Research Article

Developmental Effects of L-aspartyl-L-Phenylalanine Methyl Ester During Early Pregnancy in Female Rats

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Abstract | Early gestation is a critical period of development, during which maternal exposure to artificial sweeteners may affect embryos. The present study examined the developmental and toxicological effects of L-aspartyl-L-phenylalanine methyl ester (L-APM) during early pregnancy in a rat model. Adult female rats (*Rattus norvegicus*, Wister strain) were randomly assigned to one of three groups: control, 240 mg/kg L-APM, or 300 mg/kg L-APM. Study protocol in dams, fetal mortality, markers of oxidative stress (malondialdehyde, protein carbonyls and glutathione peroxidase activity) and reproductive indices were assessed. In offspring, COMET assay, growth parameters, inflammatory cytokines, and histopathological changes were performed. There was a significant dose-dependent increase in oxidative stress markers and a decrease in antioxidant enzyme activity, in association with impaired reproductive indices and increased fetal mortality following maternal exposure to L-APM ($P < 0.001$). Dams that received exposure to both 240 and 300 mg/kg b.w. showed a significant increase in DNA fragmentation as well as significant reductions in body weight and length of their offspring, along with elevated pro-inflammatory cytokines (IL-1β and IL-6) and mild histopathological changes in selected organs ($P < 0.05$, $P < 0.001$, and $P < 0.0001$). In conclusion, the results indicate that L-APM-induced oxidative damage, reproductive and other disturbances in the dams, as well as genotoxic, inflammatory and growth-associated effects in the offspring, could likely be linked to their exposure at early gestation. These results suggest that gestation in early pregnancy might be a sensitive period of exposure to artificial sweeteners, which will require some caution in the interpretation and further investigation.

Keywords | Artificial sweeteners, DNA integrity, Embryonic growth, Genotoxicity, Inflammatory cytokines, Oxidative stress

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INTRODUCTION

L-aspartyl-L-phenylalanine methyl ester (L-APM) is a high-potency non-nutritive sweetener (NNS) widely used in low-calorie foods and beverages, exhibiting a sweetness intensity approximately 200 times greater than that of sucrose (Magnuson *et al*., 2016; Lebda *et al*., 2017; Abbas and Nurdianti, 2025). Although L-APM has been approved for human consumption, increasing evidence suggests that maternal exposure to NNSs during pregnancy may adversely influence embryonic and fetal development.

Early gestation represents a critical developmental window encompassing implantation, placental establishment, and early embryonic patterning. During this period, the developing embryo is highly susceptible to metabolic and

xenobiotic disturbances, which may result in persistent and long-term developmental consequences (Fowler *et al*., 2023; Iskandar *et al*., 2025). Maternal intake of NNSs during early pregnancy has been associated with impaired embryonic growth, disrupted organogenesis, and early developmental alterations in offspring (Ardalan *et al*., 2017; Rovelli and Longo, 2023).

Experimental animal studies have demonstrated that maternal exposure to L-APM can induce oxidative stress, reproductive dysfunction, and early neurodevelopmental alterations, including deficits in motor coordination, learning, and memory (Ali *et al*., 2020; Cai *et al*., 2021; Jones *et al*., 2023). Following ingestion, L-APM is metabolized into phenylalanine, aspartic acid, and methanol, metabolites capable of crossing the placental barrier. These metabolites may disrupt mitochondrial function, enhance the production of reactive oxygen species (ROS), and impair antioxidant defense mechanisms, thereby compromising cellular homeostasis during early embryogenesis (Abhilash *et al*., 2011; Lofthouse *et al*., 2016; He *et al*., 2023).

Elevated oxidative stress and metabolite accumulation have been shown to promote neuronal apoptosis, interfere with early neural circuit formation, and induce epigenetic modifications, including alterations in DNA methylation and histone acetylation. Such changes may adversely affect fetal growth, organogenesis, and neurobehavioral outcomes (Anbara *et al*., 2022; Griebsch *et al*., 2023; Yin *et al*., 2025). Moreover, *in vivo* studies have reported that maternal L-APM exposure alters oxidative stress biomarkers, inflammatory cytokine levels (e.g., IL-1β and IL-6), reproductive indices, and postnatal growth parameters, highlighting its potential teratogenic effects (Chen *et al*., 2022; Atalay *et al*., 2025).

