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Comparative toxicological studies of some food additives on foetal development in rats

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Abstract

This study aimed to compare the impact of some common food additives (MSG, ACK, BHA and BHT) on fetal development. To do this, forty female albino rats weighing approximately 200g were examined to determine their estrous period and then paired with appropriate fertile males. The effects of these food additives on fetal development were then monitored throughout the remainder of the pregnancy period. On the sixth day of pregnancy, the pregnant rats were divided into five different groups: Group A (control) received saline, while Groups B-E received MSG, ACK, BHA and BHT respectively in doses of 3, 15, 25 and 14 mg kg-1 day-1. Upon morphological, visceral and skeletal examination of the fetuses, it was concluded that the food additives had a significant negative impact on fetal development. Therefore, it is advised that consumers should avoid consuming food additives during this period of gestation.

Keywords: 1. Food additives; Teratogenicity; Sweeteners; MSG; Fetal developmen

1. Introduction

Food additives usually improve food product properties such as maintaining consistency, texture, taste, colour, quality, alkalinity, or acidity. They are widely used in two main categories: the first is to assure food safety and prevent food degradation by bacteria, oxidation, or chemical reactions; while the second is to improve the texture, appearance, and all sensory properties. Food additives are classified according to their basic role into preservatives, flavour enhancers, bulking agents and colouring agents (1).

For instance, monosodium L-glutamate (MSG) is a common glutamic acid salt utilized as a food additive (flavour enhancer) used increasingly more often both at home and in the food industry since 1907. Therefore many marketed food products such as canned or fast food contain varying concentrations of MSG (2). MSG is one of the world's most extensively used food additives, ingested in processed food products as a flavour enhancer, which increases the sapidity of food (3). At the same time, many studies have proven that MSG causes symptoms such as numbness, irritability, sweating, headache and dizziness. It may exacerbate many conditions, including asthma, urticaria, atopic dermatitis, arrhythmias, neuropathy and abdominal discomfort (4). The major adverse effects reported in either humans or animals were obesity, diabetes, hepatotoxic, neurotoxic and genotoxic effects.

Acesulfame-K (ACK) is a food additive used as a sweetener. It is approximately 200 times sweeter than sucrose with zero calorie content (5). Although it was discovered in 1976, it took FDA approval in 1998 to be used in some pharmaceutical preparations and as an ingredient in varieties of food such as baked goods, beverages, candies, chocolates, dairy products, desserts and more (6).

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used for many years as antioxidants to preserve and stabilize the freshness, nutritive value, flavour, and colour of

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foods and animal feed products. BHT may be present as an indirect food additive. Approximately 40 countries reportedly permit the use of BHT as a direct or indirect food additive. The US Food and Drug Administration (FDA) currently permits BHA and BHT as food additives. Food-grade BHA, referred to as 2(3)-tert-butyl-4-hydroxyanisole, is generally a mixture of greater than 85% 3-tert-butyl-4-hydroxyanisole (3-BHA) and 15% or less 2-tert-butyl-4-hydroxyanisole (2-BHA). Food grade BHT, butylated hydroxytoluene, 2,6-di-tertiarybutylphenol, 4 methyl 2,6 ditertiarybutylphenol, is also known as food antioxidant (7).In addition, the kinetics of the two antioxidants BHA and BHT differ significantly; BHA is characterized by rapid absorption and excretion by rats, rabbits, and humans, whereas BHT is cleared less rapidly from most species, with little evidence of long-term tissue storage (8).

Teratogens can affect an embryo in a variety of ways, including physical deformities and behavioural or mental disorders as well as a reduction in the child's intellectual quotient. It can also lead to problems such as unseasonable labour, robotic revocations, and deliveries and detriment to the embryo (9).

Early pregnancy is the critical period for embryonic development, in which cell differentiation and morphogenesis are at their peak. During this period, embryos are more susceptible to any teratogen agent. Studies have demonstrated that oral intake of MSG to pregnant rats influences the body weight and hypothalamus of their offspring.

This study is designed to determine the toxicity and teratogenic effects of four food additives: Monosodium glutamate (MSG), Acesulfame-K (ACK), Butylatedhydroxy-anisole (BHA) and Butylatedhydroxytoluene (BHT) on the fetal development in pregnant rats.

2. Material and methods:

2.1. Material:

2.1.1. Food additives (tested compounds):

Monosodium glutamate (MSG), Acesulfame K (ACK), Butylated hydroxy anisole (BHA) and Butylated hydroxytoluene (BHT).

2.1.2. Chemicals used for studying the effect on developing feoti:

Ethyl alcohol (95%) (El-Gomhoria Co.Egypt), Glycerin (20, 50, 80 and 100%) (El-Gomhoria Co.Egypt), Potassium Hydroxide powder (El-Gomhoria Co.Egypt).

Bouin's solution was prepared according to [1], about 100 ml concentrated picric acid, 25 ml formaldehyde, 10 ml glacial acetic acid.

Glycerine 20, 50, 80, 100%, solution.

Mallsch's solution was prepared by adding 7.5 ml of potassium hydroxide 4% to 60 ml glycerine and distilled water up to 300 ml. [2].

Alizarin red stain used for staining of foetal skeleton [3].

2.1.3. Animals:

Forty female albino rats were weighing 200±25 g and ten male albino rats weighing 250±50g. Rats were obtained from the animal house, National research Centre, Dokki, Giza, Egypt. All animals were kept individually in stainless steel cages, the diet and distilled water were allowed ad libitum, standard laboratories condition (25º C, RT). All experimental procedures were carried out according to the Institutional Animal Ethics Committee of the NRC, Egypt. They were fed on the standard balanced normal diet according to AIN-93 [4]. The animals were accommodated to the laboratory conditions for two weeks before being experimented.

