), S-shaped heart looping (H), limb buds' appearance, wing bud (WB), leg bud (LB), the dense parallel of somites, whose aliments belong to the neural tube, and body bending and flexion.

The histological study

The 3-day-old embryos were fixed with 4 % paraformaldehyde in PBS, processed, and embedded in paraffin wax. The paraffined block was sectioned at 4-6 µM. The tissue sections of each group were stained with hematoxylin and eosin (H&E), observed under a light microscope (Olympus BX53, Japan), and photographed.

The immunohistochemistry study

To investigate the NCCs on 3-day-old embryos, sections of paraffin embedded on the 3-day-old embryonic tissue were dewaxed, rehydrated, and retrieved using 10-mM sodium citrate buffer (pH 6.0) for 20 min. Sections were then rinsed in PBS and a protein block (Vector, USA) was applied to block nonspecific background staining for 5 min. Sections were incubated with the anti-HNK-1 (1:100), a specific marker of NCCs, at 4 °C overnight. The slides were washed with PBS, followed by incubation with the secondary, tagged with HRP (1:500), for 4 h. The slides were then washed in PBS, followed by incubation with DAB Substrate (Vector, USA) for 20 min at room temperature, and counterstained in hematoxylin for 30 s. The slides were observed under a light microscope (Olympus BX53, Japan) and photographed.

Statistical analysis

Data were analyzed using Prism software version 8. All data were expressed as the mean ± SD. Differences in the measured parameters in the different studied groups were tested using the One-Way ANOVA test and Pearson's chi-squared statistical test. Significance was considered at $p < 0.05$.

Results and discussion

Folic acid reduced the risk of embryonic mortality after MSG exposure

To study MSG-induced toxicity in chick embryos, we further investigated the potential treatment effect of folic acid after MSG exposure. The results showed the highest dose used in the study was 4 mg/kg egg weight of MSG, which led to a mortality rate of over fifty percent (52.00 ± 0.03), as shown in **Figure 2**. Surprisingly, the FA can attenuate the adverse effects of MSG-induced toxicity by reducing the number of embryonic deaths, as shown in **Figure 2**. FA alone had no effects similar to the NSS group ($p > 0.05$). Moreover, there was a statistical difference between the MSG and MSG+FA groups at the lowest dose of 1 mg/kg egg weight of MSG ($p < 0.05$). Although the high concentrations of MSG, at 4 mg/kg in FA treatment groups, showed a statistically significant difference when compared to the MSG group ($p < 0.05$), this represented the high death rate for FA treatment rather than the results for MSG alone. These results suggested that the FA effectively reduced embryonic mortality at a low level of MSG.

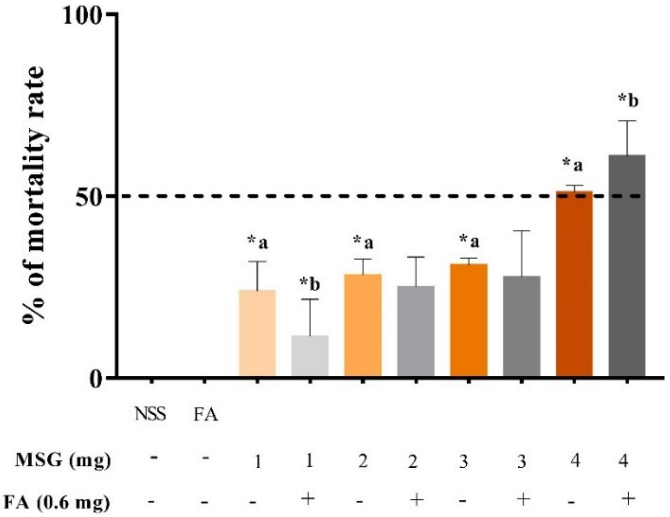

Figure 2 The bar graphs demonstrated the percentage mortality rates for day-3-old chick embryos compared to the control, FA, MSG, and FA+MSG groups. Data represent mean ± SD. [a]The data are significantly different when compared with NSS. [b]The data are significantly different when compared with MSG treatment. Significant differences $^{*}p < 0.05$. n = 250.

Folic acid reduced the risk of retardation and organ malformation after MSG exposure

We further explored the effects of MSG on growth retardation and organ malformation. The results showed that MSG induced teratogenicity by delaying growth and organ formation. The chick embryos showed developmental disruptions followed by growth retardation, which reduced the number of branchial arches, as well as causing abnormal body twitching, and delayed limb bud formation (**Figures 3(b) - 3(d)**). The craniofacial malformation was microcephaly and the anterior neuropore opened; this was found with 2 - 4 mg MSG (15.38 ± 0.3, 33.34 ± 0.4 and 57.10 ± 0.1 percent, respectively), while the absence of eye primordia was found in 3 - 4 mg MSG (20.01 ± and 42.80 ± 0.1 percent). The heart tube was irregular and U-shaped with a dilated lumen, as found in 1 - 4 mg MSG (46.15 ± 0.5, 38.46 ± 0.6, 33.34 ± 0.1 and 71.42 ± 0.3 percent, respectively). Overall, the MSG group showed significant adverse effects due to MSG, leading to embryo retardation and malformation when compared to the control group (**Figures 3(a) - 3(d)** and **Table 1**).