Accordingly, this study aimed to comprehensively evaluate the impact of maternal L-APM exposure during early pregnancy on oxidative stress status, reproductive dysfunction, and genotoxicity in dams, as well as on growth performance, inflammatory responses, and early neurodevelopmental outcomes in the offspring.

MATERIALS AND METHODS

Ethical approval and animal euthanasia

The experimental procedures were approved by the Scientific Committee of the College of Veterinary Medicine, University of Baghdad (Animal Welfare Protocol PG/723, 31/03/2024). All experiments were therefore performed in accordance with ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals. At the conclusion of the experimental period (early-pregnancy) all pregnant female albino rats (*Rattus norvegicus,* Wister strain) were humanely anesthetized by rapid inhalation of chloroform into a small chamber until unconsciousness (and within 30 s) was achieved. When surgical anesthesia had been achieved (demonstrated by the absence of pedal reflexes), maternal euthanasia could be rapidly performed and maternal tissues and fetuses subsequently and immediately collected. They made all reasonable efforts to minimize both the pain and distress of the animals used and the number of animals used to gather adequate statistical validity.

Experimental design and dosing regimen

The study was conducted in accordance with FDA guidelines for reproductive toxicity, with specific modifications. Female albino rats were randomly divided into three groups (n= 10 per group): A control group and two treated groups. Animals in the treated groups (both female and male) received L-APM at doses of 240 and 300 mg/kg/day via oral gavage for a pre-gestational period of 60 consecutive days.

At the beginning of the experiment, the animals were 14–16 weeks old and weighed between 200–250 g. Rats were housed in special cages under standard laboratory conditions and allowed a two-week acclimatization period prior to treatment initiation. Housing conditions were maintained at a controlled temperature of 20–25 °C with a 12 h light/12 h dark cycle in an air-conditioned room. Wood shavings were used as bedding and changed regularly, and cages were cleaned twice weekly. Animals were provided with a standard pellet diet and had free access to tap water ad libitum.

Mating and pregnancy confirmation

Female albino rats were separated from males one month prior to the experiment to allow acclimatization and synchronization of the estrous cycle. After completion of the 60-day pre-gestational exposure period, mating was carried out at a ratio of 2 females to 1 male. Pregnancy was confirmed by the presence of a vaginal plug and spermatozoa in vaginal smears, which was designated as gestation day 0. Administration of L-APM was continued throughout the early gestational period.

Biochemical assessments and molecular assessment

Hepatic GPx activity, MDA levels, protein carbonyl content, and serum glucose concentrations were measured using rat-specific ELISA kits (Elabscience, USA; Cat. No. E-EL-R2497 for GPx, E-EL-0060 for MDA, E-EL-R0726 for protein carbonyls and E-EL-R0166 for glucose). Additionally, inflammatory cytokines (IL-1β and IL-6) were quantified using ELISA kits specific for rats (Cloud-Clone Corp., USA; Cat. No. SEA563Ra for IL-1β and SEA079Ra for IL-6) according to the manufacturer's

instructions. Optical density (OD) for all biochemical and molecular parameters was determined in a microplate ELISA reader (e.g., BioTek ELx800, USA) at 450 nm, following the manufacturer's protocols. All assays were processed in duplicate and performed in triplicate, and quantifications were conducted according to the standard curves attached with every kit.

Reproductive and teratogenic parameter assessments

Mortality rate in pups, and fertility, gestation, viability, and lactation indices were recorded to assess reproductive performance (Klaassen *et al*., 1986; Tag *et al*., 2021).

Cytogenic parameter

DNA damage was evaluated using the alkaline COMET assay with the COMETAssay® kit (Trevigen Inc., USA; Cat. No. 4250-050-K). Low melting point agarose and SYBR Green I were used for slide preparation and DNA staining, respectively. Fluorescence microscope was used to examine the slides for the assessment of DNA damage by alkaline COMET assay. Statistical robustness was achieved by analyzing 100 randomly selected cells per animal (50 cells per duplicate slide). Quantification of DNA damage was performed using specialized image analysis by COMET score software for the assessment of parameters such as (tail length, DNA % in tail, tail moment, olive moment).

Clinical observations

Maternal body weight was monitored throughout gestation. Offspring body weights were recorded at birth (day 0), day 4, and weekly until weaning (day 21). Pups were observed for gross structural abnormalities and functional development, including eye opening, ear unfolding, incisor eruption, and independent locomotion (Adams *et al*., 1985; Nguyen *et al*., 2017).