2.2. Methods:

2.2.1. Examination of experimental rats:

They were examined periodically using vaginal smear technique to ensure that they were in regular oestrus cycle [5], each four females were paired with a male in a separate cage. The following morning, a vaginal smear was taken to verify the first day of pregnancy. The presence of spermatozoa in the obtained vaginal smear indicates zero day of pregnancy [6]. The pregnancy was confirmed by microscopic examination of the vaginal smear, the persistence of diestrus state for 5 days after mating indicates pregnancy and palpable fetal masses in the abdomen at the on 5th day after the mating.

2.2.2. Grouping of Animals:

On the sixth day of pregnancy, females were allocated into five equal experimental groups of 8 females in each group and treated in a specific period from the 5th day through the 19th day of pregnancy as follows: Group A: The pregnant dams had received saline kept as control, group B: The pregnant dams had received MSG in a dose of 3 mg kg− 1 day− 1, group C: The pregnant dams had received ACK (15 mg kg− 1 day− 1), group D: The pregnant dams had received treatment dose of BHA (25 mg kg− 1 day− 1) and group E: The pregnant dams had received treatment dose of BHT (14 mg kg− 1 day− 1). Both control and treated pregnant females are kept under observation until the 20th day of gestation. At 20th day, the pregnant rats were decapitated and dissected to examine the effect of the tested four food additives on fetal development by morphological, visceral and skeletal examination.

2.2.3. Examination of foetal developmental changes:

2.2.3.1. Morphological Examination:

The method used was described by Hayes [7]. The dam was laid on absorbent paper and the abdominal wall was reflected over the thorax. The horns of the gravid uterus were exteriorized and the ovaries were removed from the fat pad. The ovaries were immersed in saline and the number of corpora lutea on each ovary was counted. The uterus was removed without confusing the left and right horns. The isolated uterus was cut opened with a scissor and the fetuses were pulled out. The number of implantation sites, resorption sites, live and dead fetuses were examined for gross external anomalies, weighed and measured, pre-implantation deaths (equation 1) and post-implantation deaths (equation 2). These Indices were calculated according to Hayes [7].

Pre-implantation death= (number of corpora lutea - number of implantation sites)/(number of corpora lutea) x100 (Eq1)

Post-implantation death= (number of implantation sites - number of live fetuses)/ (number of implantation sites) x100 (Eq2)

The fetuses obtained from each dam were subjected to morphological examination then classified into three thirds; the first two thirds were injected intraperitoneally with 0.2 ml bouin's solution and then kept in a glass jar containing bouin's solutions for at least one week to reveal their visceral abnormalities. The other third of fetuses were eviscerated and kept in 95% ethanol to reveal skeletal malformations.

2.2.3.2. Visceral Examination:

Fetuses preserved in Bouin's fixative were rinsed with cold water and examined grossly. Sections were made using the technique described by Hayes [7]. Five transverse sections were made through the head. The first two were made between the nose and the eyes; the third through the middle of both eyes; the fourth, just behind the eyes; and the fifth, approximately 3mm behind the third cut. The neck was removed from the body of the fetus by sectioning it transversely at the level of the clavicle. Cross sections of the body were made approximately 1mm apart. All sections were examined on both sides under dissecting microscope for any visceral malformations.

2.2.3.3. Skeletal Examination:

The eviscerated fetuses were kept in ethanol for 7 days for dehydration. The dehydrated, eviscerated fetuses were placed in a 2% potassium hydroxide solution for 24-35 hours to clear the non-calcified tissues. After clearing, fetuses were stained in Mallsch's solution with alizarin red for 24 hr. Then they were transferred to Mallsch's solution. After that, these fetuses were preserved in 20, 50, 80 and 100% glycerin solution, respectively. The stained skeletons were examined under dissecting microscope for any abnormality of shape or size or the absence of a bone [3].

2.2.4. Statistical Analysis:

In the present study, all results were expressed as mean ± standard error of the mean. Data analysis was achieved by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using software program GraphPad Prism (version 5.00). The difference was considered significant when $P<0.05$.

3. Results:

3.1. Effects of tested four food additives on developing foeti:

On the 20th day of gestation, each dam was anaesthetized and a caesarian section was performed to obtain the foeti. The uterine horns were examined for possible resorption sites.

The numbers of implantation sites, viable, weakly alive and dead foeti are illustrated in table (1) and shown in figures from (1-5). The obtained data clearly demonstrate that MSG provoked a highly significant decrease ($p<0.05$) ; 36 with a percent of 64% in the mean value of the viable foeti obtained from dams given MSG in comparison to viable feoti of control dams.

ACK evoked significant decrease ($p<0.05$); 41with a percent of 59 % in the mean value of the viable foeti obtained from dams given ACK versus to ; 100 % for the control group.

While BHA and BHT showed a highly significant decrease ($p<0.05$); 43 and 57 with a percent of 57 and 43 % respectively in the mean value of the viable foeti obtained from dams given these two food additives ingredients versus ; 100 % for the control group.

Dams given MSG, ACK, BHA & BHT displayed a significant increase ($p<0.05$); 87, 134, 403 and 575% respectively in the mean values of the preimplantation death versus to control group fig. (1-2)

MSG, ACK, BHA & BHT elicited a significant increase ($p<0.05$); 519, 547,622 and 820% in the mean value of the post-implantation death in dams versus to control group fig. (1-2).

