Article

EVALUATION OF THE DEVELOPMENTAL TOXICITY OF SOME COMMONLY USED FOOD ADDITIVES

Hamid Abdulraoof Saleh
Associate Professor of Anatomy and Embryology,
Department of Anatomy, Faculty of Medicine and Princess Al-Jawhara
Center of Excellence in Research of Hereditary Disorders, King Abdul Aziz University,
P O Box 80316, Jeddah 21589, Saudi Arabia.

Tel: +966 506358995; Fax: +966 2 6231774; e-mail: hamidphd@hotmail.com

ABSTRACT

The use of food additives has increased in the last few years and many studies have linked the consumption of these additives with various health problems. This study has examined the effects of food additives on the fetal development and the pregnancy outcome. Four different food additives were chosen from various groups of additives. Pregnant adult female rats were divided into five groups: aspartame, annatto, monosodium glutamate, tartrazine and control group. All doses were given by gavage once per day, from day 0 till day 20 of gestation. The results showed that administration of these additives to pregnant rats revealed a dose-dependent toxicity in the mothers such as a decrease in maternal weight gain, water and food consumption. In addition, these additives provoked a deterioration of pregnancy and fetal growth parameters. This study provides more evidence about the harmful effects of these additives on mothers and fetuses.

Keywords: Aspartame – Annatto - Monosodium Glutamate – Tartrazine - Developmental Toxicity – Embryolethality - Food Additives

Oxford Research Forum Journal (October 2010) 3 (2): 59-71

INTRODUCTION

Food additives are substances that are used in the food industry in order to improve the food's taste, texture and appearance; by preserving its flavour and preventing it from souring. With the advance of processed foods in the second half of the 20th century, many food additives have been introduced which are either natural or artificial [1]. Food additives in use today can be divided roughly into three main types: cosmetics, preservatives and processing aids, totaling presently about 3,794 different additives, of which over 3,640 are used purely as cosmetics, 63 as preservatives and 91 as processing aids [2, 3].

With the great increase in the use of food additives, there has also emerged considerable scientific data linking food additive intolerance with various physical and mental disorders of human health, particularly with infancy and childhood [4-11].

Along with improvements in processed food production, the use of food additives has increased in the 20th century. It is well known that, depending on the consumption rate, food additives have multiple adverse effects such as eczema, urticaria, angioedema, exfoliative dermatitis, irritable bowel syndrome, nausea, vomiting, diarrhea, rhinitis, bronchospasm, hyperactivity and other behavioural disorders [12].

Young children always seem to serve as the first sentinels of any environmental contamination because of the immaturity of their enzymatic detoxifying mechanism, incomplete function of excretory organs, low levels of plasma protein capable of binding toxic chemicals and incomplete development of

physiological barriers such as the blood-brain barrier [8, 13, 14, 15].

In the last few decades, a number of scientific studies on disorders during embryonic-foetal development periods and childhood caused by increased consumption of different food additives have been reported [16]. Some research has demonstrated that several food additives have serious adverse effects such as birth defects, still births, sterility, early fetal deaths and reabsorbtion of fetuses [1].

Saudi Arabia is the largest and fastest growing market for fast food in the Gulf region, where the popularity of fast-food alternatives to traditional cuisines has prompted debate over the nutritional and cultural impacts of Westernization. 35% of the population of Saudi Arabia is under the age of 16, and adult dietary preferences are often established during early childhood years [17]. However, little is known about the developmental effects of some commonly daily used food additives on pregnant women who are exposed to these additives in addition to their usual nutrition. Therefore, in this experimental study, four commonly used additives which represent the different groups of food additives (aspartame, annatto, monosodium glutamate and tartrazine) were selected in order to study their potential toxicity on the fetal developmental and pregnancy outcome.

Aspartame is methylester of N-alpha-aspartyl-L-phenylalanine, a polypeptide widely employed as an artificial, intense synthetic sweetener, having none or insignificant caloric values and a sweetening power higher than that of sucrose. Its use in pharmaceutical products and in nearly 6000 food products has been approved and among its consumers are those that replace natural foods by "light" ones [18].

Annatto is an orange-yellow dye which, in addition to its traditional uses, has also been used worldwide as a color additive for foods, drugs and cosmetics. As a food color additive, annatto has been extensively used for coloring high-fat dairy products such as butter and cheese, and for a variety of other products such as cereals, margarines, sausages, coffee creamers, ice-cream and seasonings. Bixin, a carotenoid devoid of pro-vitamin A activity, is the main oil soluble pigment found in annatto [19].

Monosodium glutamate (MSG) is a non-essential amino acid found in food with great amounts of protein, such as meat, fish, cheese and vegetables. When detected by the taste buds, it signals the protein presence in the diet. MSG is artificially added to food to provide an expansion and extension of taste. MSG is considered a flavorizing agent, being used extensively in industrialized food. Its production increased from 200 tons/year in 1969 to 800 tons/year in 2001, which reflects its wide use in the food industry [20, 21].

Tartrazine (FD & C Yellow No. 5, Food Yellow No. 4) is principally the trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-H-pyrazol-3-carboxylate. Tartrazine is an orange-colored, water soluble powder widely used in food products, drugs, cosmetics and pharmaceuticals. The estimated amounts of tartrazine manufactured in 1996 were approximately 71.35 metric tons in Japan and 985.76 metric tons in the USA [22].

MATERIALS AND METHODS

Chemicals:
The tested food additives (aspartame, annatto, monosodium glutamate and tartrazine), as well as all other chemicals used in this study, were purchased from local dealers in Jeddah, Saudi Arabia.

