Teratogenic Effects of Aspartame Exposure of Chick Embryonic Development

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ABSTRACT

Aspartame is an artificial non-saccharide sweetener 200 times sweeter than sucrose and has the no calories which approved for use in food products**.** Its metabolites can be toxic to many organs but there are only a few studies on the use of aspartame during pregnancy which it might cause abnormality to the newborn. This study about aspartame acts as the teratogenic agent on the development by using chick embryo as a model. The 120 fertilized white leghorn eggs were randomly divided into 30 eggs of control and 90 eggs of treated groups. The control group was injected with normal saline, the 3 treated groups were injected with 10, 20, 30 mg/100 ml into the yolk sac of 24 hr incubation of 3 days. All the eggs from each group were collected in day 3 of incubation and studied total mount. The results in experimental at the low concentrations of aspartame such as 10 mg/ml and 20 mg/ml showed increasing in mortality rate and growth retardation as increasing concentration of aspartame. There were retardation of brain formation, brain flexure which appear only cephalic flexure, microphthalmia, no branchial arch. However, at high concentrations 30 mg/ml there were anencephaly, anophthalmi, incomplete fusion of otic placode, abnormal heart looping, tail degeneration, and no limb buds and somite retardation. It could be concluded that aspartame has impacted the gross structure malformations and morphological changes on the development. Therefore, should avoid on the consumption of aspartame especially during pregnancy.

Keywords: Aspartame, teratogen, chick embryo

1. Introduction

Non-sugar sweeteners have been used for centuries, mostly in natural forms derived from the Stevia plant family artificial sweeteners were primarily used to make pharmaceuticals more than palatable, and as a sugar substitute in foods designed for patients with diabetes (Talbot JM et al.,1978). Aspartame is an artificial non-saccharide sweetener 200 times sweeter than sucrose, and is commonly used as a sugar substitute in foods and beverages. It is a methyl ester of the aspartic acid phenylalanine dipeptide with the trade names, NutraSweet, Equal, and Canderel (Grenby. 1991). Approved for use in food products by the U.S. Food and Drug Administration (FDA)

in 1981. This substance may be harmful to people with the disease due to a genetic disorder called phenylketonuria in which patients with this disease the body is unable to use amino acids, phenylalanins. This brings about congestion of the phenylpruric acid in the blood causing harm to the brain. This disease occurs most often in children, therefore has to be specified on the food label that contains aspartame. There was no report about the teratogenic effect of aspartame that affect the development of embryos. The purpose of this study is to evaluate that aspartame acts as the teratogenic agent on the development.

However, this research is to study about the teratogenic effect of aspartame using animal models by using chick embryo because of short gestation period, inexpensive and rapidly increasing development in size which similar to the situation in the human embryo (Nusrat et al., 2011). Dose to the consumption of aspartame is increasing, the investigations in teratogenic effect of aspartame should be study especially in pregnant women.

2. Objectives of the study

This study was conducted to study the teratogenic effect of aspartame, in chick embryos which were exposure to different concentrations of aspartame by injection method to get informations about the gross structure malformations and morphological changes on the developing chick embryo which can be compared with normal and apply the knowledge to human.

3. Materials and methods

The 120 fertilized white leghorn eggs were divided into 2 groups which randomly; 30 eggs of control group (Normal saline injected) and 90 eggs of experimental group (Aspartame injected) was subdivided 3 groups by different concentration of aspartame 10, 20, 30 mg/100 ml. The eggs was cleaned with 70% alcohol for remove external grime circled at the blunt ends of eggshell for injection and labeled different dose at lateral of eggshell then incubated for 24 hour at 37 °C temperature, humidity was 68-70% in sterile incubator and turned manually 60 °C for 3 times a day. After that eggs were removed from the incubator and cleaned with 70% alcohol at the blunt ends of eggshell then drilled a small hole by dental drilled and injected aspartame experimental groups concentration of 10, 20, 30 at the volume of 0.1ml.into the yolk sac by sterile needle. In the control group, normal saline at the volume of 0.1 ml. injected into the yolk sac by sterile needle. After injection, the hole was sealed with scotch tape.