Table (1): Showing the morphological changes and mortality rate in foeti obtained from dam rats orally administered MSG, ACK, BHA and BHT from 5th day through 19th day of pregnancy.

Means superscripted with different letters are significantly different ($p<0.05$).

Group	Control	A	B	C	D
Corpus luteum	9.5±0.85	11.77± 0.78	9.8± 0.88	11.89± 0.48	12.33±2.44
Implantation site	8.6± 0.55	9.68± 0.44	7.63± 0.69	6.23± 1.63	4.45± 1.88
resorbed fetuses	0.2	1.88	1.81	1.97	1.6
dead fetuses	0.4	2	1.63	0.94	1.05
alive fetuses	8.00±0.02	5.80±0.69	4.19±0.54	3.32±0.59	1.8±0.73
pre-implantation deaths (%)	9.47	17.75	22.14	47.6	63.9
post-implantation deaths (%)	6.97	40.08	45.09	46.71	59.55
fetal B.W (g)	4.05± 0.47	3.67± 0.57	4.31± 0.21	3.65± 0.7	3.21± 1.56
cRL	4.46± 0.44	4.16± 0.48	4.6± 0.37	4.04± 0.58	3.59± 0.6
placental w. (g)	0.86± 0.045	0.67± 0.07	0.78± 0.05	0.64± 0.07	0.58± 0.10

3.2. Morphological examination:

MSG, ACK, BHA & BHT provoked a highly significant decrease ($p<0.05$) ; 20, 17, 31and 48% in the mean values of the foetal body weight of the obtained foeti from dams administered these food additives in comparison to the control group (fig.3).

MSG, ACK, BHA & BHT provoked a significant decrease ($p<0.05$) ; 22, 9, 26 and 33% in the mean values of the foetal placental weight versus to data obtained from the control group. Red patches with varying sizes were seen externally on different parts of the foetal body especially in the chest and fore limb in 15.76 % and hind limb in 37.12 % of the foeti obtained from dams; these patches were more prominent in feoti of dams that received ACK than MSG (fig. 4. I,II).

Red pitichae were seen externally on different parts of the foetal body especially in the head and back in 47.12% of the foeti obtained from dams which were more prominent in feoti of dams received ACK than MSG (fig. 5).

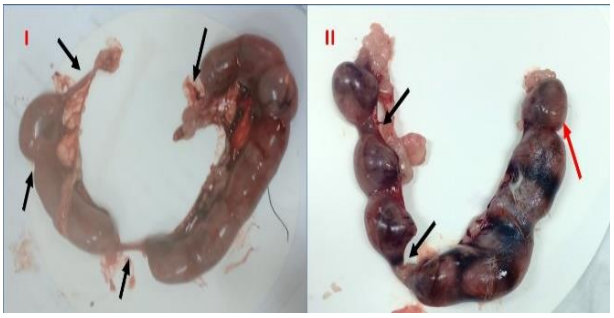


Fig. 1. Uterus of a pregnant rat orally administered MSG (I), ACK (II) respectively through 5th day of gestation showing early foetal resorption black arrows and weekly alive, dead feoti red arrow

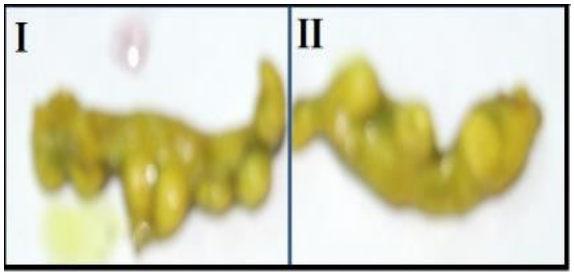


Fig. 2: Uteri of a pregnant rat fixed in bouin's solutions orally administered BHT (I), BHA (II) Through 5th day of gestation showing preimplantation death

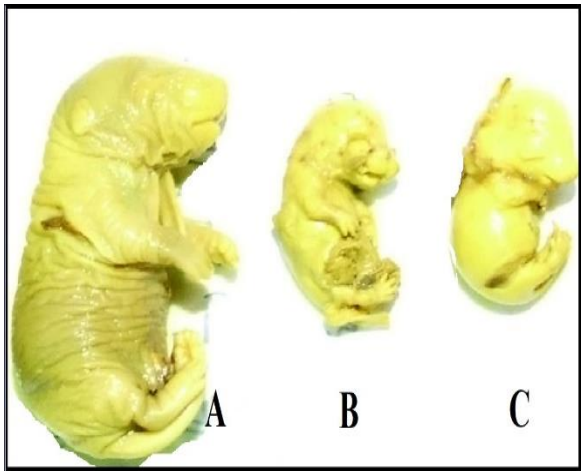


Fig. 3. B, C is Rat foeti fixed in bouin's solutions from dams orally given MSG, ACK respectively during pregnancy showing Marked decrease in fetal size compared with control rat feoti (A).