Animals and mating:
Mature nulliparous female Sprague-Dawley albino rats (weighing 175 –200 grams at the beginning of the experiment) were used in this study. They were obtained from the Animal House of King Fahd Medical Research Center. During the study the female rats were kept in separate metallic cages under standard temperature (24 ± 2ºC), humidity (55 ± 5%) and lighting (12 h: 12h Light: Dark) conditions. The rats were fed a standard chow diet *ad libitum* and had free access to water. This study was approved and registered by the Committee of Animal Research in the Department of Anatomy, Faculty of Medicine, King Abdulaziz University. Animals were cared for at King Fahd Center for Medical Research. Mating was assisted by placing the individual females overnight in the home cage of a singly-housed male of the same stock. Mating was confirmed by positive identification of spermatozoa in a vaginal lavage smear and is designated as gestation day 0 (GD0).

Animal grouping and treatment:
Once pregnancy was confirmed, the pregnant rats were randomly divided into five main groups. Treatment of all groups was given by gavage once per day from GD0-GD20.

Aspartame group: included 16 pregnant rats which were divided into two subgroups. Both subgroups

were treated with aspartame in distilled water (50 mg/ml) which was administered as 50 and 100 mg/kg body weight, respectively.

Annatto group: included 16 pregnant rats which were divided into two subgroups. Both subgroups were treated with annatto powder suspended in corn oil (250 mg/ml) which was administered as 250 and 500 mg/kg body weight, respectively.

Monosodium glutamate group: included 16 pregnant rats which were divided into two subgroups. Both subgroups were treated with monosodium glutamate in distilled water (50 mg/ml) which was administered as 50 and 100 mg/kg body weight, respectively.

Tartrazine group: included 16 pregnant rats which were divided into two subgroups. Both subgroups were treated with tartrazine (100 mg/ml) which was administered as 1000 and 2000 mg/kg body weight, respectively.

Control group: included 8 pregnant rats which received deionized water only.

Evaluation of pregnant rats:
The pregnant rats of each group were observed daily throughout gestation for body weight, food consumption, water intake and physical signs of toxicity following treatment e.g. pale coloration, bulging eyes, profuse salivation, increased lacrimation, fasciculation, tremors, weakness and drowsiness.

Extraction of fetuses and biopsy taking from the pregnant rats:
At the 20th day of pregnancy (GD20) in all groups, the pregnant rats were euthanized by an overdose of ether, between 10:00 and 12:00 A.M. to prevent the mothers from devouring any damaged offspring. The abdominal wall of each rat was opened and the liver and kidneys were excised and weighed. The full extent of both uterine horns was exposed promptly. Before opening either horn, fetal position within the horns, as well as the number of live and dead fetuses, as indicated by their movement following a gentle pressure, were recorded.

The uterine horns were excised along the antimesometrial border to reveal the fetuses, embryonic membranes and placentas. They were gently removed in totality from the uterus utilizing the blunt end of a pair of forceps. The number of "metrial glands" was counted and recorded. An incision along the dorsal surface of the membranes revealed the fetuses, then each fetus and its placenta were removed and weighed, the length of the umbilical cord was taken and all were recorded. Each fetus was examined for:

1) General morphology; where the following parameters were assessed: head size and shape, orofacial development (eye, ears and palate), limb development, vertebral column and tail, abdomen, umbilicus and external genitalia.

2) Fetal growth parameters including: fetal crown-rump length (CRL), head length (HL), and biparietal diameter (BPD), were measured by using a dial (Vernier) caliber and recorded.

Determination of Preimplantation Losses:
The abdominal wall of the mother was opened and the full extent of both uterine horns was exposed promptly. Before opening either horn, fetal position within the horns, as well as the number of live and dead fetuses, as indicated by their movement following a gentle pressure, were recorded. In addition, the number of "metrial glands", which are yellowish nodules usually found along the mesometrial margin of the uterine horns that mark any original implantation site, was counted and recorded. Thus, the metrial glands unoccupied by living or recently dead fetuses represent the number of prior resorption.

Statistical analysis:
One-way analysis of variance (ANOVA) and Student's t-test were used for the means of maternal weight, placental weight, fetal weight, crown-rump length, head length and biparietal diameter. Chi-square (X_2) test was used for rat embryolethality and abnormalities. The level of significance for all comparisons was set at $p < 0.05$.

RESULTS

I. Effects of food additives on pregnant rats:
During the experimental period of this study, no overt signs of toxicity were noted in the pregnant rats of control or different treated groups. The data on weight gain, water consumption and food consumption during pregnancy are shown in Table 1, while the data on the liver, kidney and placental weights are shown in Table 2.

1. Maternal body weight gain:
Maternal weight gain displayed a noticeable reduction in all tested food additives when compared to the

control group except for the monosodium glutamate, which showed a slight increase. In the case of aspartame, annatto and tartrazine treated groups; the reduction in weight gain was remarkable in the high doses (from GD 6 to GD11) when compared with low doses displaying various degrees of significance. Also, the female rats treated with tartrazine showed the highest reduction in weight gain among all treated groups (Table 1) (Figure 1).

2. Maternal water consumption:

There was a considerable reduction in maternal water consumption in all tested food additives when compared to the control group except for the monosodium glutamate, where there was a slight increase. In the case of aspartame, annatto and tartrazine treated groups; the reduction in water consumption was remarkable in the high doses (from GD 6 to GD11) when compared with low doses displaying various degrees of significance. Also, the female rats treated with tartrazine showed the highest reduction in weight gain among all treated groups (Table 1) (Figure 2).

3. Maternal food consumption:

Maternal food consumption showed a reduction in all tested food additives when compared to the control group except for the monosodium glutamate, which caused a slight increase. In the case of aspartame, annatto and tartrazine treated groups; the reduction in food consumption was remarkable in the high doses (from GD 6 to GD11) when compared with low doses displaying various grades of significance. Again, the female rats treated with tartrazine showed the highest reduction in weight gain among all treated groups (Table 1) (Figure 3).

4. Maternal liver and kidney weights:

The maternal liver and kidney weights were decreased in all food additives treated groups (Table 2). This decrease was remarkable in the high dose groups with statistically significant difference when compared to the control group and the low dose treatment of the same food additive. Also, the female rats treated with tartrazine showed the lowest liver and kidney weights among all treated groups.