Moreover, we found that FA can abrogate MSG-induced teratogenicity by reducing growth retardation and tissue agenesis in the MSG+FA group. A significant treatment effect on growth and development was obtained by encouraging the anterior neuropore to close among the 2 - 4 mg MSG (11.76 ± 0.3, 10.00 ± 0.1 and 9.09 ± 0.2 percent, respectively, when compared with MSG alone, $p < 0.05$), completing eye formation, and the formation of 4 pairs of branchial arches. In addition, FA also significantly reduced heart defects, as observed in the regular heart tube, which was S-shaped without dilation (1, 2 and 4 mg MSG, $p < 0.05$). The limb buds were well developed. The FA in the MSG+FA group showed a possible treatment role for craniofacial, heart, and limb defects when compared to the MSG group (**Figures 3(b) - 3(e)** and **Table 1**).

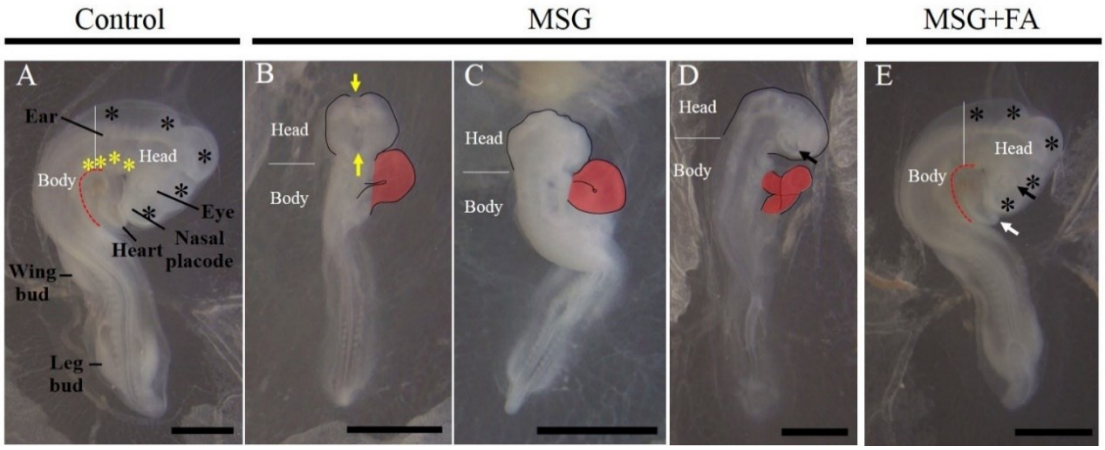

Figure 3 The photograph demonstrated the whole body of surviving 3-day-old chick embryos in the dextrodorsal view. The normal embryo is indicated in photos A and E, as follows; 5-brain vesicle prominence (black asterisk), eye, ear, nasal placode, and 4 pairs of branchial arches (yellow asterisk). The heart relies on the normal position (H), which forms the S-shaped loop (white arrow). The appearances of the limb buds were named wing bud and leg bud. The embryo from the MSG group showed an opened anterior neuropore and frontonasal prominence separation (yellow arrows) in photo B with 2 mg MSG, unidentified brain vesicles (**Figure 3(C)**, 3 mg MSG), a reducing-size head portion (**Figure 4(D)**, 4 mg MSG), and the absence of eyes (**Figures 3(B) - 3(C)**). The heart defects were identified as dilation and U-shaped looping, which are indicated in the red area in **Figures 3(B) - 3(D)**. The embryo in the treatment group, photo E, showed a normal development, similar to the control group. The pictures were captured under a stereomicroscope at 1.25x magnification, scale bar = 500 µM.

Table 1 Frequency percentages of particular developmental defects in living 3-day-old chick embryos in control and experiment groups.

Types of malformation	Groups									
	Control		MSG				MSG+FA			
	NSS (n=25)	FA (n=25)	1 (n=25)	2 (n=25)	3 (n=25)	4 (n=25)	1 (n=25)	2 (n=25)	3 (n=25)	4 (n=25)
Craniofacial defects	0	0	0	15.38±0.3^{a*}	33.34±0.4^{a*}	57.10±0.1^{a*}	0	11.76±0.3	10.00±0.1^{b*}	9.09±0.2^{b**}
Eye absence	0	0	0	0	20.00±0.1^{a*}	42.80±0.1^{a*}	0	0	0^{b**}	0^{b**}
Heart defects	0	0	46.15±0.5^{a*}	38.46±0.6^{a*}	33.34±0.1^{a*}	71.42±0.3^{a*}	10.00 ± 0.1^{b*}	17.64±0.2^{b*}	40.00±0.1	27.27±0.4^{b*}

The control groups (NSS and FA) showed 0 % embryonic malformations, and the statistical test showed no significant difference between the NSS and FA groups ($p > 0.05$). The MSG groups (1 - 4 mg MSG) showed a variety of embryonic malformations when compared to the control (NSS). [a]There was a significant different between MSG and control (NSS); [b]there was a significant difference between MGS+FA and MSG alone. $^{*}= p < 0.05$, $^{**}= p < 0.01$

Folic acid abrogated the MSG-induced tissue teratogenicity

We observed the potential treatment effect of FA on MSG-treated embryonic tissue. The results showed that the control group showed tissue and organ organization without any effects (**Figures 4(B) - 4(D)** and **Figures 4(F) - 4(H)**). The tissue sections from the MSG groups confirmed the MSG-induced toxicity in various organs (**Figures 4(J) - 4(L)**). The MSG group with 2 mg MSG demonstrated an anterior neuropore opening, resulting in neural tissues becoming exposed to the environment, as indicated by the black arrows (J - K). Eye malformation and decreased BA formation were demonstrated in photo K, as well as heart tissue dilation (L). The MSG+FA group with 2 mg MSG+FA (**Figures 4(N) - 4(P)**) showed that FA demonstrated high efficacy when treating MSG-induced tissues and cellular toxicity by causing the anterior neuropore to close. The eye and BA were completely formed (N). The heart tissues became a thick

wall; the endocardial cushion appeared and filled the pericardial sac (O - P). These results indicate that FA has a potential treatment effect on reducing tissue teratogenicity.