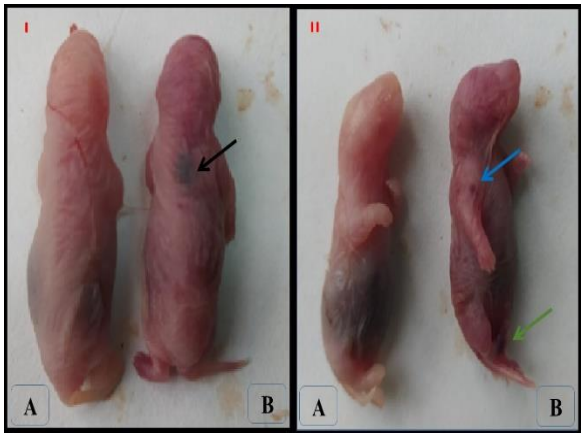


Fig. 4. (I, II): B is rat foeti orally given MSG during pregnancy showing Marked red patches on different sites of the body especially on dorsal vertebrae note right shoulder and right feet compared with control rat feoti A

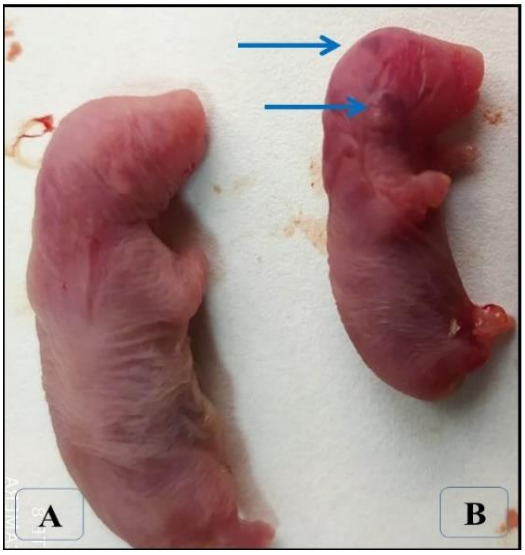


Fig. 5. B is Rat foeti orally given ACK during pregnancy showing Marked red patches on different sites of the body especially on encephalon compared with control rat feoti A.

3.3. Visceral examination:

Foeti obtained from the treated and control groups and later kept in Bouin's solution were macroscopically examined with the aid of a magnifying hand lens and the obtained results are revealed in Figure (6-9) and table (2). Oral administration of MSG, ACK and BHA to pregnant rats from the 5th through the 19th day of gestation evoked manifold visceral foetal abnormalities in naris as nostril anomalies showed in figure (6 A-C) as narrow nostril and slight congestion of nostril with variable degrees the most prominent changes are recorded in MSG.

The investigation of the cross-section through the eye showed slight congestion and Uni-lateral Anophthalmia in feoti of MSG and BHA groups from the higher record to the lower. Fig. (6 D-G).

The last section of feoti skull of MSG, ACK and BHA groups revealed different of the abnormalities including (dilatation of central and lateral ventricles, microencephaly), these abnormalities were recorded in MSG as dilatation of central and lateral ventricles while the microencephaly changes are reported in ACK and BHA groups. Fig. (6 H-J).

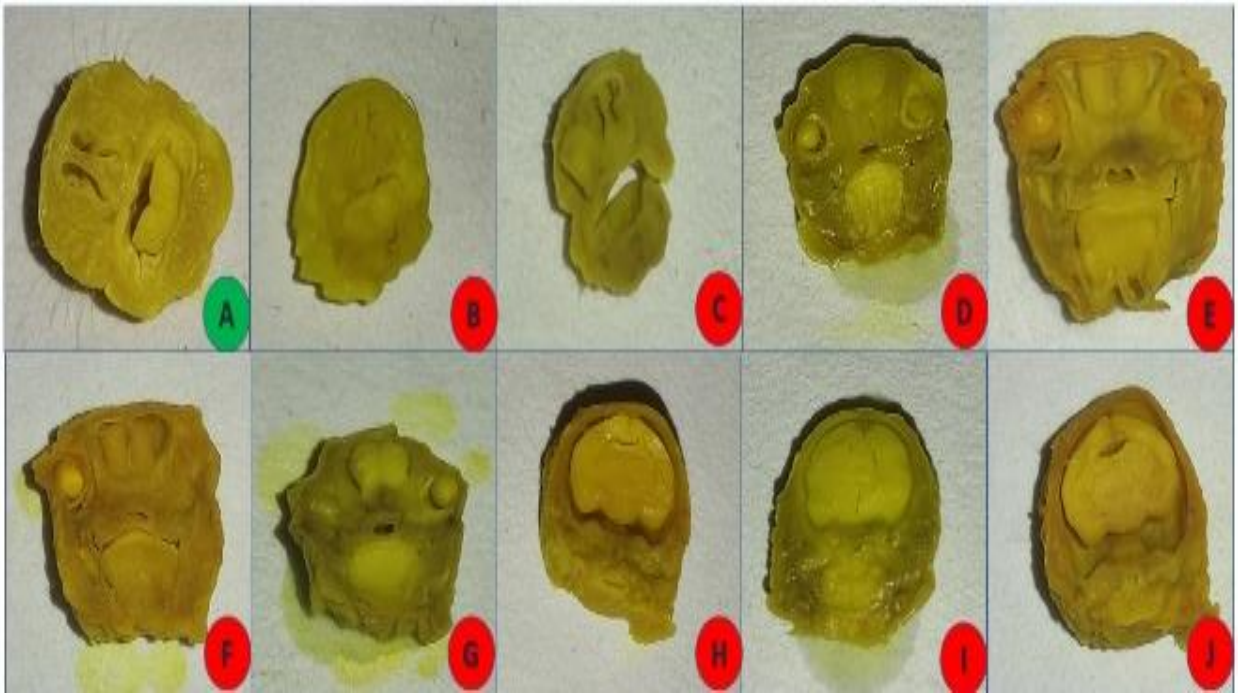


Fig. 6. Cross sections showing normal naris A, but photos from (b-c) showing nostril anomalies as narrow nostril (B), slight congestion of nostril (C). cross section through eye showing normal section D, the abnormalities have been shown (E-G) while E showed slight congestion (arrow), right Uni-lateral Anophthalmia (F), left Uni-lateral Anophthalmia (G). the last section showing normal brain in cranial cavity (H), (I-J) represented the abnormalities where I, dilatation of central and lateral ventricles, microencephaly (J).