5. Placental weight and umbilical length:

The weight of the placentas and the length of the umbilical cord were decreased significantly in all food additives treated groups when compared to the control group (Table 2). Also it was noticed this decrease was remarkable in the high dose groups with statistically significant difference when compared to the control group and the low dose treatment of the same food additive. Also, the female rats treated with tartrazine showed the lowest placental weight, and umbilical cord length among all treated groups.

II. Effects of food additives on pregnancy and embryolethality:

There is an increase in the number of preimplantation losses, resorptions, and dead fetuses in different food additives study groups (Table 3). These findings were remarkable in the high doses of food additives (Figure 4) with statistically significant reduction of the number of live fetuses when compared to the control group. Also, it was noticed that the highest percentages of preimplantation losses, resorptions, and dead fetuses were found in the high dose of tartrazine treated group.

III. Effects of food additives on fetal growth:

Fetal growth was reflected through the following parameters which include weight (gm), crown-rump length (cm), head length (cm), and biparietal diameter (cm) (Table (4). It was noticed that there was a significant reduction in fetal growth parameters in all treated groups compared with the control group. In addition, the decrease was remarkable in the high dose groups with statistically significant difference when compared to the control group and the low dose treatment of the same food additive. Moreover, the highest reduction of the above parameters was found in the high dose of tartrazine treated group.

DISCUSSION

The present study was designed to investigate the adverse effect of some commonly used food additives on mothers and their fetuses when ingested during pregnancy. The choice of food additives used in this study was based primarily on their representation to different groups of food additives e.g. annatto and tartrazine as food coloring agents, aspartame as a food sweetener and monosodium glutamate as a taste enhancer. In addition, these food additives are used in many processed and fast foods products which are distributed in most places all over the world.

The results of this study showed that administration of the above food additives to pregnant rats resulted in a dose-dependent toxicity for the mothers in the form of decreased maternal weight gain, water and food consumption except in the case of monosodium glutamate, which resulted in an increase in such parameters. In addition, all of these food additives provoked a deterioration of fetal parameters in the form of decreased placental weight and fetal

growth parameters, number of fetuses and increased resorptions.

The reduced maternal parameters of rats treated with aspartame, annatto and tartrazine could be explained on the basis of previous studies, which reported that the use of food additives may pose the risk of the loss of nutritional value of the food, which can result in inappropriate diets and subclinical malnutrition [23,24]. Those authors also mentioned that the wide use of food additives can contribute to malnutrition in the following ways; the common factor in most foods containing additives is high salt, sucrose and fat content. Pure sucrose contains literally no nutrients, but only calories. Similarly fat contains few nutrients and is very high in calories. In addition, foods containing additives are mainly processed foods, which have lost a substantial proportion of their nutritional value through the processing procedure [25]. Even though some vitamins and/or minerals are sometimes added to foods after processing, the ratio of essential nutrients to calories is usually still quite inadequate, resulting in a high calorie, but a low nutritional, intake. This type of diet, because of the high calorie and low nutritional content, can result in less than optimum nutrition and therefore subclinical and/or marginal malnutrition [26].

The toxic effects of the tested food additives on the reproductive and fetal parameters have been explained by different studies [24-26] They have shown that the inefficient diet not only affects the health and behavior of an individual, it also has serious long-term consequences on reproduction and on the future infant's health, as a good maternal diet is of paramount importance in relation to healthy fetal development and a successful pregnancy outcome. Moreover, subclinical maternal malnutrition has also been frequently associated with low birth weight infants, which in turn appears to have a clear negative effect on the infant's future health [27- 29]. The substances that the fetus utilizes are supplied by maternal blood and cross the placental membrane. Maternal malnutrition, principally in the final trimester, generally produces children with reduced weight. Furthermore, it is known that grave malnutrition, resultant from an inadequate diet, causes a reduction in fetal growth [30, 31].

Regarding the monosodium glutamate, the tested maternal parameters showed a slight increase contrary to the other food additives when compared to the control group. Findings in literature analyzing its relation to the weight gain, water and food consumption were conflictive. Some studies demonstrated decrease in the above mentioned parameters in the group that used monosodium glutamate [32-34]. However, other studies revealed an increase in food and water consumption [21,35]. These authors have also mentioned that when monosodium glutamate is administered orally in female pregnant rats, this can cause alterations in the hypothalamus of the offspring with its consequences, such as a decrease in the secretion of GH and obesity. They believed that consuming high amounts of monosodium glutamate during the gestational period might have a connection with the future obesity of the newborn [36].

Regarding the administration of aspartame to the pregnant rats, the reduction in weight gain, water and food consumption was in accordance with previous observations [37], which suggested that the aspartame caused a diminution of caloric intake. Also, the reduction in fetal and reproductive parameters could indicate that the fetuses cannot be supplied with the requirements for substrates, including glucose, either due to possible diminution in the blood of rats that were fed sweetener, or by possible reduction of the placental area, provoking a quantity of exchanges that is less than ideal for the fetus. This result was observed previously [38], where a significant diminution of weight and placenta for the groups treated with aspartame was found.

In the present study, administration of annatto to pregnant mothers resulted in a reduction of both maternal and fetal parameters, which had not been previously reported [39]. They suggested that annatto was neither maternally toxic nor embryo toxic to the female rats treated by gavage at a dose of 0, 31.2, 62.5, 125, 250 or 500 mg/kg per day on days 6–15 of gestation.