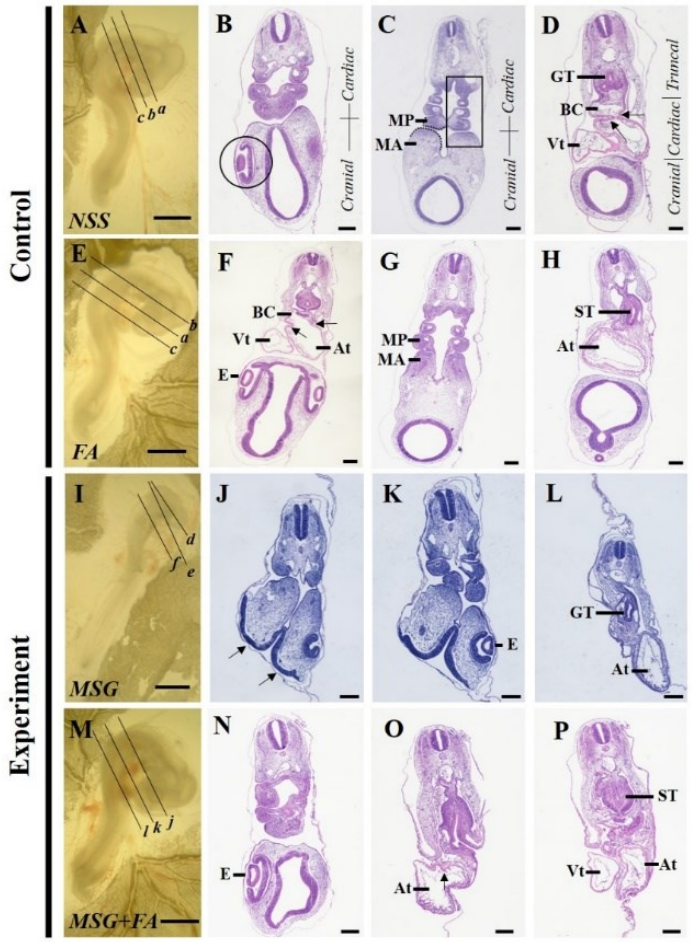

Figure 4 Photographs demonstrate the whole body of surviving 3-day-old chick embryos from the dextrodorsal view (A, E, I, M) and a serial section from the same specimens in the craniofacial, cardiac, and truncal regions (B - D, F - H, J - L, N - P). The 2 upper columns demonstrate the control group (NSS and FA), while the 2 lower columns show the experimental groups (2 mg MSG and 2 mg MSG+FA). The whole living embryo in the control group showed normal development related to the HH18 stage (A, E), as confirmed by the serial sections (B - D and F - H). The formation of the eye, optic cup (OC), and lens vesicle (LV) was observed on the cranial part, which was indicated by a circle in B. The 4 pairs of branchial arches were indicated in a square frame in C, with a maxillary prominence (MP), and a mandibular arch (MA). In addition, the heart tissues indicated in photos D, F, and H were found in the heart chambers (bulbus cordis (BC), atrium (At), and ventricle (Vt)) and primitive septum (black arrows). The gut tube (GT) is displayed in the truncal region (D, H, L, P). The pictures were captured under a stereomicroscope (2x) and a light microscope at 1.25x magnification, scale bar = 200 µM.

Folic acid diminished the destruction of neural crest cells after MSG exposure

To study the role that neural crest cells played in 3-day-old chick embryos exposed to MSG, the HNK-1 marker was used to localize NCCs. We found that the tissue sections through the cranial, cardiac, and trunco-sacral regions in the control group had a high NCC subpopulation, which were well-localized on brain vesicles, ears, eyes, heart, and nervous tissues (**Figures 5(A2) - 5(A4)**, **Figures 5(A6) - 5(A8)** and **Figures 5(C) - 5(D)**). In the MSG group (**Figures 5(A10) - 5(A12)** and **Figures 5(C) - 5(D)**), there were a few NCC populations in the tissues. The tissue section through the craniofacial region demonstrated MSG-induced cellular toxicity in the eyes and brain tissues (A10 - A11). The neural tube in the cardiac and truncal regions showed a few NCCs that formed a dorsal root and prevertebral ganglion (A12 and C). Moreover,

the notochord was found in a pair, which is indicated by black arrows in A11. A weakness was found in HNK-1 signaling in the endocardial cushion of the heart (A12 and C). Lastly, the gut tube (GT) showed a few of the NCC population on the muscular layer (A12 and D). In FA treatment in the 2-mg MSG+FA, (**Figures 5(A14)** - **5(A16)** and **Figures 5(C)** - **5(D)**), the tissue sections through the craniofacial region showed the population of NCCs that finished migrating from the origin and occupied the target organs, including the eyes, ears, and cranial nerve tissues. The frontonasal prominence of the forebrain and brain vesicles had a high NCCs subpopulation, which was well-localized (A14 - A15). The neural tube (NT) in the cardiac and truncal regions showed that the NCCs can form into a dorsal root, sympathetic and visceral ganglions (A16 and D). Furthermore, the NCCs in the cardiac region migrated well, and localized not only on the endocardial cushion but also on the great vessel (C). Lastly, the gut tube (GT) had a high NCC population in the muscular layer, where NCCs came from the truncal region of the neural tube (D). These results suggest the treatment effect of FA on MSG-induced teratogenicity reduced NCC destruction and prevented the malfunction of NCC in chick embryos.