The visceral abnormalities from figure (7) in the cross sections of the chest revealed (Slight pulmonary fibrosis, cardiomegaly, heart engorged with blood, pulmonary hypotrophy and intrathoracic haemorrhage as shown in ACK group, while BHA feoti group showed thickening of ventricular wall and interventricular septum and heart engorged with blood. The minor changes were recorded in the MSG group as congestive heart and intrathoracic hemorrhage.

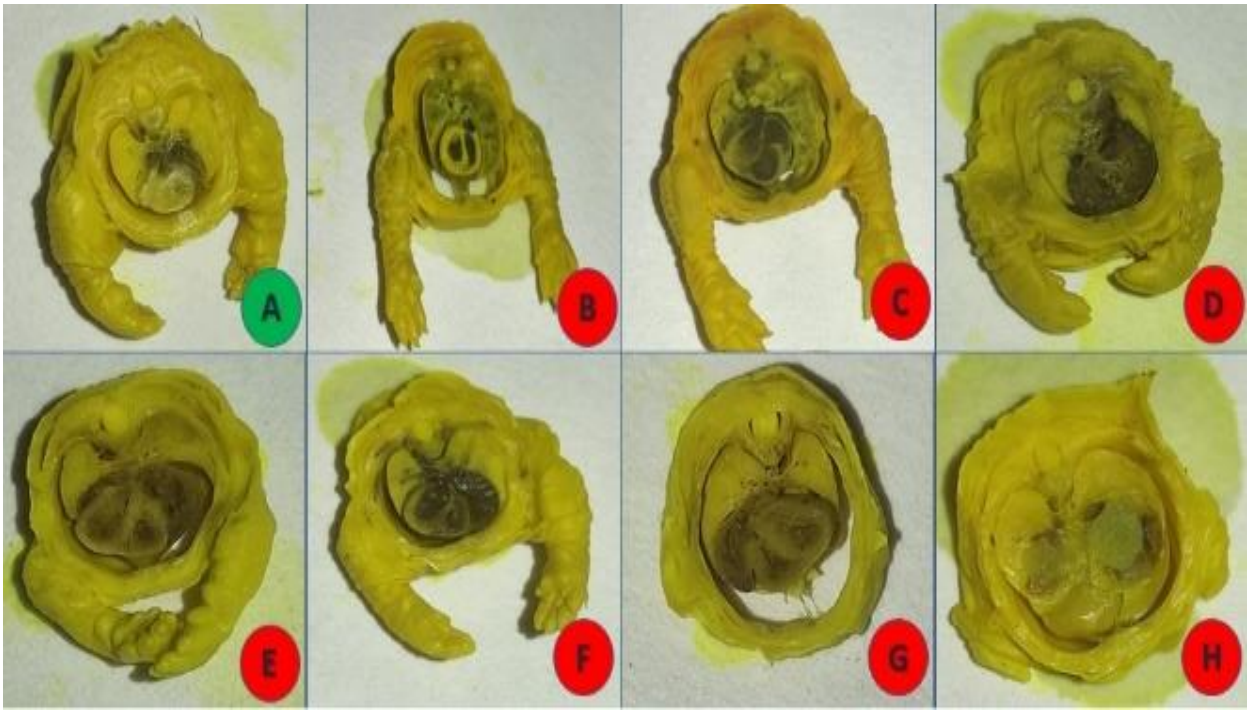


Fig. 7. Cross sections showing normal chest picture (heart and lung) A, but photos from (b-h) showing chest anomalies as pulmonary fibrosis (B), Slight pulmonary fibrosis and cardiomegaly and heart engorged with blood (C), pulmonary hypotrophy and intrathoracic hemorrhage (D), thickening of ventricular wall and interventricular septum and heart engorged with blood (E, G), congestive heart (F), intrathoracic hemorrhage (H).

The cross-section through abdomen and pelvis showed abnormal vacuoles in hepatic tissue with slight hepatomegaly with Unilateral renal agenesis(K), perihepatic haemorrhage (hemoperitoneum) in variant degrees extended from the lowest to highest degrees as order (ACK< BHA<MSG). Fig. (8-9)

On the other hand, the hepatotoxicity was recorded with variant degrees extending to hepatic fibrosis (ACK<BHA) and significant hepatomegaly (MSG). In the investigation of intestinal and pelvis images of feoti of the tested groups (MSG, ACK and BHA) , the abnormalities were seen as internal haemorrhage with variant degrees and narrow small intestine the later shown in the MSG group only. (Fig. 8).