In this study, tartrazine has shown the worst effect on the maternal and fetal parameters when compared with other food additives. These findings have not been reported in rats at the dose of (60–1000 mg/kg/day) by gavage. In addition, it has been reported that tartrazine had no teratogenic effects when ingested in drinking water (0.05–0.7%) in rats [40-41]. However, some behavioural toxicity effects of tartrazine were reported 42 where some neurobehavioural effects were observed (at the dose of 1.0–2.0% in the diet). Moreover, hyperactivity in children, behavioural changes such as irritability, restlessness, and sleep disturbance were associated with the ingestion of tartrazine in some children, and these effects showed dose-dependant (1–50 mg/child) [43]. Furthermore, it was reported that 23 children who consumed a

tartrazine beverage showed increased levels of over activity, aggressive and/or violent activity, poor speech, poor coordination, and the development of asthma and/or eczema [44].

Although the results have demonstrated clearly the harmful effects of food additives on the pregnant rats and their fetuses, it has been argued that these toxicological food additive tests on animals for the assessment for human safety levels are really not definite. First of all, experiments on animals are conducted on healthy species fed on a nutritious diet, not on the malnourished, elderly or sick. Secondly, only one agent is tested at a time, whereas humans are known to consume an elaborate cocktail of 12 to 60 different additives in the course of a single meal [1,45]. This might be a reason why we still remain ignorant of the number of people really affected by the consumption of food additives. However, a recent published report showed that small children are much more likely to react to certain foods. Although the exact numbers are not known, surveys suggest that one child in 10 may be affected in some way [46].

In conclusion, the data from this study provides proof and a warning, especially to pregnant mothers, against the abuse of different food additives used in our daily foods. Further dose–response and toxicokinetic studies are needed to translate the results of this work into public health recommendations.

ACKNOWLEDGEMENTS

This project was funded by the KAU Waqf (Scientific Endowment) under Grant No: 429/011-5. The authors acknowledge with thanks KAU Waqf support for Scientific Research and also the services provided by the Deanship of Scientific Research, King Abdulaziz University.

REFERENCES

1. **Miller M.** Danger! Additives at Work. London: London Food Commission, 1985.

2. **Industrial Aids Ltd.** Depth Study of the food additives industry, London 1980, updated by discussions with market researchers, and Key Note Food Flavourings and Ingredients, London, 1985.

3. **The London Food Commission.** Food Adulteration and how to beat it. London: Unwin Paperbacks, 1988.

4. **Weiss B, Williams JH, Margen S, Abrams B, Caan B et al.** Behavioral responses to artificial food colors. Science 1980; 207: 1487-89.

5. **Feingold BF.** Dietary Management of Behavior and Learning Disabilities. In: Miller SA ed. Nutrition & Behavior. Philadelphia, Pennsylvania, USA: Franklin Institute Press, 1981: 37.

6. **O'Banion DR.** An Ecological and Nutritional Approach to Behavioral Medicine. Springfield, Illinois, USA: Charles C Thomas, 1981.

7. **Silbergeld EK, Anderson SM.** Artificial food colors and childhood behavior disorders. Bull New York Acad Med, Second Series 1982; 58/3: 275-95.

8. **Weiss B.** Food additives and environmental chemicals as sources of childhood behaviour disorders. J Am Acad Child Psych 1982; 21/2: 144-52.

9. **Rippere V.** Food additives and hyperactive children: A critique of Conners. Br J Clin Psych 1983; 22: 19-23.

10. **King DS.** Psychological and behavioral effects of food and chemical exposure in sensitive individuals. Nutrition and Health 1984; 3: 137-51.

11. **Schoenthaler SJ, Doraz WE, Wakefield JA.** The impact of a low food additive and sucrose diet on academic performance in 803 New York City public schools. Int J Biosocial Res 1986; 8/2: 185-95.

12. **Smith JM.** Adverse reactions to food and drug additives. European J Clin Nutr 1991; 45/l: 17-21.

13. **Harley J, Ray R, Tomasi L et al.** Hyperkinesis and food additives: Testing the Feingold hypothesis. Pediatrics 1978; 61: 818-28.

14. **Stare FJ, Whelan EM, Sheridan M.** Diet and hyperactivity: Is there a relationship? Pediatrics 1980; 66: 521-25.

15. **JECTA - Joint Expert Committee on Food Additives of the United Nations Food and Agriculture Organisation and the World Health Organisation.** Environmental Health Criteria Geneva 1987; 70: 46-65.

16. **Feingold BF.** Food additives and child development. Hospital Practice 1973; 21: 11-12, 17-18.

17. **Collison KS, Zaidi MZ, Subhani SN, Al-Rubeaan K, Shoukri M, Al-Mohanna FA.** Sugar-sweetened carbonated beverage consumption correlates with BMI, waist circumference, and poor dietary choices in school children. BMC Public Health 2010; 9: 1-234.

18. **AFSSA.** Assessment Report: Opinion on a possible link between exposition to aspartame and the incidence of brain tumours in humans. Maisons-Alfort , French Food Safety Agency, 2002.

19. **Morrison EY, Thompson H, Pascoe K, West M, Fletcher C.** Extraction of a hyperglycemic principle from annatto (Bixa orellana): a medicinal plant in West Indies. Tropical and Geographical Medicine 1991; 43: 184-88.

20. **Taylor-Burds CC, Westburg AM, Wifall TC, Delay ER.** Behavioral comparisons of the tastes of L-alanine and monosodium glutamate in rats. Chem Senses. 2004; 29/9: 807-14.

21. **Diniz YS, Faine LA, Galhardi CM, Rodrigues HG, Ebaid GX et al.** Monosodium glutamate in standard and high-fiber diets: metabolic syndrome and oxidative stress in rats. Nutrition 2005; 21/6: 749-55.

22. **Ishimitsu S, Mishima I, Tsuji S, Shibata T.** Comparison of estimated production of coal-tar dyes in Japan and USA. Food Sanitat Res 1998; 48/5: 33–40.

23. **Wynn M, Wynn A.** The prevention of handicap of early pregnancy origin: Some evidence for the value of good health before conception. London: Foundation for Education and Research in Childbearing, 1981.