Neonatal functional and morphological evaluation

- Structural evaluation: Detectable malformations such as craniofacial, limb, or tail deformities were recorded (Mazarati *et al*., 2018).
- Functional evaluation: Neurobehavioral assessments including Negative Geotaxis, Swimming Rank Test, and Cleft Avoidance were conducted at regular intervals to monitor sensorimotor development (Hill *et al*., 2018; Frazier *et al*., 2021).
- All neurobehavioral assessments (Negative Geotaxis, Swimming Rank Test, and Cleft Avoidance), COMET assay, and cytokine measurements were conducted in offspring after weaning (postnatal day 21).

Histopathological Scoring and Statistical Analysis

The severity of histopathological alterations in the offspring's brain, heart, and liver was evaluated using a semi-quantitative 4-point scoring system: (0) Normal, (1) Mild, (2) Moderate, and (3) Severe. Scoring was based on the percentage of tissue involvement and the presence of degenerative changes, including neuronal gliosis, myofibrillar fragmentation, and hepatocellular necrosis.

Data are presented as mean ± SEM (n = 5 per group). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test to compare each treated group directly with the control group. Differences were considered statistically significant at $P < 0.05$ (Gibson-Corley *et al*., 2013; Dunnett, 1955; Landmann *et al*., 2021).

Ethical considerations and data analysis

All handling minimized stress, pain, and potential injury. Observations and measurements were performed gently and efficiently. Data were analyzed using IBM SPSS Statistics v22. Results are presented as mean ± SE. One-way and two-way ANOVA was used to evaluate treatment effects, with $P < 0.05$ considered statistically significant (Rosenfield and Melley, 1980; Field, 2024).

RESULTS

Maternal administration of L-aspartyl-L-phenylalanine methyl ester (L-APM) during early gestation significantly affected reproductive performance and maternal physiological parameters. As shown in Table 1, fetal mortality was significantly increased in the treated groups, whereas fertility, gestation, viability, and lactation indices were markedly reduced compared with the control group ($P < 0.001$).

In addition, oxidative stress markers were significantly altered. Serum glutathione peroxidase (GPx) activity was significantly decreased, while malondialdehyde (MDA) and protein carbonyl (PCO) levels were significantly elevated in treated dams, indicating enhanced oxidative stress status (Table 1).

Offspring born to L-APM–treated dams exhibited significant neurobehavioral impairments. As summarized in Table 2, latency times in the Negative Geotaxis test were significantly prolonged, cleft avoidance times were increased, and swimming ranks were significantly reduced compared with control offspring ($P < 0.0001$).

Table 1: Reproductive performance and oxidative stress parameters.

	Groups				
Early Stage	**Control**	**300 mg/kg**	**240 mg/kg**	**LSD**	**P-value**
Total Number of Dead Fetuses	2.80 ± 0.37 b	31.80 ± 0.58 a	30.40 ± 0.51 a	1.53	<0.001
Fertility Index of Dams	90.00 ± 0.32 a	69.20 ± 1.11bc	70.40 ± 1.08 b	2.81	<0.001
Gestation Index of Dams	98.60 ± 0.87a	76.00 ± 0.45c	78.00 ± 0.45 b	1.92	<0.001
Viability Index of Pups	97.20 ± 0.37a	69.60 ± 1.69 b	70.40 ± 1.47 b	4.04	<0.001
Lactating Index (L.I.)	95.40 ± 0.51a	65.20 ± 0.74 b	65.60 ± 0.68 b	1.99	<0.001
GPX Concentrations (µmol/l) in Dams	84.80 ± 1.50 a	25.60 ± 1.36 c	34.40 ± 3.04 b	6.50	<0.0001
MDA (Nanomol/ml) in Dams	0.65 ± 0.05 c	8.14 ± 0.35a	7.10 ± 0.25 b	0.77	<0.0001
Protein Carbonyl (Nanomol/ml) in Dams	0.44 ± 0.051c	10.30 ± 1.01 a	6.15 ± 0.23 b	1.84	<0.0001

Values are means ± SEM, n = 5 per treatment group. Means in a row without a common small letter differ significantly ($P < 0.05$). Means in a column without a common capital letter differ significantly ($P < 0.05$).