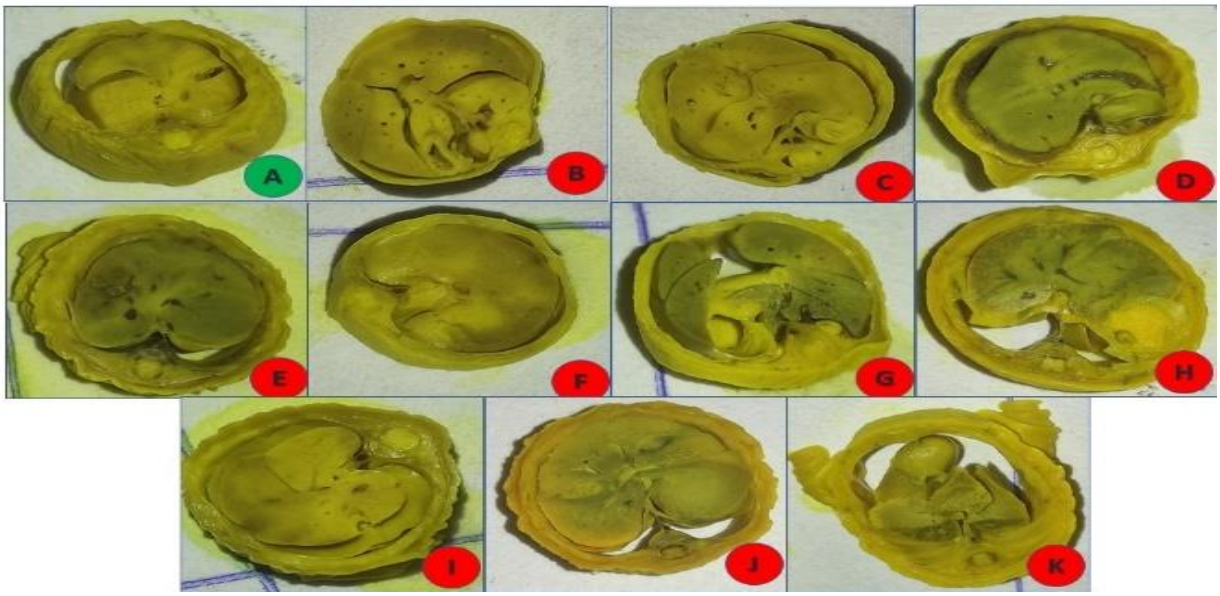
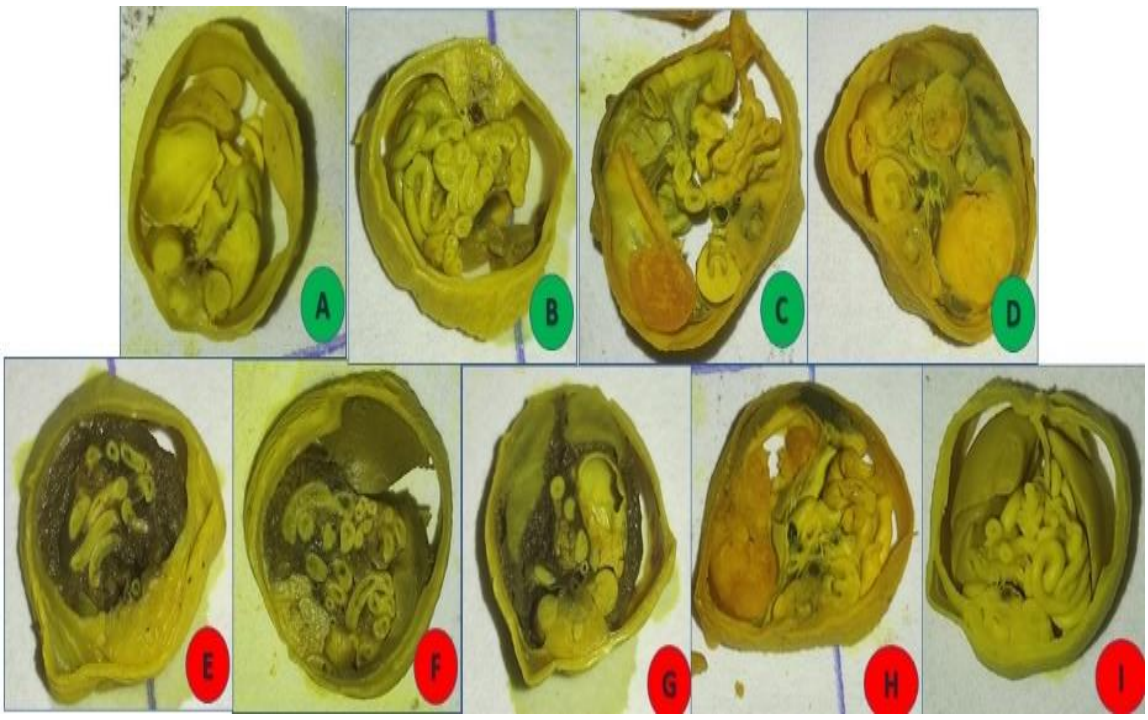


Fig. 8. Cross sections showing normal control liver (A), while (B-K) showing abnormalities as B-C: abnormal vacuoles in hepatic tissue with slight hepatomegaly with Unilateral renal agenesis(C), perihepatic hemorrhage (hemoperitoneum) D, photos (E-K) showing hepatotoxicity with variant degrees extending to hepatic fibrosis (H) and significant hepatomegaly (J).

Fig.9. Cross sections (A-I) showing intestinal and pelvis image revealing (A-D) normal sections , while the



abnormalities were seen in (E-I) where (E-H) showed internal hemorrhage with variant degrees and (I) showed narrow small intestine.

Table (2): Showing the visceral anomalies rate in foeti obtained from dam rats orally administered MSG, ACK, BHA and BHT from 5th day through 19th day of pregnancy.