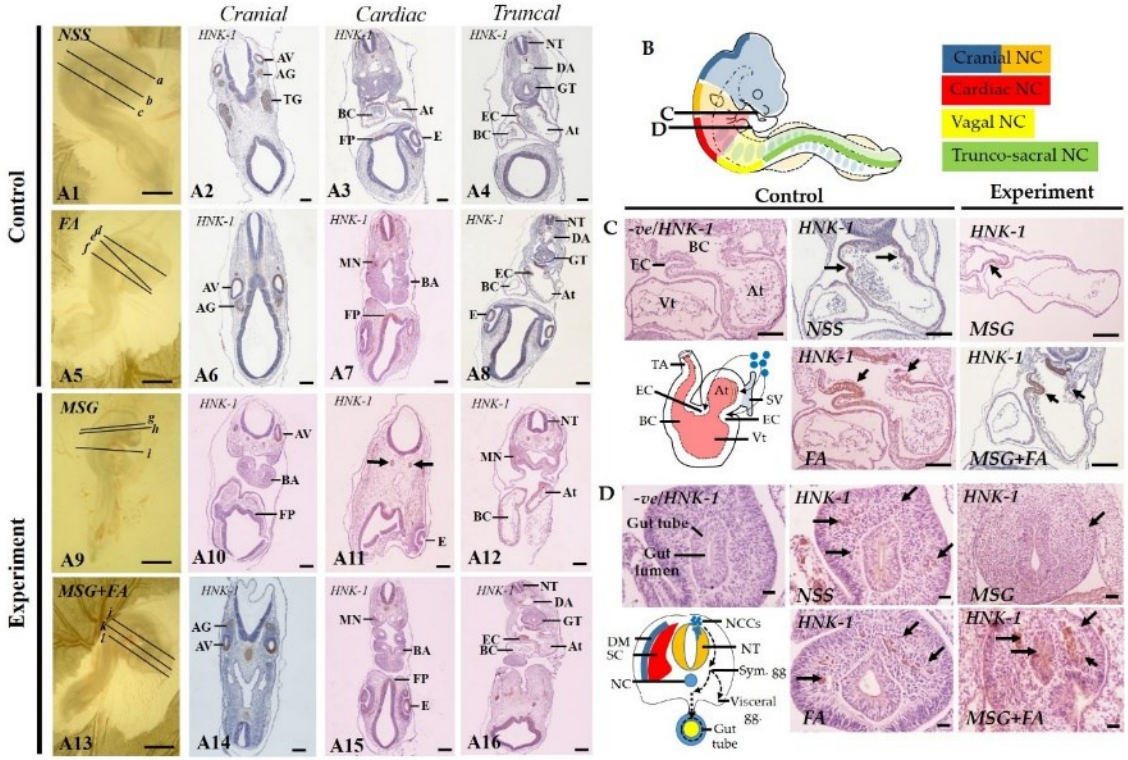

Figure 5 Photographs demonstrated the whole body of surviving 3-day-old chick embryos from the dextrodorsal view (A1, A5, A9, A13) and a serial section from the same specimens through the craniofacial, cardiac, and trunco-sacral regions (A2 - A4, A6 - A8, A10 - A12, A14 - A16). B is the diagram of the NCCs' origin and migration route in several regions, while photos C and D are the tissues sectioning through the heart and stomach related to photo B. The tissues from the control (NSS and FA) and experiment groups (2 mg MSG and 2 mg MSG+FA) were stained with a specific antibody of monoclonal anti-HNK-1. The whole living embryo from the control showed normal development related to the HH18 stage, as confirmed by the serial sections (A2 - A4, A6 - A8 and C - D). The NCC populations in the brain (frontonasal prominence (FNP)), neural tube (NT), ears (auditory vesicle (AV)), eye (optic cup (OC), lens vesicle (LV)), and cranial ganglions (aortic facial ganglion (AG), and trigeminal ganglion (TG)) were observed on the cranial part (A2 - A3, A6 - A8). In addition, the heart tissues indicated in photos C showed the NCCs in heart chambers (bulbus cordis (BC), atrium (At), ventricle (Vt)) with a high affinity with the primitive septum (endocardial cushion (EC)) (shown in black arrows). The gut tube (GT) and dorsal aorta (DA) were well-developed and many NCCs were found in the truncal region (D). The pictures were captured under a stereomicroscope (2x) and a light microscope at 1.25x magnification; scale bar = 200 µM.