24. **Doyle W, Crawford MA, Wynn AHA, Wynn SW.** Maternal nutrient intake and birth weight. J Human Nutr and Dietetics 1989; 2: 415-22.

25. **Barnes B, Bradley SG.** Planning for a Healthy Baby. London: Ebury Press, 1990.

26. **Wynn SW, Wynn AH, Doyle W, Crawford MA.** The association of maternal social class with maternal diet and the dimensions of babies in the population of London women. Nutrition and Health 1994; 9: 303-15.

27. **Barker DJP.** Fetal and Infant Origins of Adult Disease. London: BMJ Books, 1992.

28. **Barker DJP, Hales CN, Clark PMS.** Fetal nutrition and cardiovascular disease in adult life. The Lancet 1993(a); i: 938-41.

29. B**arker DJP, Martyn CN, Osmond C, Hales CN, Fall CHD.** Growth in utero and serum cholesterol concentrations in adult life. Br Med J 1993(b); 307: 1524-27.

30. **Moore KL.** Embriologia clínica. 4th ed. Rio de Janeiro: Guanabara Koogan, 2000.

31. **Sadler TW.** Langman's Medical Embryology, 11th edition. North American Edition. Philadelphia, Pennsylvania, USA: Lippincott, Williams and Wilkins, 2009.

32. **Nakagawa T, Ukai K, Ohyama T, Gomita Y, Okamura H.** Effects of chronic administration of sibutramine on body weight, food intake and motor activity in neonatally monosodium glutamate-treated obese female rats: relationship of antiobesity effect with monoamines. Exp Anim 2000; 49/4: 239-49.

33. **Perello M, Gaillard RC, Chisari A, Spinedi E.** Adrenal enucleation in MSG-damaged hyperleptinemic male rats transiently restores adrenal sensitivity to leptin. Neuroendocrinology 2003; 78/3: 176-84.

34. **Kim YW, Choi DW, Park YH, Huh JY, Won KC et al.** Leptin-like effects of MTII are augmented in MSG-obese rats. Regul Pept 2005; 127/1-3: 63-70

35. **Fernandez-Tresguerres Hernandez JA.** Effect of monosodium glutamate given orally on appetite control (a new theory for the obesity epidemic). An R Acad Nac Med (Madr) 2005; 122/2: 341-55.

36. **Hermanussen M, Garcia AP, Sunder M, Voigt M, Salazar V, Tresguerres JA.** Obesity, voracity, and short stature: the impact of glutamate on the regulation of appetite. Eur J Clin Nutr 2006; 60/1: 25-31.

37. **Vermunt SH, Pasman WJ, Schaafsma G, Kardinaal AF.** Effects of sugar intake on body weight: A review. Obes Rev 2003; 4/2: 91-9.

38. **Portela, GS, Azoubel R, Batigàlia, F.** Effects of aspartame on maternal-fetal and placental weights, length of umbilical cord and fetal liver: a kariometric experimental study. Int J Morphol 2007; 25/3: 549-54.

39. **Paumgartten FJR, De-Carvalho RR, Araujo IB, Pinto FM, Borges OO et al.** Evaluation of the developmental toxicity of annatto in the rat. Food Chem Toxicol 2002; 40: 1595-1601.

40. **Collins TFX, Black TN, Brown LH, Bulhack P.** Study of the teratogenic potential of FD & C Yellow No. 5 when given by gavage to rats. Food Chem Toxicol 1990; 28: 821-7.

41. **Collins TFX, Black TN, O'Donnell Jr MW, Bulhack P.** Study of the teratogenic potential of FD & C Yellow No. 5 when given in drinking water. Food Chem Toxicol 1992; 30: 263-8.

42. **Sobotka TJ.** Estimation of Average, 90th Percentile and Maximum Daily Intakes of FD & C Artificial Food Colors in One Day's Diets Among Two Age Groups of Children. USA: Division of the Consumer Studies, Food and Drug Administration, 1976.

43. **Rowe KS, Rowe KJ.** Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo-controlled, repeated-measures study. J Pediatr 1994; 125: 691-98.

44. **Ward NI.** Assessment of chemical factors in relation to child hyperactivity. J Nutri Environ Med 1997; 7/4: 333-42.

45. **Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R.** A population study of food intolerance. The Lancet 1994; 343: 1127-29.

46. **National Dairy Council.** Adverse Reactions to Food. Topical Update 2. London: National Dairy Council, 1994.

Table (1): Effects of food additives on the pregnant rats

Groups	Maternal Wt. Gain (gm) Mean ± SD	Maternal water consumption (ml) Mean ± SD	Maternal food consumption (gm) Mean ± SD
Control (8)	99.6 ± 11.9	51.5 ± 4.3	32.1 ± 2.3
Aspartame 50 mg (8)	88.5 ± 10.8	49.1 ± 3.9	30.1 ± 2.5
Aspartame 100 mg (8)	74.5 ± 9.1 [b,d]	38.2 ± 3.1 [c,f]	25.2 ± 3.4 [b,e]
Annatto 250 mg (8)	85.4 ± 10.2 [a]	47.9 ± 3.8	29.5 ± 2.7
Annatto 500 mg (8)	73.2 ± 8.9 [c,d]	37.1 ± 3.1 [c,f]	23.9 ± 3.7 [c,e]
Monosodium glutamate 50 mg (8)	108 ± 12.9	52.9 ± 4.5	35.5 ± 2.1
Monosodium glutamate 100 mg (8)	101 ± 12.2	50.7 ± 4.4	30.2 ± 2.4 [d]
Tartrazine 1000 mg (8)	83.5 ± 9.9 [b]	47 ± 3.8 [a]	29.8 ± 2.9
Tartrazine 2000 mg (8)	70.5 ± 8.8 [c,d]	35.1 ± 3.2 [c,f]	23.2 ± 3.8 [c,e]

ANOVA test:

a- $P < 0.05$ compared to control group.

b- $P < 0.01$ compared to control group.

c- $P < 0.001$ compared to control group.

d- $P < 0.05$ compared to the low dose group of the same food additive.

e- $P < 0.01$ compared to the low dose group of the same food additive.

f- $P < 0.001$ compared to the low dose group of the same food additive.

