Egyptian Journal of Zoology (EJZ) p-ISSN: 1110-6344
Publisher: The Zoological Society of A. R. Egypt e-ISSN: 2682-3160
DOI: 10.21608/ejz.2024.248255.1110

RESEARCH ARTICLE

THE EFFECTS OF ASPARTAME ON SOME FETAL TISSUES OF FEMALE ALBINO RATS

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Article History:
Received: 12 November 2023
Revised: 9 February 2024
Accepted: 12 February 2024

Published Online:
21 February 2024

Keywords:
Aspartame
Kidneys
Liver
Rat fetuses
Skeletal development

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ABSTRACT

Aspartame (ASP), a low-calorie artificial sweetener, is a methyl ester of a dipeptide. It can cause serious health problems as its metabolites can be toxic to many organs. The current study aimed to evaluate the toxicity of ASP on the rats' fetal tissues. Thirty-five pregnant female albino rats (*Rattus norvegicus domestica*) were randomly allotted into 5 groups (n=7): the control group received orally distilled water; groups "1 and 2" received orally/daily 250 and 500 mg ASP/kg body weight (dissolved in distilled water), respectively, on the 1^{st} – 7^{th} days of pregnancy; and groups "3 and 4" received orally/daily 250 and 500 mg ASP/kg body weight (dissolved in distilled water), respectively, on the 8^{th} – 20^{th} days of pregnancy. The fetal body weight and length decreased significantly in groups "2, 3, and 4" only. However, the mortality rate increased especially in groups "2 and 4", which received the high doses of ASP. Major skeletal abnormalities seen in the fetuses included inadequate ossification of the skull, vertebrae, and pectoral and pelvic girdles with their fore and hind limbs in all groups. The ASP changed the usual architecture of the hepatic parenchyma tissue in all treated groups. The kidneys of ASP-treated groups demonstrated histopathological alterations. The high doses in the first and the second periods of pregnancy had the most chronic effect than the low doses.

INTRODUCTION

Aspartame (ASP) has been classified as potentially carcinogenic to humans (group 2B: means possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC) in June 2023[1,2]. ASP is a low-calorie artificial sweetener ingested by over 200 million people worldwide[3]. It is used in many soft drinks, desserts, jams, canned fruit, chewing gum, candies, cosmetic products, vitamins, and medications to replace sucrose and minimize calories[4,5]. It decreases rates of obesity and is used for diabetics with high sugar levels in their blood. Its popularity as a weight-loss aid is growing in health-conscious societies[6]. The ASP ($C_{14}H_{18}N_2O_5$) is a methyl ester of a dipeptide consisting of two amino acids, aspartic acid, and phenylalanine[7-10]. When consumed, the ASP molecule contains around 50% phenylalanine, 40% aspartic acid, and 10% methanol[11].

The toxicity of artificial sweeteners has been investigated in several experimental animal studies. Artificial sweeteners have been linked by some researchers to health issues like hepatotoxicity and malignan-

cies[12]. A huge controversy concerning artificial sweeteners still exists. The ASP has been the most contentious of all artificial sweeteners due to its probable toxicity and carcinogenicity[13], even at the approved daily intake in humans[1,2]. Consuming ASP over an extended period causes a gradual concentration of formaldehyde compounds, which are the cause of DNA alterations and changes in protein function[6,14,15]. These consequences include cell death, autoimmunity, and malignant transformation. Additionally, some studies have revealed that excessive ASP intake might pass the placenta and accumulate in fetal tissue, perhaps leading to overweight, premature birth, or other abnormalities after a lengthy period of exposure[16,17]. Its metabolites can cross the placenta and cause changes in its structure[16,17]. ASP consumption causes hepatocellular damage and changes in the liver, hepatotoxicity, and cancer in adult albino rats especially due to the most chronic metabolism of ASP into methanol[18,19]. In addition, ASP's impact on the kidney when administered by a mother showed the chronic effect of ASP on the fetal kidney structure[20]. Therefore, the present study aimed to assess how ASP affected fetal body weight and length, fetal mortality, the skeletal structure, and the histological structure of fetuses' vital organs such as liver and kidneys on the 20th day of pregnancy.

MATERIAL AND METHODS

Chemicals

The ASP (Molecular weight: 294.307 g/mol, purity: 98%, and catalogue number: 22874) was received from Sigma Pharmaceutical Industries (Cairo, Egypt). The median lethal dose (LD_{50}) of aspartame in mice and rats is >5 g/kg body weight[21]. The ASP doses used in the present study were 250 and 500 mg ASP/kg body weight dissolved in distilled water, which equals 1/20 and 1/10 of LD_{50}, respectively. All other used stains and laboratory chemicals such as alizarin red S, alcian blue, Harris hematoxylin and eosin (H&E), glycerin, 95% ethyl alcohol, and 10% neutral formalin solution were purchased from Sigma Pharmaceutical Industries.

Animals

Pure-strain virgin female and male albino rats (*Rattus norvegicus domestica*, weight: 200 ± 20 g, ages: 8-12 weeks) were picked up from Theodor Bilharz Research Institute (Giza, Egypt). They were kept in clean ventilated cages with unlimited food and water under regulated conditions of temperature (25°C), humidity (50%), and a 12-hour light/dark cycle. Female and male rats were mated with a ratio of 3:1 after a week of acclimatization to the laboratory setting. The day "1" of gestation was marked by identifying vaginal plug presence.

Experimental design

The pregnant rats were arranged into five groups (7 rats each): the control group (C) received orally distilled water; group "1" (G1) and G2 received orally/daily 250 and 500 mg ASP/kg body weight dissolved in distilled water, respectively, on the 1st – 7th days of pregnancy; and G3 and G4 received orally/daily 250 and 500 mg ASP/kg body weight dissolved in distilled water, respectively, on the 8th – 20th days of pregnancy.

External morphological and mortality analysis

Cesarean sections are the birth of fetuses *via* a uterine incision (hysterotomy) and an open abdominal incision (laparotomy), which were used to remove the uterus of the pregnant rats on the 20th day of pregnancy. In each horn, the number of fetal swellings, survival, and mortality, as well as early and late resorptions, were counted. Early and late resorptions were distinguished by their size. For morphological examinations, the fetuses were closely examined for morphological anomalies, and photographs were obtained of the control fetuses and treated fetuses with high and low dosages of ASP. The weight and length of the living and deceased fetuses were measured, and their

morphology was examined for any external deformities such as red patches, swellings, and morphologically prominent deformities were photographed for detailed evaluation.