Discussions

MSG is widely used as a flavor enhancer. It does not cause much toxicity in adults; however, whether it impairs embryonic development in the prenatal period is still controversial. We used a chick embryo to study its teratogenesis and the potential role of FA treatment. In the present study, we found that the highest lethal concentration of MSG is 4 mg. This finding was higher than that in the 2018 study by Roongruangchai and colleagues [23], who studied the topic using chick embryos as a model. However, our results differed from the study of Abu Elnaga *et al.* [39], which used rodents as a model. The results are different because the MSG concentration used was lower than that in our study, at 1×10^{-6} to 2.5×10^{-6} mg/kg. This MSG concentration only reveals the malformations in embryos. In addition, MSG-induced teratogenicity was confirmed in our study, which showed congenital malformations and death. These malformations include growth retardation and organ malformations. The craniofacial malformation is one of the defects represented by the brain vesicle opening, facial unfusing, and microphthalmia. The highest rate of congenital disabilities occurred in the heart, where looping and dilation caused heart-shaped irregularities. In humans, the neurulation process was disturbed, and the neural tube was incompletely formed. This resulted in the anterior neuropore opening, which caused anencephaly and facial defects [40]. Moreover, the anomalies in the development of the branchial arches affect facial formation, resulting in diseases such as Treacher Collins syndrome (TCS) [28], or cleft lip and cleft palate [29]. Moreover, these defects were also found in our study using chick embryos. In humans, this process has occurred for from 3 to 8 weeks, and it occurred for 3 days in avians (HH stage 4 - 10). The heart is an organ with a complicated formation process, and takes a long time to develop. In humans, this process takes almost 2 weeks, and in avians, about 2 days are needed for the organogenesis processes [41-43]. One piece of evidence was congenital heart defects in the baby, caused by the mother being exposed to a teratogen during pregnancy [44]. This evidence was similar to our results, so the heart may be sensitive to teratogen. Additionally, there were slight disturbances that could result in abnormal heart development. Most of the abnormalities were septal defects, named ventricular, atrioventricular septal defect, and Tetralogy of Fallot, and the great vessel defects, named conotruncal defects [45,46].

The negative effects of MSG are based on an all-or-none theory. During organogenesis, the embryos are exposed to high or low concentrations of MSG. The embryonic cells cannot eliminate their toxicity, resulting in immediate and irreversible abnormalities, so the embryo slows its growth or dies. However, if the embryo can get rid of all the toxins, then it will develop normally, without any effects [47]. These results correspond with the incidence of death and the malformation rate in pregnant rats that received MSG [48,49]. At the cellular level, MSG-induced toxicity and teratogenicity due to MSG caused the NCCs' dysfunction, which led to fewer NCCs being present in the target organ. The teratogenicity of MSG disturbed the neural crest cells' ability to function, so these cells were reduced when exposed to MSG. Therefore, our results reveal few cell localizations on the target area. TMSG showed a significant adverse effect on NCC functions during the developmental processes, as was found in our study. From the experiment, we found that a high affinity to NCCs' signaling was present for the optic vesicles, multiple ocular and periocular structures of the eyes, dorsolateral and ventromedial of the brain vesicle and neural tube, sensory and motor ganglia, pre-and post-autonomic ganglia, branchial arches, layers of great vessels and the heart. NCC dysfunction results in phenotypic effects and craniofacial defects. Our results revealed that MSG is a non-genetic factor that interferes with NCC function, resulting in craniofacial defects. According to the results, we hypothesize that MSG may interfere with NCCs, resulting in decreased cell proliferation and increased DNA-damage-induced cell death caused by oxidative stress. Few of the NCC sub-population in the craniofacial region were able to affect the craniofacial bone, muscle, special sensory organs, and subsets of cranial sensory receptor neuron generation.

The use of FA has been well established in the prevention of congenital anomaly [50], reduction in neuron death, and hematopoiesis [51,52]. Additionally, it has been reported to help reduce infant mortality after birth [53]. To date, animal studies have not provided sufficient information to establish the metabolic and genomic mechanisms underlying human FA responsiveness in neural tube defects. Our study found that the administration of 600-mcg of FA in 1-day-old chick embryos showed no embryo mortality, so it can be concluded that FA does not have any side effects during embryogenesis. Furthermore, FA can effectively reduce mortality in MSG-induced toxicity. However, the FA's efficacy will be decreased if the embryo is exposed to higher amounts of MSG, and this could lead to an increase in mortality during maturation. FA showed high efficacy for the treatment of growth retardation, craniofacial and heart defects, and other organs or tissues injured by MSG exposure that we found from the experiment. Moreover, FA could abrogate MSG-induced toxicity and teratogenicity by reducing the mortality rate and congenital malformations. The lethality rates and the severity of development were reduced, as they were prevented by folate supplementation [54]; therefore, in this study, we looked at the effect of folate supplementation

regarding the dose-dependent manner of MSG exposure. This was consistent with the meta-analysis studied by Goh *et al.* [55], which gathered information on pregnant women after they received FA-fortified plus with multivitamins.