The eggs were returned to the incubator and turn manually 60 °C 3 times a day until day 3. 30 eggs of control group and each experimental groups were opened for observing and recording the results of the survival and mortality rates. After that, the embryos were removed from the yolk sac, washed with normal saline, keep them on filter paper and fixed in Dietrich's FAA solution for 4 hour, transferred to 70% alcohol and processed of tissue to examine by total mount**.** several times until the embryo changed to transparent or white color, and stained the embryo with Mayer' s carmine for 10 minutes to studied morphological malformations.

4. Results

The effects of aspartame on chick embryo day 3 were recorded on percentage of survival and mortality rate compared with control group. Survival chick embryos were examined by observing heartbeat and blood circulation that compared to stage 18 of Hamburger and Hamilton in normal development chick embryo (Hamburger et al.,1992) showed in Table 1

Table 1 The survival and mortality rate of day 3 chick embryos in each group.

Group	n (%)	Survival n (%)	Mortality n (%)
Control group	30 (100)	30 (100)	0 (0)
10 mg/ml	30 (100)	22 (73.33)	8 (26.67)
20 mg/ml	30 (100)	17 (60.00)	13 (40.00)
30 mg/ml	30 (100)	14 (46.67)	16 (53.33)

The result showed fertilized eggs were exposed to aspartame which different concentrations, a decrease in number of survival chick embryos was observed as increasing concentration. The percentage of survival chick embryo in 3 experimental groups included 10 mg/ml, 20 mg/ml and 30 mg/ml showed 73.33%, 60.00% and 46.67% survival rate respectively and compared with control group resulted in 100% of survivor.

The total mount of day 3 chick embryo

The effect of aspartame on chick embryo day 3 studies by total mount showed that experimental group were morphological anomalies in several structures and showed growth retardation when compared with the control group.