Insights on the endoskeleton

Fetuses were immersed in 95% ethyl alcohol for 4 days, and then acetone was added for one day to eliminate any remaining fat. Both alizarin red S and alcian blue staining were used to detect cartilage and bone in fetuses. The skeleton of each fetus was stained using 10 mL of staining solution, which was made up of 17 volumes of 70% ethanol, 0.1% of 95% ethanol, and an equivalent volume of 0.3% filtered alcian blue in 70% ethanol, along with 1.0 mL of acetic acid was added to the mixture. After a water wash, the stained fetus was put in an increasing succession of glycerol and 1% aqueous KOH solution before being stored in 100% glycerin[22].

Histopathological examination

To quickly remove the liver and kidney tissues, a dorsal midline incision was done to get access to the renal bed. The tissue samples were stored in a 10% neutral formalin solution. Sections (5 µm) were stained with H&E for regular histopathological investigation by light microscope[23].

Statistical analysis

The Kruskal-Wallis H test was used for statistical analysis[24] to detect the significant differences in the independent variable of fetal weights and lengths between the various groups, proceeded by the *post-hoc* Dunn's test[25]. Pearson's Chi-square test[26,27] with simulated *P*-value (based on 2000 repeats) indicated *P*-values for mortality percent.

RESULTS

Effect of ASP on the external morphology of rats' fetuses

Morphology on the 20th day showed that ASP causes developmental delays in fetuses, which leads to more or less a decrease in body weight and length (Figure 1A-C). The body weights of the fetuses were significantly decreased in ASP-treated groups, except G1, compared with the control group (Figure 1A). The Kruskal-Wallis H test indicated a significant difference between control and G2-4 in a dependent variable, $\chi2(4) = 29.93$, $P<0.001$, with a mean rank score of 31.93 for C, 23.57 for G1, 10.14 for G2, 19 for G3, and 5.36 for G4. The mean rankings of the following pairs were significantly different, depending on the *post-hoc* Dunn's test with a Bonferroni corrected alpha of $P<0.005$: C-G2, C-G3, and C-G4.

The body lengths of the fetuses were significantly decreased in ASP-treated groups compared with the control group (Figure 1B). The Kruskal-Wallis H test indicated that there was a significant difference in the dependent variable between the different groups, $\chi2(4) = 22$, $P<0.001$, with a mean rank score of 31.29 for C, 16.86 for G1, 16.21 for G2, 6.14 for G3, 19.5 for G4. The *post-hoc* Dunn's test using a Bonferroni corrected alpha of $P<0.005$ indicated that the mean rank of the following pair is significantly different: C-G1, C-G2, C-G3, C-G4. G2 and G4 were the most affected group. The morphology of ASP-administered fetuses revealed the presence of a pattern of convexity body, aberrant tail, and production of superficial hematomas as examples of congenital abnormalities in parts of the body. On the other hand, there were red patches on the body of the fetuses of G2 and G4 (Figure 1C).

Effect of ASP on the fetuses' mortality and the shape of rats' uteri

Administration of ASP raised the ratio of the lifelessness of embryos in comparison with the control group (Figure 2A). Pairwise comparison using Pearson's Chi-squared test with simulated *P*-value (based on 2000 replicates) showing *P*-value test of equal proportion resulted in $P<0.001$, X-squared = 18.449.

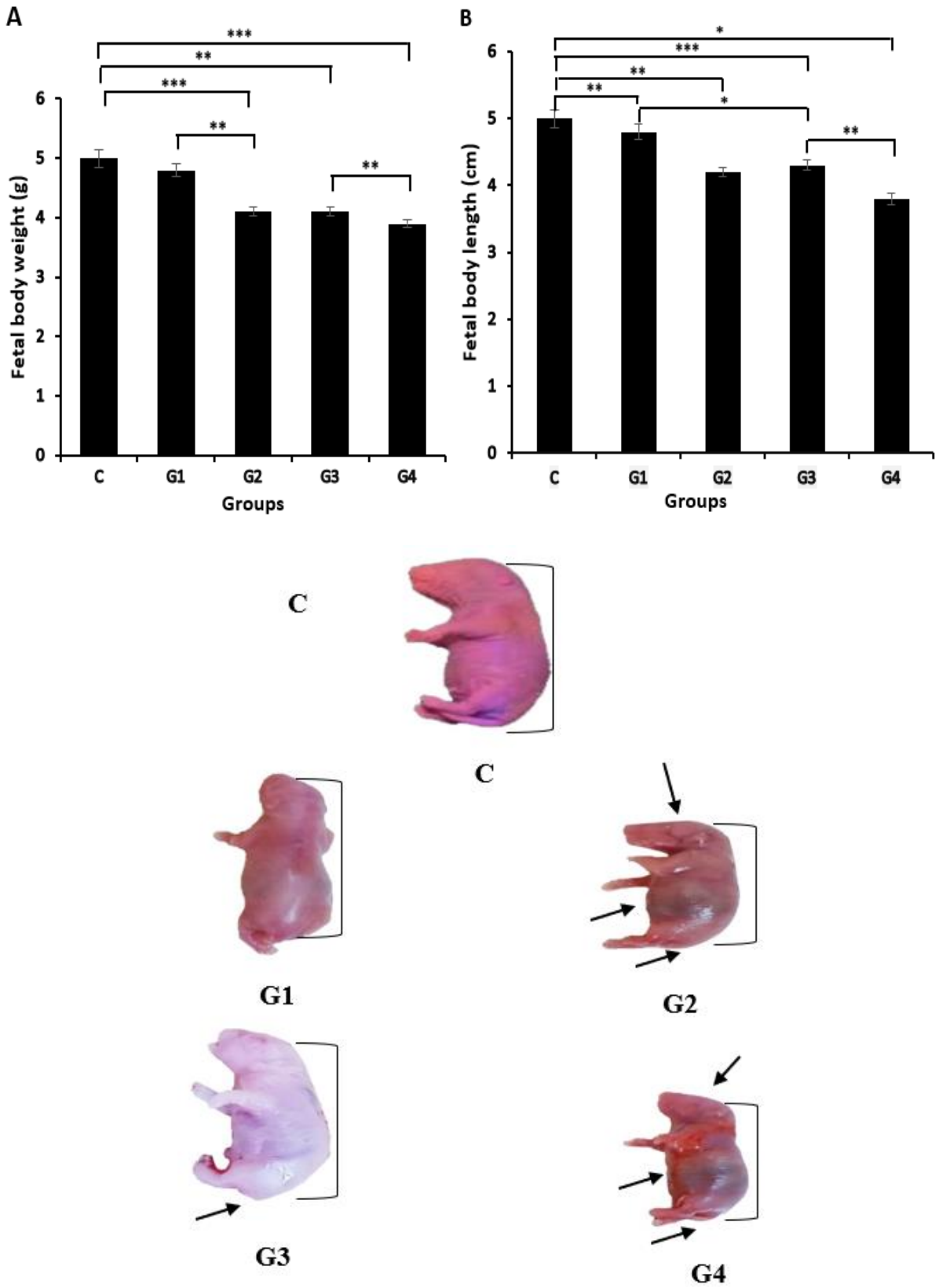

Figure 1: The effect of aspartame on the morphology of fetuses. **(A)** The fetal body weight. **(B)** The fetal body length. Data are expressed as mean ± standard error. ***P*<0.05, ****P*<0.01, and *****P*<0.001. **(C)** Photographs showing the fetal external morphology at more or less 3 weeks of age. The arrows referred to fetuses' length differences, red patches on the body of the fetuses of G2 and G4, and aberrant tail G2-4.