Table (2): Effects of food additives on the pregnant rats

Groups	Maternal Liver Wt. (gm) Mean ± SD	Maternal Kidney Wt. (gm) Mean ± SD	Placental Wt. (gm) Mean ± SD	Umbilical cord length (cm) Mean ± SD
Control (8)	10.08 ± 0.35	1.1 ± 0.07	0.64 ± 0.06	1.96 ± 0.25
Aspartame 50 mg (8)	9.7 ± 0.34 [a]	1.05 ± 0.06 [a]	0.60 ± 0.14 [a]	1.93 ± 0.28
Aspartame 100 mg (8)	9.1 ± 0.29 [c,e]	0.95 ± 0.05 [c,f]	0.49 ± 0.07 [c,f]	1.78 ± 0.19 [b,d]
Annatto 250 mg (8)	9.8 ± 0.33	1.05 ± 0.04 [a]	0.57 ± 0.1 [b]	1.89 ± 0.32
Annatto 500 mg (8)	9.0 ± 0.28 [c,f]	0.95 ± 0.07 [c,e]	0.50 ± 0.06 [c,f]	1.79 ± 0.26 [b,d]
Monosodium glutamate 50 mg (8)	9.75 ± 0.35	1.05 ± 0.08 [a]	0.61 ± 0.09 [a]	1.92 ± 0.18
Monosodium glutamate 100 mg (8)	9.05 ± 0.27 [c,e]	0.95 ± 0.04 [c,f]	0.52 ± 0.08 [c,f]	1.74 ± 0.23 [b,e]
Tartrazine 1000 mg (8)	9.68 ± 0.29 [a]	1.0 ± 0.06 [a]	0.59 ± 0.09 [a]	1.84 ± 0.24 [a]
Tartrazine 2000 mg (8)	8.95 ± 0.21 [c,f]	0.9 ± 0.03 [c,f]	0.47 ± 0.08 [c,f]	1.74 ± 0.17 [b,d]

ANOVA test:

a- $P < 0.05$ compared to control group.

b- $P < 0.01$ compared to control group.

c- $P < 0.001$ compared to control group.

d- $P < 0.05$ compared to the low dose group of the same food additive.

e- $P < 0.01$ compared to the low dose group of the same food additive.

f- $P < 0.001$ compared to the low dose group of the same food additive.

Oxford Research Forum Journal, October 2010, Vol 3, No 2

Table (3): Effects of food additives on pregnancy and embryolethality

Groups (No. pregnant rats)	Total No. of corpora lutea	Total implantation	Pre-implantation loss		Resorptions		Dead fetuses		Live fetuses	
			No.	%	No.	%	No.	%	No.	%
Control (8)	80	80	-	0.0	2	2.5	-	0.0	78	97.5
Aspartame 50 mg (8)	84	82	2	2.4	4	4.8	-	0.0	78	92.8
Aspartame 100 mg (8)	80	76	4	5.0	6	7.5	2	2.5	68 [a]	85.0
Annatto 250 mg (8)	82	80	2	2.4	6	7.3	-	0.0	74	90.3
Annatto 500 mg (8)	80	76	4	5.0	6	7.5	2	2.5	68 [a]	85.0
Monosodium glutamate 50 mg (8)	84	80	4	4.8	2	2.4	-	0.0	78	92.8
Monosodium glutamate 100 mg (8)	82	76	6	7.3	3	3.7	1	1.2	72 [a]	87.8
Tartrazine 1000mg (8)	86	84	2	2.3	4	4.7	-	0.0	80	93.0
Tartrazine 2000mg (8)	80	74	6	7.5	8	10.0	2	2.5	64 [a]	80.0

Chi-square test:

a- $P<0.05$ compared to control group

Table (4): Effects of food additives on fetal growth

Groups	Fetal Weight (gm) Mean ± SD	Crown-Rump Length (cm) Mean ± SD	Head Length (cm) Mean ± SD	Biparietal Diameter (cm) Mean ± SD
Control (78)	3.33 ± 0.49	3.23 ± 0.25	1.45 ± 0.06	0.77 ± 0.05
Aspartame 50 mg (78)	3.15 ± 0.51	3.04 ± 0.22	1.4 ± 0.05	0.76 ± 0.05
Aspartame 100 mg (68)	2.43 ± 0.56 [c,f]	2.69 ± 0.19 [c,e]	1.29 ± 0.06 [b,e]	0.68 ± 0.06 [c,f]
Annatto 250 mg (74)	3.11 ± 0.52	3.1 ± 0.29	1.38 ± 0.07	0.74 ± 0.06
Annatto 500 mg (64)	2.39 ± 0.43 [c,f]	2.55 ± 0.24 [c,f]	1.26 ± 0.07 [c,e]	0.65 ± 0.06 [c,f]
Monosodium glutamate 50 mg (78)	3.19 ± 0.49	3.08 ± 0.18	1.43 ± 0.06	0.77 ± 0.06
Monosodium glutamate 100 mg (72)	2.51 ± 0.39 [c,f]	2.67 ± 0.17 [c,e]	1.25 ± 0.06 [c,e]	0.65 ± 0.05 [c,f]
Tartrazine 1000 mg (80)	2.95 ± 0.41 [b]	2.98 ± 0.18 [a]	1.36 ± 0.08 [a]	0.72 ± 0.03 [b]
Tartrazine 2000 mg (68)	2.35 ± 0.32 [c,f]	2.64 ± 0.19 [c,e]	1.25 ± 0.08 [c,d]	0.64 ± 0.04 [c,f]

ANOVA test:-

a- P <0.05 compared to control group.

b- P <0.01 compared to control group.

c- P <0.001 compared to control group.

d- P <0.05 compared to the low dose group of the same additive.

e- P <0.01 compared to the low dose group of the same additive.

f- P <0.001 compared to the low dose group of the same additive.