FA supplementation was able to prevent embryonic or fetal death, which occurred during neurulation, after approximately 24 h in chick embryos (HH 4 - 10) [56]. Compared to humans, the gestational age was 3 - 4 weeks of gestation (CS 7 - 9) [52]. Throughout the neurulation process, the FA allowed for the 4 germ layers to regenerate, including the NCC layer, which is important in the organogenesis of the embryo. If the neurulation process fails, the embryo will become disabled, and this failure eventually leads to embryonic death, as FA is not only used in the synthesis, repair, and functioning of DNA [57], but cell apoptosis is also prevented through various FA mechanisms [58]. In this experiment, the cells were abnormal. This was caused by MSG toxicity, resulting in cell damage and death. FA could repair damaged cells, resulting in normal organogenesis. Our study proved that FA was able to reduce the severity of MSG-induced teratogenicity in chick embryos. However, there were still limitations to the research, which influenced the explanation of the mechanisms of FA-reduced inflammation at the tissue and cellular levels, resulting in cell death. Therefore, further studies will focus on the mechanisms of FA-reduced oxidative stress, cellular inflammation, and death after MSG exposure. This is a significant pathologic function of MSG teratogenicity.

In addition, we found that FA was necessary to treat vagal and cardiac NCCs functions during heart formation, which reduced organ agenesis after the embryo was exposed to MSG. The proliferation and migration of cells from the posterior hindbrain and anterior spinal cord gave rise to parts of the heart tissues, such as the aorticopulmonary septum, outflow endocardial cushion, cardiac septum, and conducting system, whose genesis confirmed the cardiac NCCs' functions. Research by Kirby *et al.* [59], indicated that NC migration disorders caused failure in cardiac outflow tract septation, which is a relatively common form of congenital heart disease in humans. Many reports of molecular studies indicated that Pax3 may also play a role in modulating neural crest migration, survival, proliferation, and/or interaction with other tissues [60-64]. However, the molecular pathways regulating cardiac neural crest function and their interaction with cardiac tissues remain unclear [65]. In our research, we confirmed that FA abrogated the heart defects in chick embryos in our research, which reversed to normal. However, the molecular pathways regulating cardiac NCC function and their interaction with cardiac tissues remain largely unknown. This is a limitation of our research because we could not ascertain whether FA was involved in the expression of particular genes, which mechanisms were used, and how they affected NCCs. Therefore, further research will be conducted at the molecular level to understand the mechanisms of the pathological mitigation of FA treatment.

Conclusions

In summary, the MSG was used as a teratogen in a chick embryo model during the first 3 days of development. The chick embryos were used as models for 2-month pregnant women. The teratogenicity and tissue toxicity were shown to be dose-dependent, causing growth retardation and craniofacial defects, as well as leading to the absence of eyes and heart defects. Histologically, MSG led to the destruction and malformation of NCCs. FA treatment alleviated the teratogenicity and toxicity induced by MSG at concentrations lower than LD50 by reducing NCC destruction and preventing the malfunctioning of NCC in chick embryos. Therefore, these experimental protocols were beneficial for teratogenic studies, and the results can be applied to future studies to elucidate the causes of birth defects that are harmful to the embryos.

Acknowledgements

We would like to thank the research staff of the Anatomy Department and Pathobiology Department for supporting each technique performed in this research. The facilities are supported by the Pathology Information and Learning Center, Department of Pathobiology, Faculty of Science, Mahidol University, Thailand.

References

[1] LE Mitchell. Maternal effect genes: Update and review of evidence for a link with birth defects. *HGG Adv.* 2022; **3**, 100067.

[2] A Christianson, CP Howson and B Modell. *March of dimes: Global report on birth defects, the hidden toll of dying and disabled children*. March of Dimes Birth Defects Foundation. Virginia, 2005.

[3] Y Zhou, X Mao, H Zhou, L Wang, Z Qin, Z Cai and B Yu. Birth defects data from population-based birth defects surveillance system in a district of Southern Jiangsu, China, 2014-2018. *Front. Publ. Health* 2020; **8**, 378.

[4] ML Feldkamp, JC Carey, JL Byrne, S Krikov and LD Botto. Etiology and clinical presentation of birth defects: Population based study. *BMJ* 2017; **357**, j2249.

[5] GE Wachholz, BD Rengel, N Vargesson and LR Fraga. From the farm to the lab: How chicken embryos contribute to the field of teratology. *Front. Genet.* 2021; **12**, 666726.

[6] D Zosen, MG Hadera, JS Lumor, JM Andersen and RE Paulsen. Chicken embryo as animal model to study drug distribution to the developing brain. *J. Pharmacol. Toxicol. Meth.* 2021; **112**, 107105.

[7] MN Vergara and MV Canto-Soler. Rediscovering the chick embryo as a model to study retinal development. *Neural Dev.* 2012; **7**, 22.

[8] RF Gasser, RJ Cork, BJ Stillwell and DT McWilliams. Rebirth of human embryology. *Dev. Dynam.* 2014; **243**, 621-8.

[9] AV Hoffbrand and DG Weir. The history of folic acid. *Br. J. Haematol.* 2001; **113**, 579-89.

[10] HK Mitchell, EE Snell and RJ Williams. The concentration of "folic acid". *J. Am. Chem. Soc.* 1941; **63**, 2284.

[11] IH Rosenberg. A history of the isolation and identification of folic acid (folate). *Ann. Nutr. Metabol.* 2012; **61**, 231-5.

[12] JG Donnelly. Folic acid. *Crit. Rev. Clin. Lab. Sci.* 2001; **38**, 183-223.

[13] K Sato. Why is folate effective in preventing neural tube closure defects? *Med Hypotheses* 2020; **134**, 109429.

[14] D Rothman. Folic acid in pregnancy. *Am. J. Obstet. Gynecol.* 1970; **108**, 149.