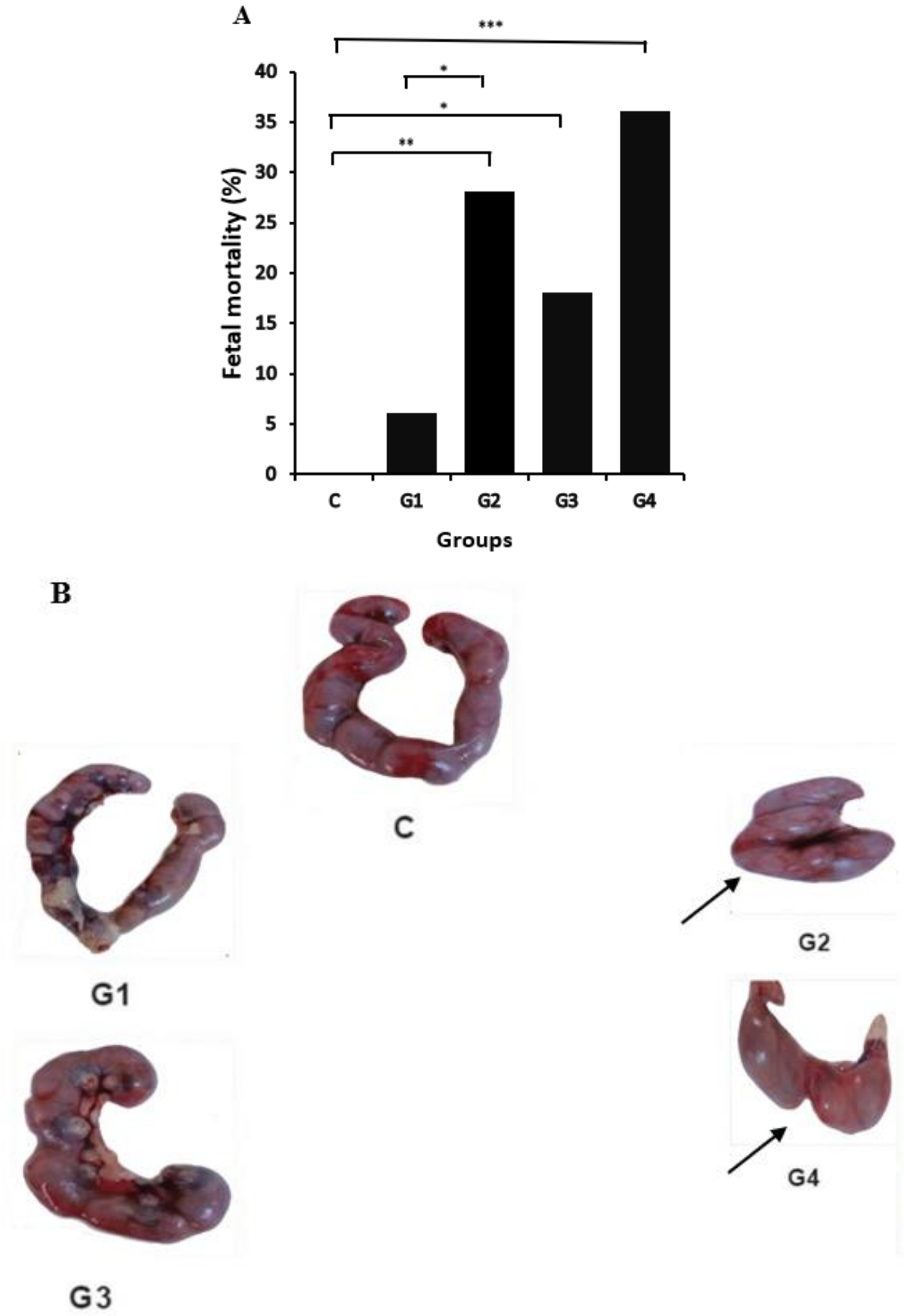

Figure 2: The effect of aspartame on the fetal mortality **(A)** and the shape of uteri (**B)**. *****P<0.05, **P<0.01, and *** P<0.001. The arrows referred to resorbed embryos.

The control group had a big uterus that naturally encapsulated huge embryos. The uterus of G4 was quite tiny, with several resorbed embryos. The G2 had a smaller number of implantation sites, with a few resorbed embryos. The uteri of G1 and G3, who received lower doses of ASP, were larger and contained more live embryos than those of G2 and G4 (Figure 2B).

Effect of ASP on the endoskeleton of rats' fetuses

Albino rats' skeletal structure is divided into two parts: the axial and appendicular skeletons. The skull, spinal column, ribs, and sternum are all part of the former. The pectoral girdle and forelimbs, as well as the pelvic girdle and hind limbs, make up the latter (Figure 3A). The administration of varying doses of ASP to the mothers generated several undesirable consequences ranging from moderate to severe deformities in the 20th day fetuses according to the osteological abnormalities (Figure 3A).

The control albino rat fetuses' skulls were examined on the 20th day of gestation and revealed ossification of all its constituents (Figure 3B) and (Figure 4A). The skulls of fetuses that were given varying amounts of ASP by their mothers had malformations. Osteorability is more or less in the components of the skull (Figure 3B). There was a marked shortfall in the volume and length of the skull with several defects (Figures 3B and 4A). Modest ossification showed in the lower jaw bones in fetuses from all ASP-treated groups with a gradual lack of ossification, as indicated in Figure "4A", and a slight ossification of the administered group's dentition.