Table 2: Neurobehavioral test.

Early stage	**Groups**				
	Control	**300 mg/kg**	**240 mg/kg**	**LSD**	**P-value**
Negative Geotaxis Test (60 second) of Pups	6.20 ± 2.54 b	25.40 ± 2.48 a	23.20 ± 1.11 a	6.62	<0.0001
Cleft Avoidance Test (per 60 second) of Pups	13.00 ± 0.84c	44.40 ± 1.86 a	36.80 ± 2.48 b	5.71	<0.0001
Swimming Rank Test/ Grade of Pups (per 60 second)	4 ± 0 a	2.60 ± 0.25 b	2.80 ± 0.20 b	0.56	0.003

Values are means ± SEM, n = 5 per treatment group. Means in a row without a common small letter differ significantly ($P < 0.05$). Means in a column without a common capital letter differ significantly ($P < 0.05$).

Table 3: Blood glucose levels (mg/dl) of dams before and at the end of gestation.

Early Stage	**Group**				
	Control	**300 mg/kg**	**240 mg/kg**	**LSD**	**P-value**
Before	79.90 ± 0.69 Bb	86.5 ±1.88 Ba	82.10 ±0.61 Bb	3.45	0.002
End	123 ± 1.67 Ac	162 ± 2.21 Aa	154 ± 0.81 Ab	4.76	<0.0001
LSD	3.71	5.95	2.07		
P-value	<0.0001	<0.0001	<0.0001		

Values are means ± SEM, n = 30 per treatment group. Means in a row without a common small letter differ significantly (P < 0.05). Means in a column without a common capital letter differ significantly (P < 0.05).

Table 4: Inflammatory markers and histopathology scores.

Early Stage	**Groups**				
	Control	**300 mg/kg**	**240 mg/kg**	**LSD**	**P-value**
IL-1 β (pg/ml) in Pups	74.50 ± 2.01 c	110.00 ± 1.95a	97.10 ± 2.40 b	6.56	<0.0001
IL-6 (pg/ml) in Pups	9.80 ± 0.38 b	16.80 ± 1.19 a	15.90 ± 0.79 a	2.63	0.0001
Brain Histopathology Score	0.00 ± 0.00	2.00 ± 0.00*	1.00 ± 0.00*		
Liver Histopathology Score	0.00 ± 0.00	0.40 ± 0.00*	0.40 ± 0.00*		
Heart Histopathology Score	0.00 ± 0.00	0.50 ± 0.00*	0.17 ± 0.00*		

Values are means ± SEM, n = 5 per treatment group. Means in a row without a common small letter differ significantly ($P < 0.05$). Means in a column without a common capital letter differ significantly ($P < 0.05$).*Significantly different from Control group at same pregnancy stage ($P < 0.001$, * Dunnett's test). GPX; glutathione peroxidase, MDA: malondialdehyde, IL: interleukin.

Maternal exposure to L-APM resulted in significant alterations in metabolic balance. Blood glucose levels showed a marked and significant elevation in treated groups compared with controls ($P < 0.0001$), as demonstrated by two-way ANOVA analysis (Table 3).

Inflammatory biomarkers were significantly elevated in pups born to treated dams. Serum levels of interleukin-1β (IL-1β) and interleukin-6 (IL-6) were markedly higher compared with controls ($P < 0.0001$), as presented in Table 4.

Histopathological lesion scores also revealed significant structural alterations in offspring tissues. Brain sections showed mild to moderate cortical disturbances (Figure 1), liver tissues exhibited hepatocellular vacuolation and sinusoidal congestion (Figure 2), and cardiac sections showed myofibrillar disorganization and focal myocardial degeneration (Figure 3). Semi-quantitative histopathological scoring demonstrated a dose-dependent increase in tissue damage across all examined organs (Table 4). Neuronal degeneration and gliosis scores in the brain were significantly higher in the 300 mg/kg group compared with the 240 mg/kg group ($P < 0.001$). Similarly, liver and heart tissues showed significantly elevated injury scores, reflecting severe hepatocellular vacuolation and myocardial hyaline degeneration, respectively ($P < 0.05$).