Oxford Research Forum Journal, October 2010, Vol 3, No 2

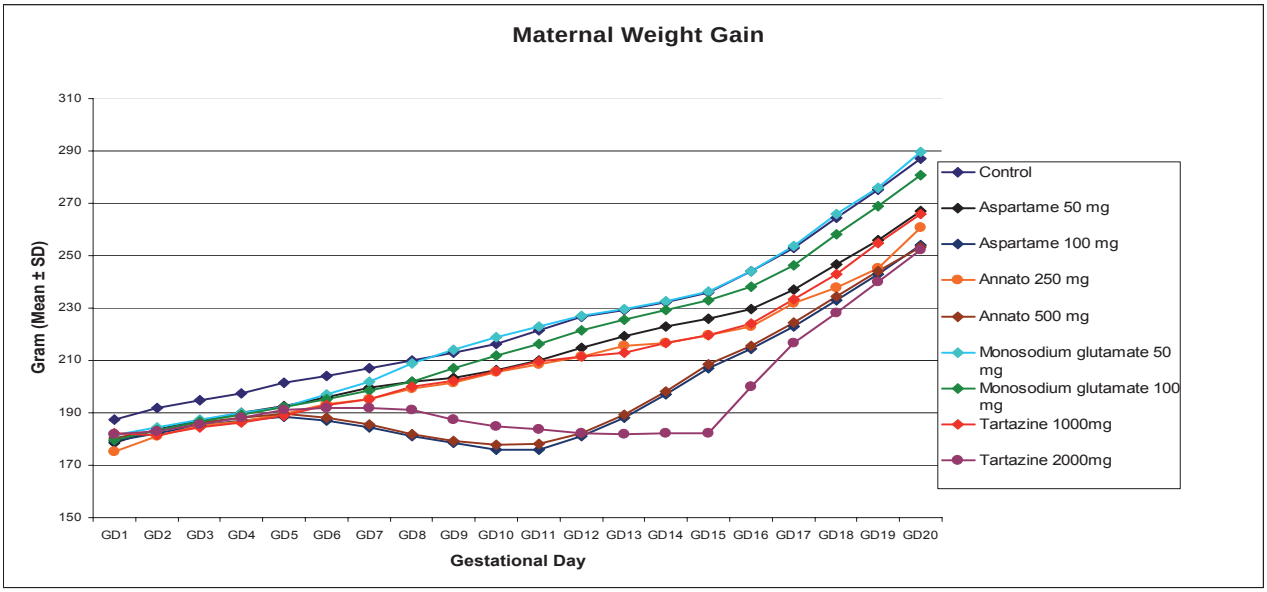

Figure (1): showed maternal weight gain in the different treated groups in comparison with that in control group.

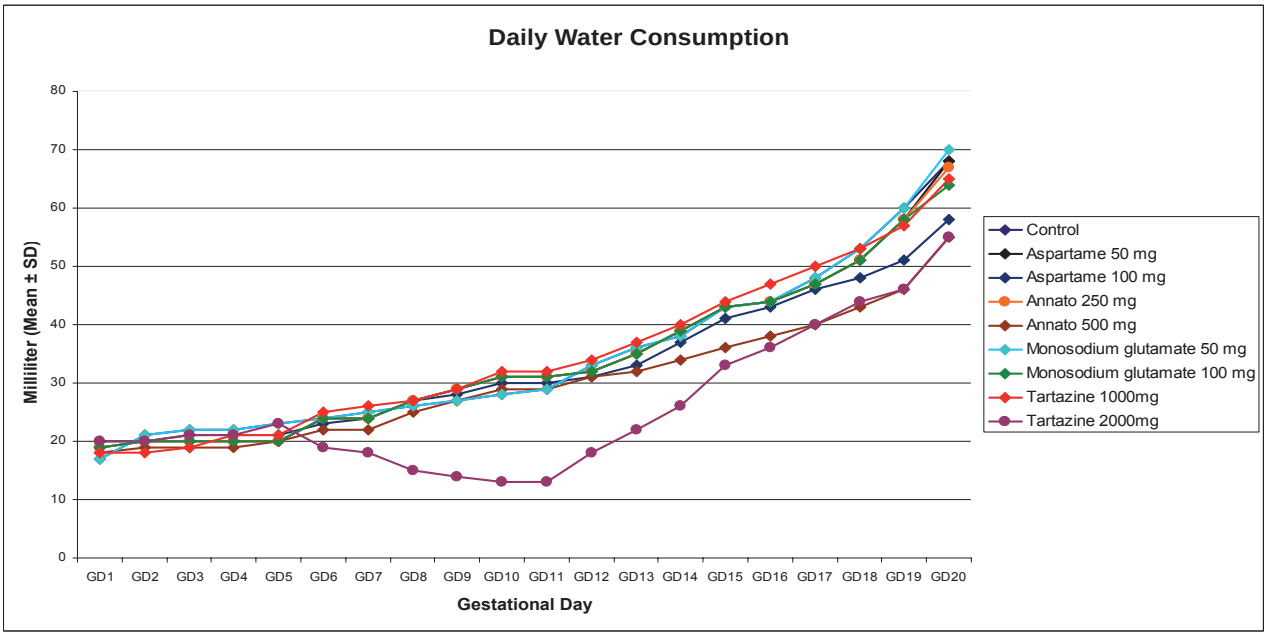

Figure (2): showed maternal water consumption in different treated groups in comparison with that in control group.