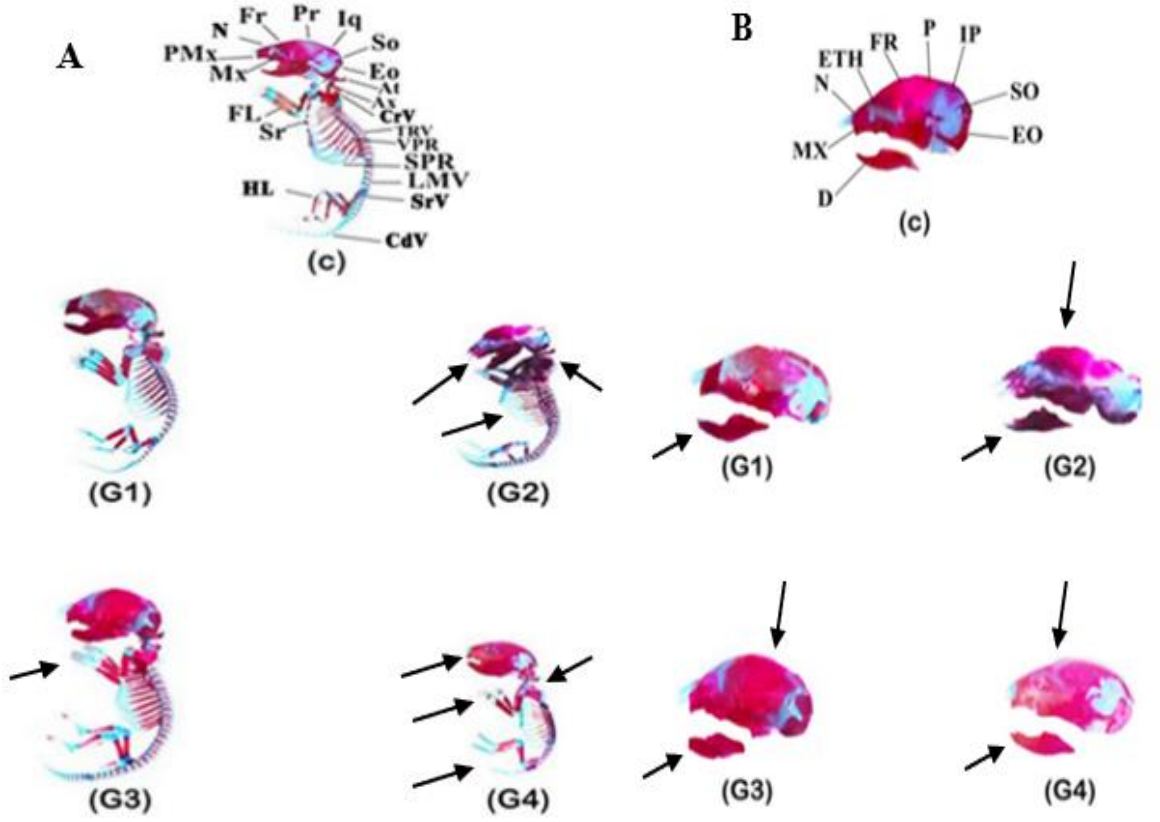

Figure 3: Lateral views on the 20th day of gestation of rat fetuses of the control and aspartame-treated groups. **(A)** Fetal skeletal system. The arrows referred that osteorability is less in the components in skeletal system. **(B)** Fetal skull. The arrows referred to abnormal ossification of the supra-occipital, parietal, interparietal, and zygomatic processes of the squamosal, tympanic bulla, squamosal, periotic, supra-occipital, palatine, and pterygoidmoid bones and lower jaw G2-4. The abbreviations cited in the figure were identified in Table "1.

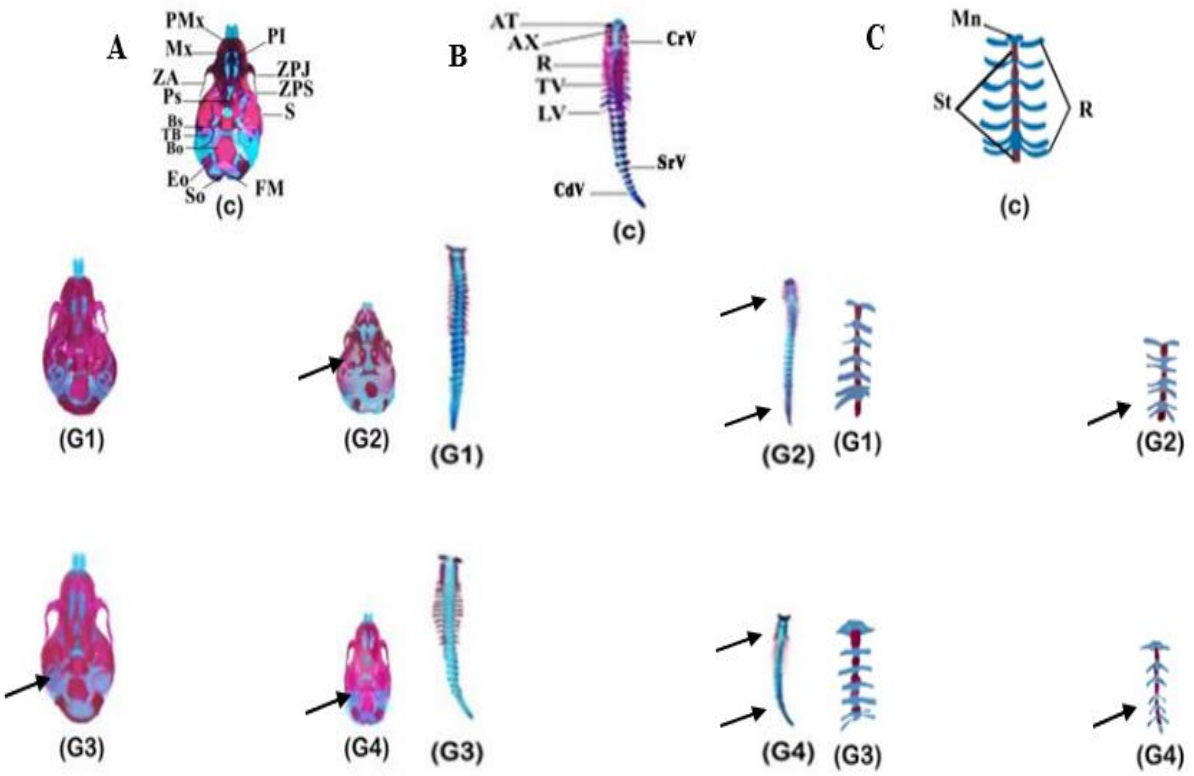

Figure 4: Ventral views on the 20th day of gestation of rats' fetuses of the control and aspartame-treated groups. **(A)** Fetal skull. The arrows referred abnormal ossification zygomatic processes of the squamosal, tympanic bulla, squamosal, periotic, and pterygoidmoid bones. (B) Fetal vertebral column. The arrows referred that ossification of the atlas and axis was significantly reduced. **(C)** Fetal sternum. The arrows referred exhibited more or fewer ossifications. The abbreviations cited in the figure were identified in Table "1".