	Control		MSG		ACK		BHA		BHT	
No. of examined fetuses	45		37		35		27		21	
	No	%	No	%	No	%	No	%	No	%
Microencephaly	0	0.00	1	2.70	2	5.71	3	11.11	0	0.00
Dilatation of central and lateral ventricle	0	0.00	3	8.11	0	0.00	0	0.00	0	0.00
Anophthalmia	0	0.00	5	13.51	1	2.86	3	11.11	0	0.00
Nostril anomalies	0	0.00	4	10.81	3	4.29	1	3.70	0	0.00
Chest anomalies	2	4.44	2	5.41	4	11.43	1	3.70	1	4.76
Intrathoracic hemorrhage	1	2.22	2	5.41	3	8.57	1	3.70	1	4.76
Cardiac anomalies	0	0.00	3	8.11	1	2.86	4	14.81	1	4.76
Hepatic anomalies	0	0.00	5	13.51	2	5.71	5	18.52	0	0.00
Renal anomalies	0	0.00	6	16.22	2	5.71	3	11.11	0	0.00
Internal hemorrhage	0	0.00	7	18.92	2	5.71	4	14.81	0	0.00
Intestinal anomalies	0	0.00	3	8.11	0	0.00	0	0.00	0	0.00

3.4. Skeletal examination:

Alizarin red stained skeletons of rat foeti obtained from both treated and control dams were macroscopically examined by the naked eye and a magnifying hand lens for detecting possible skeletal malformations.

The encountered malformations in the cranium bone, sternebrae, coccygeal vertebrae and digits were depicted in figures (10) and table (3). The investigators recorded these malformations as incomplete tarsal bone ossification and absence of coccygeal vertebrae was recorded in ACK group. The graph C shows variation of size, the lowest size was reported with BHA, MSG, ACK respectively. Also, MSG group showed trisomy 13 syndrome (an increase number of ribs to 13 couples of ribs) and incomplete ossification of cranial bone

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Table (2): Showing the Skeletal anomalies rate in foeti obtained from dam rats orally administered MSG, ACK, BHA and BHT from 5th day through 19th day of pregnancy.

	Control		**A**		**B**		**C**		**D**	
No. of examined fetuses	23		19		17		14		11	
	No	%	No	%	No	%	No	%	No	%
skull	1	4.35	2	10.53	2	11.76	1	7.14	1	9.09
ribs	1	4.35	3	15.79	1	5.88	1	7.14	1	9.09
xyphoid	0	0.0	2	10.53	1	5.88	1	7.14	0	0.00
Sternbrae	1	4.35	3	15.79	0	0.00	0	0.00	0	0.00
fore digit	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
limbs and metacarpal	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
hind digit	0	0.0	1	5.26	4	23.52	1	7.14	0	0.00
limbs and metatarsal	0	0.0	2	10.53	5	29.40	2	14.29	0	0.00
coccygeal vertebrae	0	0.0	0	0.0	5	29.40	1	7.14	0	0.00

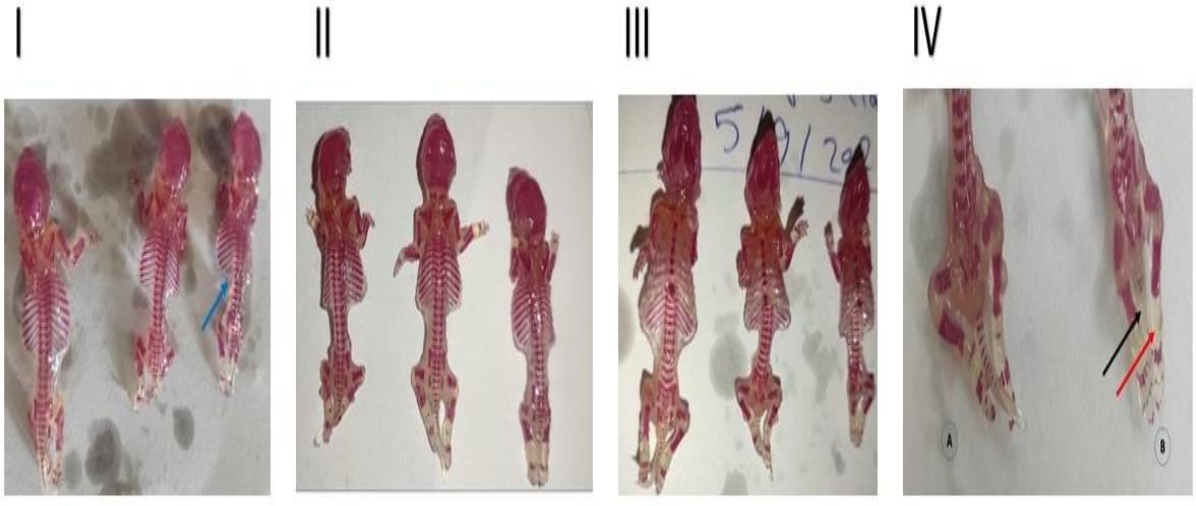

Fig. (10). (A) showing normal digit left while arrow refer to incomplete tarsal bone ossification right, but photos from (b) showing normal skeleton of tail left and absence of coccygeal vertebrae right (arrow). The graph C showing variation of size, D graph showing trisomy 13 syndrome (increase numbers of ribs to 13 couples of ribs) arrow . E incomplete ossification of cranial bone

2. 4. Discussion:

Pregnancy is a sensitive period considered to be a major life event and characterized by several physical and psychological changes such as eating behaviors, habits, and eating disorders related to pregnancy [8,9]. Some pregnant women tend to tasty and food with flavor than other ordinary food. The food additives are the best choice to encourage for consuming unpalatable food. Nausea and vomiting are the primary pregnancy related complications in 50% of women [10]. These changes lead most women to change their tastes and food preference during pregnancy develop an increased preference the tendency toward flavoring agent containing food such as spicy or sweety diet [11–13]. The safety of some food additives has been studied but their impact on fetal development has not been resolved yet.