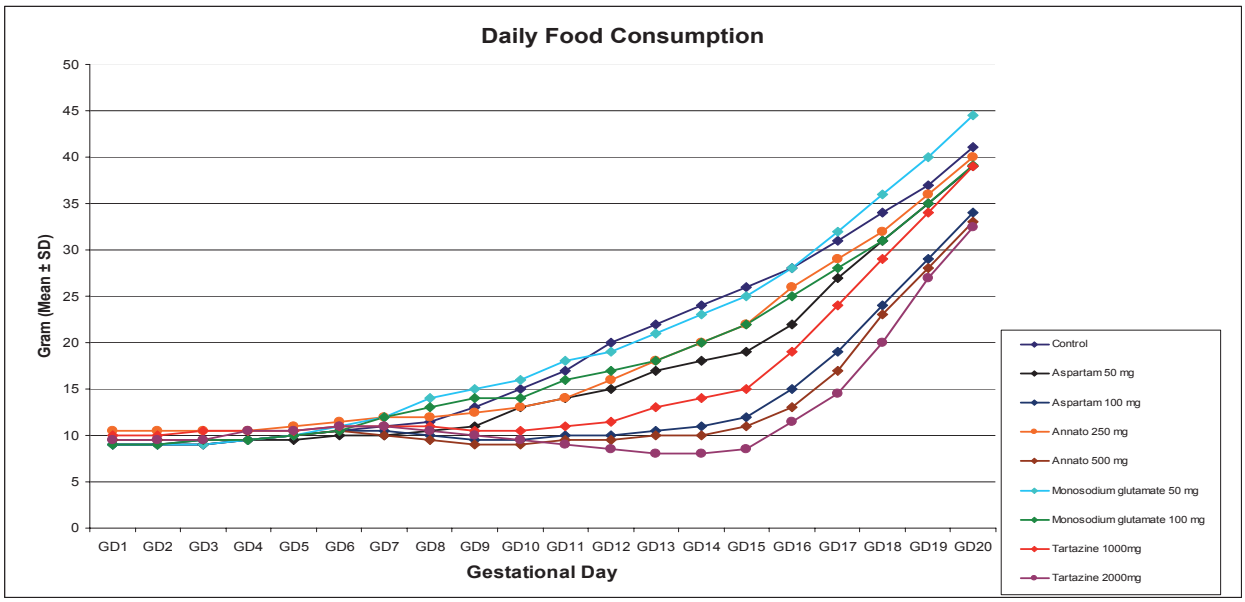

Figure (3): showed maternal food consumption in different treated groups in comparison with that in control group.

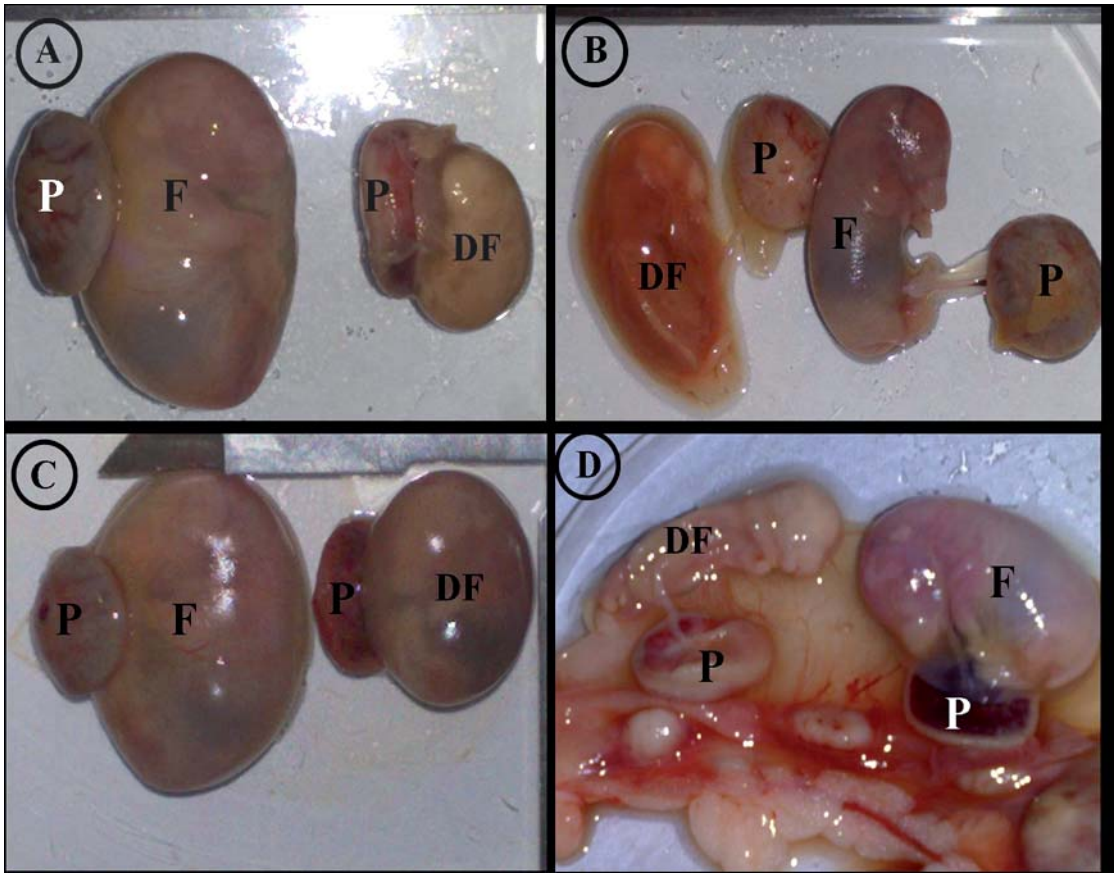

Figure (4): Showed dead fetuses recovered from high doses of treated groups: (A) Tartrazine 2000 mg, (B) Monosodium glutamate 100 mg, (C) Aspartame 100 mg, and (D) Annatto 500 mg. (DF= dead fetuses, F = fetus, P = placenta).