The spinal column of control fetuses had well-ossified vertebrae, with seven cervical, twelve thoracics, seven lumber, four sacral, and ten caudal vertebrae. Examination of the spinal column in fetuses maternally given ASP at two doses showed that the ossification of the atlas and axis was significantly reduced; vertebrae had ossified to less degrees (Figure 4B). Each pair of ribs in the control group is divided into bony and cartilaginous sternal regions. Except for the last 3 pairs, the sterna portion of the ribs communicates with the sternum (Figure 4C). There were no differences in the number of ribs or their ossification in the whole treated groups. The control fetuses' sternum is made up of 6 rod-like ossified sternebrae organized in a straight line, the ending of which is the xiphisternum (Figure 4C). The sternebrae of fetuses given ASP by their mothers exhibited more or fewer ossifications than the control group (Figure 4C).

The pectoral girdle of the control fetuses on the 20th day of gestation exhibited a well-ossified scapula and clavicle stained with alizarin red S and a cartilaginous supra-scapula stained with alcian blue. The control fetuses have a well-developed fore limb, in addition to cartilaginous carpalia and meta-carpalia (Figure 5A). Comparing the ASP-treated groups with the control group, the pectoral girdle and fore limb of the fetuses obtained from maternally supplied ASP at different doses showed a diminution of size, length, and ossification level (Figure 5A). At high ASP doses, the pectoral girdle and forelimbs of all G2 and G4 fetuses revealed a severe absence of ossification (Figure 5A). Furthermore, the phalanges and metacarpalia of the forelimbs seemed to be arched in G2 and G4.

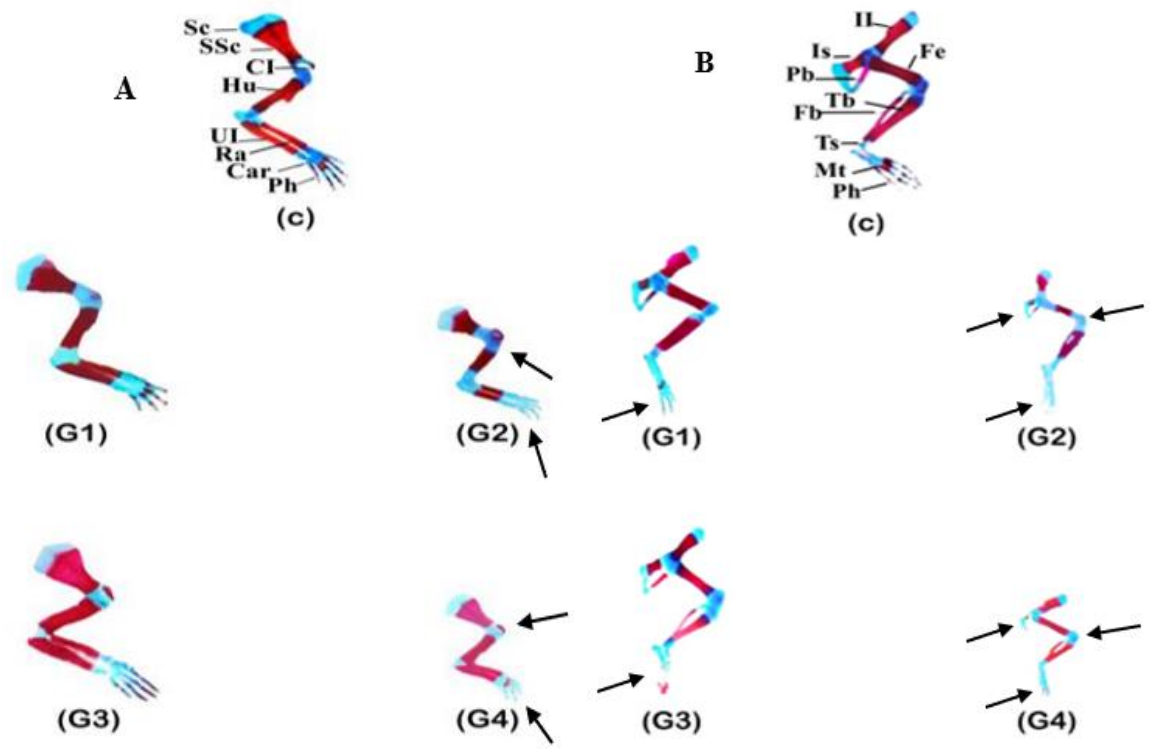

Figure 5: Lateral views on the 20th day of gestation of rat fetuses of the control and aspartame-treated groups. **(A)** Fetal pectoral girdle and fore limb. **(B)** Fetal pelvic girdle and hind limb. The arrows referred to a reduction in size, length, and ossification level. The abbreviations cited in the figure were identified in Table "1".

Table 1: Abbreviations cited in Figures "3-5".

Abbreviations	Bone name	Abbreviations	Bone name
At	Atlas	N	Nasal
Ax	Axis	Pb	Pubis
Bo	Basi-occipital	Ph	Phalanges
Bs	Basi-sphenoid	Pl	Palatine
Car	Carpales	PMx	Pre-maxilla
Cd.V	Caudal-vertebrae	Pr	Parietal
Cl	Clavicle	Ps	Pre-sphenoid
CP	Condyle-process	Ra	Radius
Cr. V	Cervical-vertebrae	R	Ribs
D	Dentery	S	Squamosal
ETH	Ethmoid	St	Sternebrae
Eo	Exo-occipital	Sc	Scapula
Fb	Fibula	So	Supra-occipital
Fe	Femer	Sr	Sternum
FL	Fore-limb	Sr.V	Sacral-vertebrae
FM	Formaen-magnum	SSc	Supra-scapula
Fr	Frontal	SPR	Sternal-portion-ribs
HL	Hind-limb	TB	Tympanic-bulla
Hu	Humerus	Tb	Tibia
Il	Ilium	TV	Thoracic-vertebrae
Ip	Interparietal	Ts	Tarsalia
Is	Ischium	Ul	Ulna
LV	Lumbar-vertebrae	VPR	Vertebral-portion-ribs
Mt	Metatarsalia	ZA	Zygomatic-arch
Mn	Manubrium	ZPJ	Zygomatic-process-jugal
Mx	Maxilla	ZPS	Zygomatic-process-squamosal

Control fetuses have a pelvic girdle consisting of 3 bones (ilium, ischium, and pubis). In nature, the pubic symphysis is cartilaginous. The femur, tibia, fibula, tarsals, metatarsals, and phalanges represent the hind limbs of control fetuses (Figure 5B). The pelvic girdle and hind limbs of ASP-maternally treated fetuses revealed significant shortening and trimming, as well as incomplete and absent ossifications of their components. Distortion was seen in the metacarpal bone and phalange cartilage drawings in fetuses from the administered group (Figure 5B).