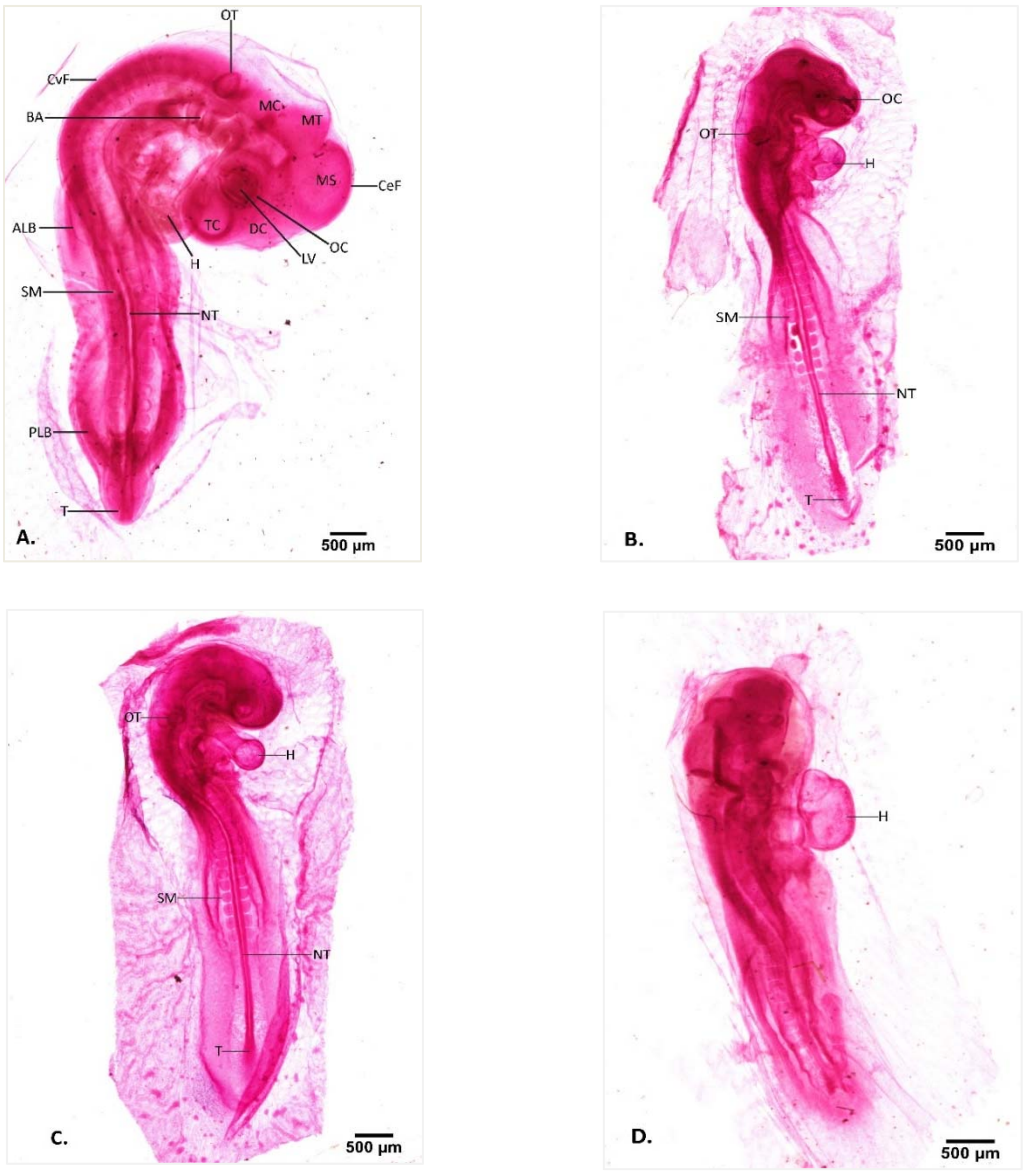

Figure 1 The micrograph of day 3 chick embryos by total mount preparation in each group including A. control group, B. 10 mg/ml, C. 20 mg/ml and D. 30 mg/ml of aspartame treated groups. (TC = Telencephalon, DC = Diencephalon, MS = Mesencephalon, MT = Metencephalon, MC = Myelencephalon, OT = Otocyst, OC = Optic cups, LV = Lens vesicle, CeF = Cephalic flexure, CvF = Cervical flexure, H = Heart loop, BA= Branchial arch, ALB = Anterior limb bud, PLB = Posterior limb bud, NT = Neural tube, SM = Somite, Vv = Vitelline vessel, T = Tail fold)

The control group (Figure 1 A.) showed normal development of the chick embryo on day 3 of incubation. There were about approximately 36 somites or HH stage 18 as characterized by Hamburger Hamilton stage (Hamburger et al., 1992). The general appearances were head and tail fold, the cephalic end twisted longitudinal axis of 2/3 part of head and flexed about 90 degrees to the right side. There were 2 flexures including cephalic flexure at mesencephalon and cervical flexure between myelencephalon and spinal cord . Spinal cord proceeded from myelencephalon extended to caudal end. The neural tube appeared 2 dense lines parallel along the midline of the body and lateral sides of the neural tube with approximately 36 pairs of somite from cephalic which extended to the caudal end. Caudal part of chick embryo were appeared anterior and posterior limb buds, and tail fold. Tail fold located at the caudal end. Head region developed secondary brain vesicles, which showed prominently observed including telencephalon and diencephalon of forebrain, mesencephalon of midbrain, metencephalon and myelencephalon of hindbrain. There were sense organs including optic cup which was large size like horse-shoe shaped and there was a lens vesicle located at the center and otocyst was closed vesicle located at myelencephlon. Branchial arch appeared between head and cervical regions which divided into 4 archs, mandibular arch (arch 1), hyoid arch (arch 2,3,4) The heart loop appeared S-shaped. The lateral side found that vitelline vessels which supplied the wall of yolk.