[15] BDL Fournière, F Dhombres, P Maurice, SD Foucaud, P Lallemant, M Zérah, L Guilbaud and J Jean-Marie. Prevention of neural tube defects by folic acid supplementation: A national population-based study. *Nutrients* 2020; **12**, 3170.

[16] CD Dolin, AL Deierlein and MI Evans. Folic acid supplementation to prevent recurrent neural tube defects: 4 milligrams is too much. *Fetal Diagn. Ther.* 2018; **44**, 161-5.

[17] KM Appaiah. Chapter 13 - monosodium glutamate in foods and its biological effects. *Ensuring Global Food Saf.* 2010; **2010**, 217-26.

[18] K Ganesan, K Sukalingam, K Balamurali, SRBS Alaudeen, K Ponnusamy, IA Ariffin and SB Gani. A studies on monosodium L-Glutamate toxicity in animal models-a review. *Int. J. Pharmaceut. Chem. Biol. Sci.* 2013; **3**, 1257-68.

[19] M Freeman. Reconsidering the effects of monosodium glutamate: A literature review. *J. Am. Assoc. Nurse Pract.* 2006; **18**, 482-6.

[20] Z Kazmi, I Fatima, S Perveen and SS Malik. Monosodium glutamate: Review on clinical reports. *Int. J. Food Properties* 2017; **20**, 1807-15.

[21] K Niaz, E Zaplatic and J Spoor. Extensive use of monosodium glutamate: A threat to public health? *EXCLI J.* 2018; **17**, 273.

[22] K Beyreuther, HK Biesalski, JD Fernstrom, P Grimm, WP Hammes, U Heinemann, O Kempski, P Stehle, H Steinhart and R Walker. Consensus meeting: Monosodium glutamate-an update. *Eur. J. Clin. Nutr.* 2007; **61**, 304-13.

[23] J Roongruangchai, Y Viravud, V Plakornkul, K Sripaoraya, W Boonmark and K Roongruangchai. The teratogenic effects of monosodium glutamate (MSG) on the development of chick embryos. *Siriraj Med. J.* 2018; **70**, 514-22.

[24] F Al-Qudsi and A Al-Jahdali. Effect of monosodium glutamate on chick embryo development. *J. Am. Sci*. 2012; **8**, 499-509.

[25] J Casale and AO Giwa. *Embryology, branchial arches*. StatPearls, Florida, 2022.

[26] R Hunt and PN Hunt. The role of cell mixing in branchial arch development. *Mech. Dev*. 2003; **120,** 769-90.

[27] MB Carbonell, RF Bayona, Z Garavito-Aguilar, BC Parada, GH Arboleda and C Infante-Contreras. Hey1 gene expression patterns during the development of branchial arches and facial prominences. *Rev. MVZ Córdoba* 2018; **23**, 6813-25.