Effect of ASP on fetuses' liver tissues

The liver sections of the control group of fetuses were examined, and they were composed of hepatocytes that split by radially distributed blood sinusoids. The blood sinusoids contain Kupffer cells (Figure 6A). The central vein is positioned in the center of tubules, and triades (portal spaces) containing hepatic artery branches. The ASP-maternally treated groups sections exhibited considerable distortion and disorganization, with the hepatic lobules entirely deteriorated (Figure 6B-E). Hepatocytes were so severely injured that they almost lost their distinctive appearance and had fatty modifications. Many necrotic cells had histopathological necrosis signs, including pyknosis, karyorrhexis, and karyolysis in the nucleus. Changes were also easily met within the individual hepatocyte (Figure 6B-E). This hepatocyte damage was evident in the form of severe cytoplasmic vacuolization in certain cells, and edema particularly in (Figure 6C) with spaces dilation, congestion of the central vein in (Figure 6D,E), and lymphatic infiltration (Figure 6B,C).

Effect of ASP on fetuses' kidneys tissues

The components of the kidneys are proximal, distal, and collecting tubules and besides that Bowman's capsule partly surrounds the glomerulus, which is formed of an inner or visceral and an exterior or partial layers (Figure 7A,B). Histopathological changes with light microscopic examination of the fetuses' kidneys were found in the proximal and distal convoluted tubules of ASP-treated groups. The renal tubular cells showed a foamy appearance with more or less injured nuclei (Figure 7C,D,F). They also showed advanced symptoms of necrosis. The glomeruli were fused (Figure 7C-F), as well as the tubules appeared as compact layers (Figure 7C,F). The glomerulonephritis of the renal tissue was observed; the glomeruli were shrunken, and the endothelial cells were swollen. The renal tubular cells showed early autolytic breakdown of cytoplasm (Figure 7C-F).

DISCUSSION

It was found that among the aspartame metabolites, methanol is the toxicant that causes systemic toxicity[6,19]. In rats, embryogenesis begins on day eight and continues for a total of fourteen days during the twenty-one-day gestation period. Throughout this stage, malformations of several developing organs can be specifically induced by exposure to a teratogenic substance. In the current study, ASP was given from the 1^{st} to 7^{th} days and from the 8^{th} to 20^{th} days of pregnancy, which fell inside the teratogenic window; and the data showed that ASP affected the rat fetuses. The maintenance of pregnancy and the growth/development of the fetus are typically compromised by drug- or chemical-induced placental malfunction or injury[28,29]. ASP intake during pregnancy may influence pregnant rats and their fetuses, as evidenced by decreased fetal and placental weights and shorter umbilical cords. It was observed that ASP-treated adult pregnant albino rats had a considerable impact on the normal structure and function of the placenta, which was most likely mediated by its methanol metabolite[16,30]. This may induce a significant impact on the ossification of the fetuses[16,30].

The present study showed a statistically significant reduction in the length of fetuses in ASP-treated groups in comparison with

the normal control group. G2 and G4 showed the highest reduction. Both ASP-treated groups have lower weights for viable fetuses than the control group. Studies with different high or low doses showed a highly significant decrease in fetal weight, placental weight reduction, and altered placental structure in both ASP-treated groups compared with the control group[12,17,29-31]. This decrease could indicate that the fetuses weren't receiving enough substrates, especially glucose, which could be attributed to a decrease in substrates in the blood of maternal rats that were given ASP. In contrast, it was reported that a low dose of ASP is safe for all individuals, including pregnant women and children, except those suffering from genetic disorders[6].