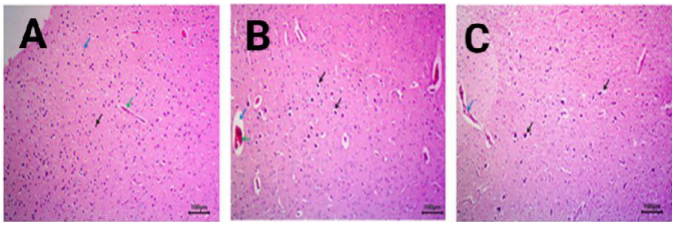

Figure 1: Histological Section of Rat's Brain Tissue Showing Different Degrees of Severety.
(A) Control group: Histological section shows the cortex with normal architecture (black arrows), glial cells (blue arrow) and blood vessels (green arrow). H and E stain, 100X. (B) 300 mg/kg group: Histological section shows the cortex with cytotoxic edema (black arrow), vasogenic edema (blue arrows), and congestion of blood vessels (green arrow). H and E stain, 100X. (C) 240 mg/kg group: Histological section shows the cortex with mild cytotoxic edema (black arrows) and mild congestion of blood vessels (blue arrow). H and E stain, 100X.

Genotoxic evaluation using the COMET assay demonstrated significant DNA damage in offspring of

Table 5: DNA damage level in blood lymphocyte using COMET assay.

Damage		Groups				
Early Stage		**Control**	**300 mg/kg**	**240 mg/kg**	**LSD**	**P-value**
COMET Assay Analysis Values	No	44.30 ± 0.52 a	40.70 ± 0.84 b	41.10 ± 0.79 b	2.25	0.009
	Low	42.70 ± 0.39a	36.60 ± 0.70b	36.90 ± 0.79 b	2.02	<0.0001
	Medium	5.39 ± 0.21b	11.10 ± 0.29 a	11.00 ± 0.41 a	0.98	<0.0001
	High	7.62 ± 0.19b	11.60 ± 0.29 a	11.00 ± 0.28a	0.79	<0.0001
Tail Length Values/Micrometer		3.80 ± 0.12 b	8.90 ± 0.33 a	8.20 ± 0.37 a	0.92	<0.0001
DNA% in tail (Percent)		2.40 ± 0.19 b	8.60 ± 0.29 a	8.10 ± 0.33 a	0.85	<0.0001
Tail Moment Values (Percent)		2.10 ± 0.10 b	8.30 ± 0.37a	8.10 ± 0.33 a	0.91	<0.0001
Olive Moment (Percent)		1.60 ± 0.19 b	8.10 ± 0.33 a	7.60 ± 0.25 a	0.81	<0.0001

Values are means ± SEM, n = 5 per treatment group. Means in a row at each time point without a common small letter differ significantly ($P < 0.05$). Means in a column at each time point without a common capital letter differ significantly ($P < 0.05$).

Table 6: Physical growth parameters of offspring.

Time		Groups				
Early Stage		**Control**	**300 mg/kg**	**240 mg/kg**	**LSD**	**P-value**
Body Weight of Pups	First day	5.55 ± 0.20 a	3.10 ± 0.10 b	3.60 ± 0.19 b	0.50	<0.0001
	Week 1	15.20 ± 1.13 a	10.10 ±0.88 b	11.10 ± 0.26 b	2.40	0.0003
	Week 2	25.10 ± 0.67 a	16.20 ±0.63 b	16.90 ± 0.26 b	1.29	<0.0001
	Week 3	32.70 ± 0.72 a	22.20 ±0.47b	22.70 ± 0.52 b	1.68	<0.0001
	Week 4	50.40 ± 0.66 a	31.80 ±0.42 b	31.80 ± 0.39 b	1.46	<0.0001
Body Length/cm of Pups.	First day	5.11 ± 0.07a	4.90 ± 0.07 ab	4.70 ± 0.17 b	0.33	0.017
	Week 1	9.40 ± 0.52 a	8.60 ± 0.46 a	8.98 ± 0.08 a	1.15	0.390
	Week 2	12.60 ± 0.16 a	12.00 ± 0.37 a	12.20 ± 0.13 a	0.45	0.208
	Week 3	16.60 ± 0.17 a	14.40 ± 0.19 b	14.40 ± 0.22 b	0.57	<0.0001
	Week 4	24.80 ± 0.15 a	18.30 ± 0.27 b	18.60 ± 0.18 b	0.59	<0.0001

Values are means ± SEM, n = 5 per treatment group. Means in a row at each time point without a common small letter differ significantly ($P < 0.05$). Means in a column at each time point without a common capital letter differ significantly ($P < 0.05$).