[28] MR Passos-Bueno, CC Ornelas and RD Fanganiello. Syndromes of the first and second pharyngeal arches: A review. *Am. J. Med. Genet.* 2009; **149**, 1853-9.
[29] JM Johnson, G Moonis, GE Green, R Carmody and HN Burbank. Syndromes of the first and second branchial arches, part 1: Embryology and characteristic defects. *Am. J. Neuroradiol.* 2011; **32**, 14-9.
[30] MED Bellard, Y Rao and M Bronner-Fraser. Dual function of Slit2 in repulsion and enhanced migration of trunk, but not vagal, neural crest cells. *Int. J. Cell Biol.* 2003; **162**, 269-79.
[31] ME Bronner and MN LeDouarin. Development and evolution of the neural crest: An overview. *Dev. Biol.* 2012; **366**, 2-9.
[32] P Noisa and T Raivio. Neural crest cells: From developmental biology to clinical interventions. *Birth Defect. Res. C Embryo Today Rev.* 2014; **102**, 263-74.
[33] TL Creazzo, RE Godt, L Leatherbury, SJ Conway and ML Kirby. Role of cardiac neural crest cells in cardiovascular development. *Annu. Rev. Physiol.* 1998; **60**, 267.
[34] EM Siismets and NE Hatch. Cranial neural crest cells and their role in the pathogenesis of craniofacial anomalies and coronal craniosynostosis. *J. Dev. Biol.* 2020; **8**, 18.
[35] S Cerrizuela, GA Vega-Lopez and MJ Aybar. The role of teratogens in neural crest development. *Birth Defect. Res.* 2020; **112**, 584-632.
[36] JP Saint-Jeannet, P Blader, LA Taneyhill. Cranial placodes and neural crest interactions in craniofacial development. *Front. Physiol.* 2021; **12**, 681397.
[37] Y Shi, J Li, C Chen, M Gong, Y Chen, Y Liu, J Chen, T Li and W Song. 5-Mehtyltetrahydrofolate rescues alcohol-induced neural crest cell migration abnormalities. *Mol. Brain* 2014; **7**, 67.
[38] NAA Elnaga, M Sarhan and H Mansour. Teratogenicity of monosodium glutamate on the pregnant rats and their fetuses. *Egypt. J. Hosp. Med.* 2019; **74**, 1737-47.
[39] CP Chen. Syndromes, disorders and maternal risk factors associated with neural tube defects (V). *Taiwanese J. Obstet. Gynecol.* 2008; **47**, 259-66.
[40] GJ Mahler and JT Butcher. Cardiac developmental toxicity. *Birth Defect. Res. C Embryo Today Rev.* 2011; **93**, 291-7.
[41] AS Mahaliyana, MFA Fasmina, AMTB Alahakoon and GMGMM Wickrama. Toxicity effects of monosodium glutamate (MSG) on embryonic development of zebrafish (Danio rerio); a promising model to study excitotoxins. *Int. J. Sci. Res*. 2016; **6**, 229-34.
[42] TA Lynch and DE Abel. Teratogens and congenital heart disease. *J. Diagn. Med. Sonography* 2015; **31**, 301-5.
[43] J Tikkanen and OP Heinonen. Congenital heart disease in the offspring and maternal habits and home exposures during pregnancy. *Teratology* 1992; **46**, 447-54.
[44] E Gilbert-Barness. Teratogenic causes of malformations. *Ann. Clin. Lab. Sci.* 2010; **40**, 99-114.
[45] T Kuribayashi and WC Roberts. Tetralogy of fallot, truncus arteriosus, abnormal myocardial architecture and anomalies of the aortic arch system induced by bis-diamine in rat fetuses. *J. Am. Coll. Cardiol.* 1993; **21**, 768-76.
[46] A Sharma. Monosodium glutamate-induced oxidative kidney damage and possible mechanisms: A mini-review. *J. Biomed. Sci.* 2015; **22**, 1-6.
[47] KR George, NG Shibija and NA Malini. Monosodium glutamate (MSG) induced developmental dysfunction in female albino rats (*Rattus norvegicus*). *Bioscan* 2013; **8**, 73-6.
[48] FA Eid, NA Abu Elnaga, M Sarhan and H Mansour. Effect of monosodium glutamate on liver of pregnant rats and their fetuses (Histological and histochemical studies). *Egypt. J. Hosp. Med.* 2018; **73**, 8091-8.
[49] CE Butterworth and A Bendich. Folic acid and the prevention of birth defects. *Annu. Rev. Nutr.* 1996; **16**, 73-97.
[50] RA Swain and LS Clair. The role of folic acid in deficiency states and prevention of disease. *J. Fam. Pract.* 1997; **44**, 138-44.
[51] J Safi, L Joyeux and GE Chalouhi. Periconceptional folate deficiency and implications in neural tube defects. *J. Pregnancy* 2012; **2012**, 295083.
[52] AO Lucas, BJ Stoll and JR Bale. *Improving birth outcomes: Meeting the challenge in the developing world*. National Academies Press, Washington, 2003.
[53] R Zhao, RG Russell, Y Wang, L Liu, F Gao, B Kneitz, W Edelmann and ID Goldman. Rescue of embryonic lethality in reduced folate carrier-deficient mice by maternal folic acid supplementation reveals early neonatal failure of hematopoietic organs. *J. Biol. Chem.* 2001; **276**, 10224-8.
[54] YI Goh and G Koren. Folic acid in pregnancy and fetal outcomes. *J. Obstet. Gynaecol.* 2008; **28**, 3-13.

[55] EO Farombi and OO Onyema. Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. *Hum. Exp. Toxicol.* 2006; **25**, 251-9.
[56] DAE Hassan, MAA Alim, SMZ Sharkawi and S Nabil. Detection of cardiac tissues toxicity caused by monosodium glutamate and the protective role of vitamin c by immunohistochemical method, heart tissue oxidative stress biomarkers and cardiac dysfunction biomarkers. *Egypt. J. Forensic Sci. Appl. Toxicol.* 2020; **20**, 13-21.
[57] W Li, Y Ma, Z Li, X Lv, X Wang, D Zhou, S Luo, JX Wilson and G Huang. Folic acid decreases astrocyte apoptosis by preventing oxidative stress-induced telomere attrition. *Int. J. Mol. Sci.* 2019; **21**, 62.
[58] ML Kirby and KL Waldo. Role of neural crest in congenital heart disease. *Circulation* 1990; **82**, 332-40.
[59] M Maroto, R Reshef, AE Münsterberg, S Koester, M Goulding and AB Lassar. Ectopic Pax-3 activates MyoD and Myf-5 expression in embryonic mesoderm and neural tissue. *Cell* 1997; **89**, 139-48.
[60] S Tajbakhsh, D Rocancourt, G Cossu and M Buckingham. Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. *Cell* 1997; **89**, 127-38.
[61] AG Borycki, J Li, FUZI Jin, CP Emerson and JA Epstein. Pax3 functions in cell survival and in pax7 regulation. *Development* 1999; **126**, 1665-74.
[62] FG Barr, N Galili, J Holick, JA Biegel, G Rovera and BS Emanuel. Rearrangement of the PAX3 paired box gene in the paediatric solid tumour alveolar rhabdomyosarcoma. *Nat. Genet.* 1993; **3**, 113-7.
[63] N Galili, RJ Davis, WJ Fredericks, S Mukhopadhyay, FJ Rauscher, BS Emanuel, G Rovera and FG Barr. Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. *Nat. Genet.* 1993; **5**, 230-5.
[64] DN Shapiro, JE Sublett, B Li, JR Downing and CW Naeve. Fusion of PAX3 to a member of the forkhead family of transcription factors in human alveolar rhabdomyosarcoma. *Canc. Res.* 1993; **53**, 5108-12.
[65] JA Epstein, J Li, D Lang, F Chen, CB Brown, F Jin, MM Lu, M Thomas, E Liu, A Wessels and CW Lo. Migration of cardiac neural crest cells in splotch embryos. *Development* 2000; **127**, 1869-78.