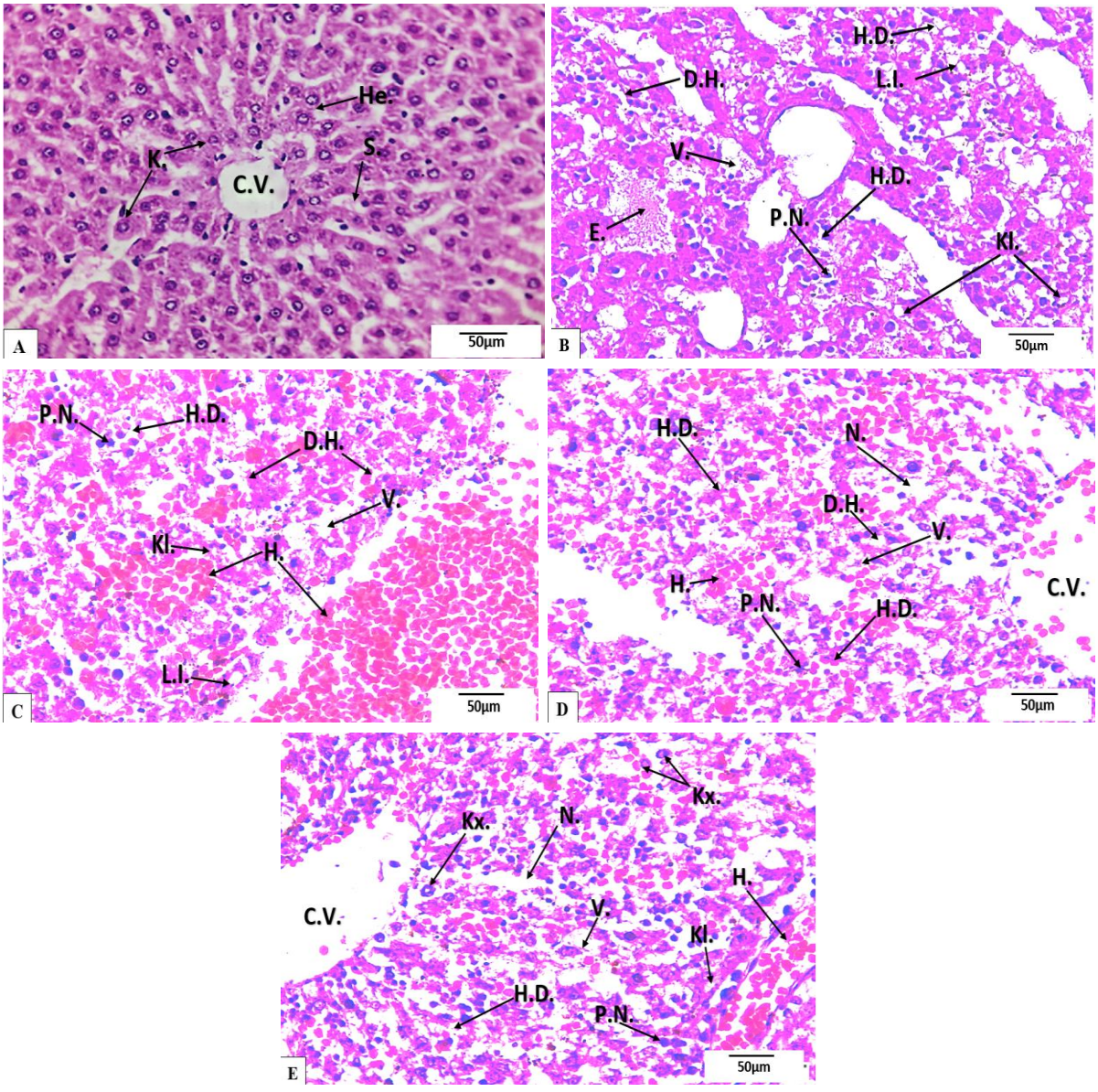

Figure 6: Photomicrograph of sections of the liver of rats' embryos on the 20th day of pregnancy of the control and aspartame (ASP)-treated groups. **(A)** The control group showing a central vein (C.V.), hepatocyte (He.), some of the Kupffer cells (K.); the hepatocytes appear in rows separated by hepatic sinusoids (S.). **(B-E)** ASP-treated groups (G1, G2, G3, and G4, respectively) showing a central vein (C.V.), degeneration of hepatocytes (D.H.), hemorrhage (H.), hydropic degeneration (H.D.), karyolysis (Kl.), karyorrhexis (Kx.), lymphatic infiltration (L.I), necrosis (N.), edema (E.), pyknotic nucleus (P.N.), and vacuoles (V.).

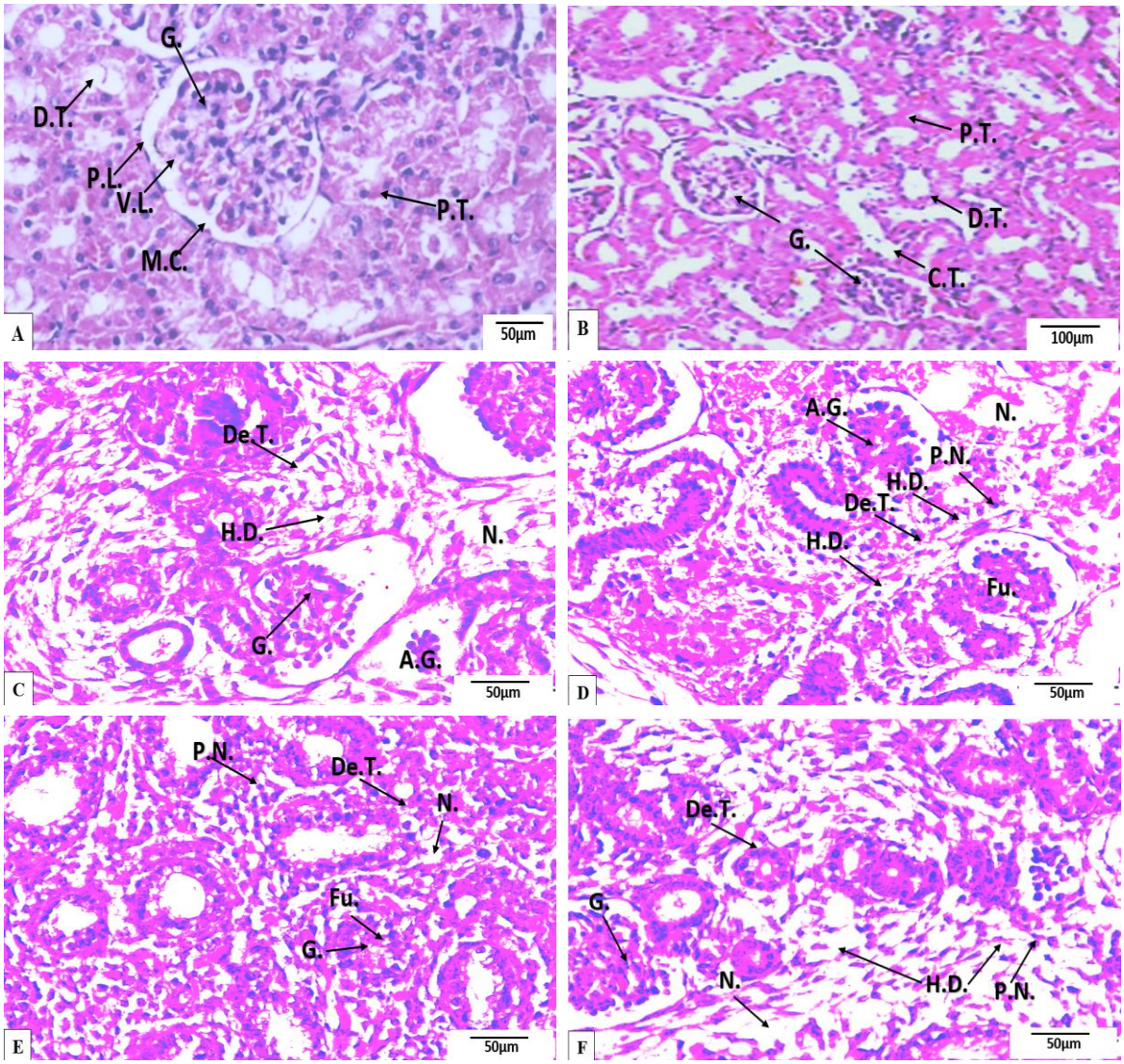

Figure 7: Photomicrograph of sections of the renal cortical area of rats' embryos on the 20th day of pregnancy of the control and aspartame (ASP)-treated groups. **(A,B)** The control group showing distal tubules (D.T.), renal corpuscles consisting of glomeruli (G.), Malpighian corpuscles (M.C.), parietal layer (P.L.), proximal tubules (P.T.), and visceral layer (V.L.). **(C-F)** ASP-treated groups (G1, G2, G3, and G4, respectively) showing atrophic glomeruli (A.G.), degenerated tubule (De.T.), fusion of the parietal and visceral layer of bowman`s capsule (Fu.), glomeruli (G.), hydropic degeneration (H.D.), necrosis (N.), and pyknotic nucleus (P.N.).

Compared with the control, a lower number of live fetuses was obtained from maternally administered ASP. The percentage of decline was higher in G2 and G4, and the number of dead fetuses was higher in G4 that received orally ASP high dose between the 8th -20th days of pregnancy. A very high decrease in the number of alive fetuses, as well as fetal body weights and lengths, was obvious in studies with different high doses of ASP[12,30,31].