L-APM–treated dams. Tail length, percentage of DNA in the tail, tail moment, and olive tail moment were all significantly increased in treated groups compared with controls ($P < 0.0001$), following a clear dose-dependent pattern (Table 5, Figure 4).

Postnatal growth and development were adversely affected by maternal L-APM exposure. Offspring from treated dams exhibited significantly reduced body weights and shorter body lengths from birth through the fourth week of lactation ($P < 0.05–0.0001$), as presented in Table 6.

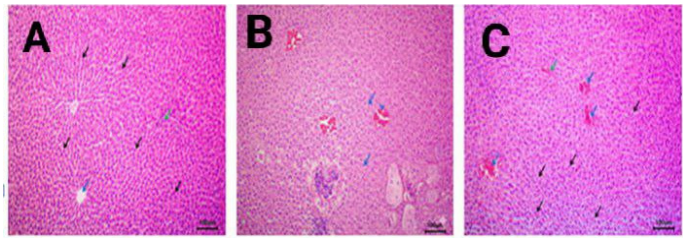

Figure 2: Histological section of rat's liver tissue showing different degrees of severety.
(A) Control group: Histological section shows normal architectures of the hepatocytes (black arrows), central vein (blue arrow) and portal area (green arrow). H and E stain, 100X. (B) 300 mg/kg group: Histological section shows mild vascular degeneration of the hepatocytes (black arrow), congestion of the central vein (blue arrows) and sinusoids (green arrow). H and E stain, 100X. (C) 240 mg/kg group: Histological section shows mild vascular degeneration of the hepatocytes (black arrow), congestion of the central vein(blue arrows) and sinusoids (green arrow). H and E stain, 100X.

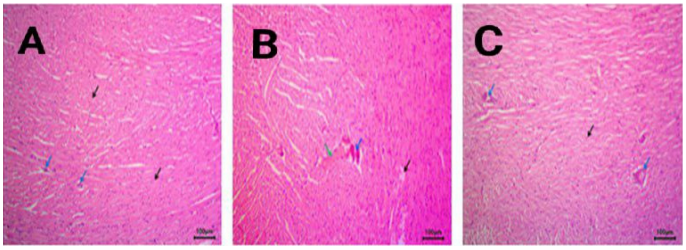

Figure 3: Histological section of rat's heart tissue showing different degrees of severity.
(A) Control group: Histological section shows normal architectures of the myocardial muscles cells (black arrows), and blood vessels (blue arrows). H and E stain, 100X. (B) 300 mg/kg group: Histological section shows mild necrosis (black arrow), and mild hyaline degeneration of the myocardial muscles cells (blue arrows), and edema (green arrow). H and E stain, 100X. (C) 240 mg/kg group: Histological section shows intact structure of the myocardial muscles cells (black arrows), with mild congestion of blood vessels (blue arrows). H and E stain, 100X.

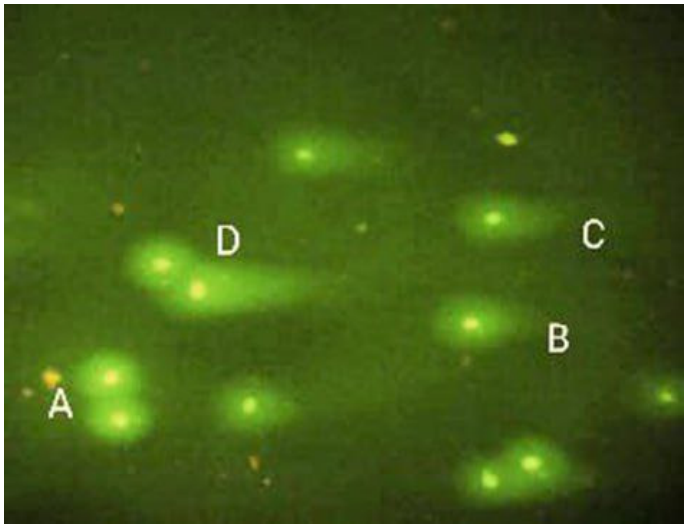

Figure 4: Microscopic images of the comet assay showing different levels of DNA damage: (A) no comet, (B) low comet, (C) moderate comet, and (D) high comet (SYBR Green stain, 10× magnification).