The effects of aspartame on day 3 of chick embryo treated with 10 mg/ml and 20 mg/ml (**Figure 1** B., C.) showed that the embryo were retardation of brain formation of all 3 parts can be recognised (prosencephalon, mesencephalon and rhombencephalon) but brain flexure which appeared only cephalic flexure with microphthalmia and no branchial arch, retardation of heart looping (only U shape loop can be seen), no limb bud, no tail fold and retardation of somite formation.

The effects of aspartame on day 3 of chick embryo treated with 30 mg/ml (**Figure 1** D.) revealed that the embryo was anencephaly, anophthalmia, incomplete fusion of otic placode, abnormal heart loop, no branchial arch and tail degeneration and no limb buds, and somite retardation of only upper half body development.

5. Discussion

The present study, the total mount of chick embryo day 3 in all experimental groups showed retardation of brain formation of all 3 parts and brain flexure which appeared only cephalic flexure, microphthalmia, no branchial arch. anencephaly, anophthalmia, incomplete fusion of otic placode, abnormal heart looping, tail degeneration, no limb buds and somite retardation of only half body development. (M.S.Weerasooriyagedara. 2018) at high concentrations of aspartame there were distinguishable negative alterations such as growth retardation, shrinkage, tail deformities in developing embryos and physiological changes. (Marielza R.et al., 2007) administration of aspartame on rat suggests that administration of aspartame on pregnancy period delays fetal growth as expression by cell damage during this period. Moreover, (A.M. Shalaby et al., 2019) degenerative changes have been reported in preeclampsia these cells are most likely to be affected by placental injury and intrauterine growth retardation

placentae. (Elfatah et al., 2012) the accumulation of the aspartame derived methanol and its metabolite formaldehyde adducts that exerted their cytotoxicity through the functional alteration of proteins and DNA mutations, thus leading to cell which can produce brain damage, growth retardation, abnormalities death. Further, the nuclear damage could be induced by oxidative stress.

6. Conclusion

The results of this study clearly indicated that with the increase of the aspartame concentrations, different observable deformities are formed has malformations in chick embryonic development, at high concentrations. Thus, the pregnant women should avoid aspartame consuming this during pregnancy for safety.

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References

Talbot JM, & Fisher KD. (1978) *The need for special foods and sugar substitutes by individuals with diabetes mellitus*. Diabetes Care. 1(4) :231–240.

Grenby, T.H. (1991) *Update on low calories sweeteners to benefit dental health*. Int. Dent. J. 41(4):217-24.

Nusrat, Z. and Uzma, S. (2011) *Reproducing windowing technique for naked eye observation of chick embryo morphogenesis*. AI Nafees Medical College, Islamabad: Ann. Pak. Inst. Med. Sci. 7(3):146-149

Hamburgert V, Hamilton HL. (1992) *A series of normal stages in the development of the chick embryo*. Developmental dynamics. 195: 231-72.

M.S.Weerasooriyagedara. (2018) *Toxicity Effects of Aspartame on Embryonic Development of Zebrafish (Danio Rerio).* International Journal of Engineering and Management Research.183-188.

Marielza R. Ismael Martins. (2007) *Effects of aspartame on fetal kidney: A morphometry and stereological study.* Int. J. Morphol. 25(4): 689-694.

Amany Mohamed Shalaby. (2019) *Effect of Aspartame on the placenta of adult albino rat. A histological and immunohistochemical study.* Annals of Anatomy. 224: 133–141.

Elfatah, A.A., Ghaly, I.S., Hanafy, S.M. (2012) *Cytotoxic effect of aspartame (diet sweet) on the histological and genetic structures of female albino rats and their off-spring.* Pak. J. Biol. Sci. 15: 904–918.