In the present investigation, skeletal dysfunction and vascular abnormalities, including coagulated blood in various parts of the fetus's body, were seen in G2 and G4 that could be linked to ASP intake with higher doses. Different experiments in adults showed the effect of ASP on bone and skeletal system[32-34]. One of the possible

hazards of ASP in the blood and digestive system is the absorption of necessary cations that can influence the body's calcium levels; long-term usage of it may cause osteoporosis and has the potential to cause cancer especially in female adult rats even at relatively low daily dosages (20 mg/kg/day)[32,35]. Relative to the degree of the red color, which indicates a reduction in the osteogenesis process, skull bones showed ossification retardation, especially in G2 and G4. The histological findings on adult albino rats given daily high doses of ASP over 12 weeks indicated significant destructive alterations in the alveolar bone in the form of enlarging marrow gaps with thin interconnecting bone trabeculae[33]. In the current study, the G1 and G3 vertebrae showed no differences from the control. The fetuses in the second and fourth groups had acute skeletal abnormalities in the cervical, lumbar, sacral, and caudal vertebrae. The ribs and sternebrae were shorter in the treated groups than in the control one. The blue coloration of the cartilaginous part of the ribs was lower than the control, indicating a decrease in chondrification. Diet soft drinks can cause bone demineralization and reduced density in humans due to ASP and phosphoric acid, potentially increasing the risk of future bone fracture[34].

The fore girdle and the limbs of the fetuses revealed a reduction in size and intensity of ossification as compared with the control group, especially in "G2" and "G4". Additionally, several phalanges were damaged, especially in the second and fourth groups. The pubic symphysis, tarsals, and metatarsals all had different degrees of chondrification, especially G2 and G4. The pelvic girdle and hind limb components were reduced than in the control group. Osteoporosis, caused by ASP consumption, may result in more than 8.9 million fractures per year[32]. The present study supported that ASP has a toxic nature when ingested in large doses over time during pregnancy.

After absorption, ASP is broken down into three components in the digestive system: aspartic acid, phenylalanine, and methanol, as well as other breakdown products such as formaldehyde and formic acid. The effects of methanol on the liver and kidneys are the most severe because of its slow rate of metabolism[19,36]. In the current investigation, successive transverse slices of the treated fetus' liver revealed that the hepatic tissue had changed its usual structure, as most of the hepatic cords had lost their typical radial distribution around the central vein. Despite several toxicological investigations, the doses 500 mg/kg body weight and 1000 mg/kg body weight of ASP changed hepatic parenchyma tissue and hepatocellular injury[37]. In the G1, G2, G3, and G4 treated groups, the hepatocytes had mild to extensive degenerative alterations, respectively. The centrilobular zone of the hepatic lobule showed less of these degenerative changes than the peripheral and intermediate zones. ASP treatment of adult male rats for 6 and 12 weeks resulted in cloudy swelling of the hepatocytes[38], also it was noticed degenerative alterations in hepatocytes in the form of cellular edema and necrosis[39]. Iman[40] found a significant rise in lipid peroxidation (LPO) levels in the liver tissue of adult male rats after four and six weeks of oral ASP administration and saw ill-defined and ruptured cell borders. LPO is an autocatalytic process that starts by removing a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane and then proceeds to oxidatively destroy the membrane[40]. Othman and Bin-Jumah[41] discovered that ASP has a direct toxicological effect on hepatic cells as found in the current study. Because of limited proof of cancer in humans, especially hepatocellular carcinoma, the IARC categorized ASP as probably carcinogenic to humans (group 2B). Additionally, the classification was based on limited evidence regarding the possible mechanisms for causing cancer[1,13,42].

The kidneys of ASP-treated groups demonstrated substantial retardation in renal development, as well as destructive

histological alterations. Administration of ASP during the ninth, tenth, and eleventh days of pregnancy caused developmental changes during this time; kidney architecture and embryonic development were slowed, as evidenced by cell damage[20]. ASP treatment during pregnancy in different doses retarded fetal growth, enhanced cell volume, and reduced the density of cells in the fetal kidneys of treated rat[38]. The histological investigation revealed infection, necrosis, penetration, and aggressive inflammatory cells, as well as renal pellet shrinkage and renal tissue infection, as reported by the study by Othman and Bin-Jumah[41]. Azez *et al*.[43] discovered that long-term ASP ingestion caused histopathology changes in the rat kidney.

The current study concluded that the ASP consumption of pregnant rats at the high dose of 500 mg/kg body weight in the first and second periods of pregnancy affected their fetuses. It minimized body weight and length, increased the mortality rate, and caused various skeletal malformations, and histopathological alternations in the liver and kidneys of fetuses

COMPLIANCE WITH ETHICAL STANDARDS

The experimental design and animal handling of the current study were approved by the local ethics committee for animal use in research of the Faculty of Science, Benha University, Egypt (approval numbers: ZD/FSc/BU-IACUC/2022-9 and BUFS-REC-2023-63ZOO), which follow the National Institutes of Health's worldwide standards for using and caring for laboratory animals.

FUNDING SOURCE DISCLOSURE

This research received no funds.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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تأثير الأسبارتام على بعض أنسجة أجنة إناث الجِرذان المهقاء

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الأسبارتام – بديل صناعي للسكر منخفض السعرات الحرارية – هو إستر ميثيل ثنائي الببتيد. يمكن أن يسبب الأسبارتام مشاكل صحية خطيرة لأن نواتج الأيض يمكن أن تكون سامة للعديد من الأعضاء. هدفت الدراسة الحالية إلى تقييم سُمية الأسبارتام على أنسجة أجنة الجِرذان. تم توزيع 35 من إناث الجِرذان المهقاء الحوامل (*Rattus norvegicus domestica*) بشكل عشوائي إلى 5 مجموعات، كل مجموعة 7 جِرذان: تلقت المجموعة الضابطة الماء المقطر عن طريق الفم؛ وتلقت المجموعتين "1 و 2" عن طريق الفم/يوميًا 250 و 500 ملجم من الأسبارتام/كجم من وزن الجسم (مذابة في الماء المقطر)، على التوالي، في الأيام 1-7 من الحمل؛ وتلقت المجموعتينن "3 و 4" عن طريق الفم/يوميًا 250 و 500 ملجم من من الأسبارتام/كجم من وزن الجسم (مذابة في الماء المقطر)، على التوالي، وذلك من اليوم الثامن إلى اليوم العشرين من الحمل. انخفض وزن وطول جسم الجنين بشكل ملحوظ في المجموعات "2، 3، 4" فقط. إلا أن معدل الوفيات ارتفع خاصة في المجموعتين "2 و 4" اللتين أُعطيتا جرعات عالية من الأسبارتام. تضمنت التشوهات الهيكلية الرئيسية التي شوهدت في الأجنة عدم كفاية التعظم في الجمجمة والفقرات وحزام الحوض والصدر والأطراف الأمامية والخلفية في جميع المجموعات. كما تسبب الأسبارتام في تغيير البنية المعتادة للبرانشيما الكبدية في جميع المجموعات المعالجة. أظهرت الكُلى في المجموعات المعاملة بالأسبارتام تغيرات نسيجية مرضية. كان للجرعات العالية للأسبارتام في الفترتين الأولى والثانية من الحمل تأثير مزمن أكثر من الجرعات المنخفضة.