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are synthetic phenolic antioxidants that are widely used as food preservatives. BHA and BHT are indicated in many documents as not carcinogenic. Many reports have affirmed the antioxidants activities of these food additives and also aimed to their anti-carcinogenic properties." They have permitted at low concentrations in food, became a part of the human diet for many years without evidence of adverse effects [14].

In spite of, the mentioned above about safety studies of BHA and BHT. Other documents mentioned that BHA has been proven to produce neoplasms of the non-glandular squamous cell portion of the stomach in rats, mice and hamsters [15] also, BHT has been reported to produce hepatocellular tumors in rats [16] and mice [17].

The results of current study indicated the toxicity of the food additives MSG and ACK and the extremely toxic effect of the BHT and BHA. But these deleterious effects need further studies to be affirmed. The current study showed clear teratogenic impact of these synthetic food additives on fetal, notably related to BHA & BHT. These incidence harmful teratogenic effect on the number of corpora luteae, implantations, number of dead fetuses and ones with gross malformations, as well as their weight which was affected extreme deleteriously.

In addition, the red patches with varying sizes were observed externally on different parts of the foetal body especially on the chest and fore limb and hind limbs; These patches were more prominent in feoti of dams that received ACK than MSG. Red patches were seen externally on different parts of the foetal body especially in the head and back of the foeti obtained from dams which gain were more prominent in the feoti of dams which received ACK than MSG.

This red petechial haemorrhages may be related to MSG and ACK's ability to decrease platelets [18,19]. These authors have reported that the administration of MSG has significant effect on platelets count that also explains intrathoracic bleeding and abdominal and pelvic internal hemorrhage.

Our result revealed neural abnormalities including the dilatation of central and lateral ventricles that were recorded in MSG. in the same way, the previous work has reported that MSG is neurotoxic, capable of producing degeneration of population of neurons, accompanied by pathological conditions, such as stroke, epilepsy, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis [32]. Moreover, our result reported presence of microencephaly changes in ACK treated dams. The previous published work focused on neurotoxic changes related to chronic ingestion of ACK in mice (at doses within the expected exposure range for humans ingesting ACK) that resulted in impairments in learning and memory in tasks localizable to the hippocampus.(5).

Also, the current visceral examination showed foetal abnormalities in naris as nostril anomalies particularly in the group of dams which ingested MSG. while the visceral examination of the chest revealed slight pulmonary fibrosis, cardiomegaly, heart engorged with blood, pulmonary hypotrophy and intrathoracic hemorrhage in the group of dams which took by gavage of ACK. in addition to, BHA group showed thickening of feotal ventricular wall and interventricular septum and heart engorged with blood. Previous similar studies have shown that dams which exposed to BHA could act as a weak endocrine disruptor, adversely affecting on the reproductive function, maturation, and hormonal secretion at every stage of fetus development[20].

The fetal hepatotoxicity that was registered had various degrees extending to hepatic fibrosis in the groups of dams were orally administered ACK and BHA, also significant fetal hepatomegaly in the group of dams that ingested MSG. This is in line with reports that BHT produce hepatocellular tumours in rats [16] and mice [17]. In the intestinal and pelvis image in feoti of dams treated with (MSG, ACK and BHA), the abnormalities were seen as internal hemorrhage with differing degrees and narrow small intestine. The later was observed in the experimental group of dams which exposed to MSG only. Many previously published research established that the foetal hepatocyte are most

susceptible cells to harmful exposures. The MSG intake was a risk factor for major birth-related liver diseases [23]. As it can pass through the placenta causing a fetal defects [24].

Moreover, the skeletal examination revealed fetal malformations as incomplete tarsal bone ossification and absence of coccygeal vertebrae that reported in the ACK group. Also, MSG group showed trisomy 13 syndrome (increase numbers of ribs to 13 couples of ribs) and incomplete ossification of cranial bone. Calcification malformations may be regarded as disruption of fetal development in ACK and MSG treated dams. Also, ACK is considered the worst artificial sweetener approved by the FDA[25]. In the FDA final report 59 FR 61538 on ACK methylene chloride is mentioned as a compound formed in the initial manufacturing step and is also known as a toxic carcinogen [26].

BHA and BHT have been suspected of inducing health risks such as child hyperactivity, damage to the lungs, liver, and kidneys, and most importantly, cancer [27]. Studies have shown that BHA and BHT can be carcinogenic at high doses and a concentration greater than 3000 ppm, it has been known to induce forestomach squamous cell carcinomas in rodents while BHT at 250 mg/kg/day increases spontaneous neoplasms and tumor-promoting activity[28]. Various mechanism studies suggested that BHT toxicity is related to an electrophillic metabolite [29].

Finally, BHT and BHA are highly volatile and instable at elevated temperature. The strict legislation on the use of synthetic food additives and consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants. Most of these natural antioxidants come from fruits, vegetables, spices, grains, and herbs [30].

5. Conclusion:

This work threw the light on the impact of the using the synthetic food additives during pregnancy and toxological impacts that shown either in visceral or skeletal as well as morphological examination. Therefore, it is advised that consumers should avoid consuming food additives during this period of gestation.

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Ethics approval and consent to participate:

Rats experiment and the study protocol was Informed consent was obtained from approved by the Ethics Committee of the National Research Centre.

Competing interests

The authors confirm that they have no competing interests and the authors have no conflicts of interest to report.

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