DISCUSSION

Early gestational exposure to L-APM was associated with measurable genotoxic and developmental alterations in offspring, although the magnitude of these effects was generally less pronounced than those reported following exposure during later stages of gestation. COMET assay parameters, including tail length, percentage of DNA in the tail, tail moment, and olive tail moment, were significantly increased in treated pups compared with controls, indicating early DNA strand breaks likely linked to oxidative stress (Fındıklı and Türkoğlu, 2014; Hamza *et al.*, 2019). Greater DNA migration observed in the 300 mg/kg group relative to the 240 mg/kg group suggests a dose-dependent genotoxic effect even during this early developmental window (Collins, 2004; Hassan *et al.*, 2023). In the present study, male rats were exposed to L-APM for two months prior to mating, a period encompassing a complete spermatogenic cycle. This paternal exposure may have contributed to the observed genotoxic and developmental alterations in the offspring. Increasing evidence indicates that oxidative stress–inducing xenobiotics can compromise sperm DNA integrity and induce epigenetic modifications, which may be transmitted to the embryo and influence early developmental processes (Aitken and Curry, 2011; Soubry *et al.*, 2014). Accordingly, the contribution of paternal exposure cannot be excluded and may partially explain the presence of measurable effects despite exposure occurring during early gestation. Mechanistically, reactive oxygen species generated during L-APM metabolism, particularly from methanol and formaldehyde, may compromise genomic integrity before organogenesis is complete (Ruszkiewicz and Albrecht, 2015; Agarwal *et al.*, 2022).

Maternal exposure to L-APM also induced early oxidative stress, as evidenced by decreased glutathione (GSH) and increased malondialdehyde (MDA) in both dams and offspring tissues. Within the same context, maternal blood glucose was modestly but significantly elevated, particularly in the 300 mg/kg group, suggesting early impairment of carbohydrate metabolism. This hyperglycemia may result from oxidative stress affecting pancreatic β-cell function and disrupting insulin secretion (Aboshanady *et al*., 2018; Ayala *et al*., 2014). These findings indicate that even during early gestation, L-APM metabolites can interfere with maternal metabolic homeostasis in a dose-dependent manner.

Inflammatory responses were also evident, with significant elevations in pro-inflammatory cytokines IL-1β and IL-6 in exposed offspring, particularly at the higher dose. Early activation of inflammatory pathways may disrupt fetal immune homeostasis and contribute to later developmental vulnerability (Dinarello, 2011; Oummadi, 2023; Mohammed *et al*., 2024; Ashish *et al*., 2025).

Offspring growth was adversely affected, as demonstrated by reductions in body weight and crown–rump length. Mild alterations were observed at 240 mg/kg, whereas 300 mg/kg exposure produced more pronounced growth retardation, suggesting early metabolic disruption prior to critical organ development. Elevated maternal phenylalanine levels may impair placental transport of essential amino acids, further exacerbating oxidative stress in embryonic tissues (Lubos *et al*., 2011; Bhoumik *et al*., 2023).

Neurobehavioral testing revealed subtle but measurable deficits. Prolonged response latencies in the Negative Geotaxis test indicated impaired vestibular and sensorimotor integration, while modest increases in Cleft Avoidance time and decreased Swimming Rank scores reflected early hippocampal- and cerebellar-associated functional compromise (Smart and Dobbing, 1971; Whishaw and Kolb, 2004; Vorhees and Williams, 2006; Lebda *et al*., 2017).

Histopathological assessment supported these functional and molecular findings. Brain sections exhibited mild neuronal vacuolation, focal gliosis, and early vascular congestion, consistent with oxidative stress-mediated injury. Liver tissues showed mild hepatocellular vacuolation and sinusoidal congestion, while cardiac tissue displayed early cardiomyocyte vacuolation and minor myofibrillar disorganization, reflecting early organ-specific sensitivity to oxidative and metabolic stress (Trocho *et al*., 1998; Zhu *et al*., 2018; Rana *et al*., 2018). The adoption of a standardized histopathological scoring system in this study provides an objective quantitative framework to evaluate L-APM's early-stage toxicity. Although the lesion scores in the brain, liver, and myocardium were less severe than those typically observed in later gestation, they exhibited a significant, dose-dependent increase compared to controls ($P < 0.05$). These initial structural alterations marked by mild neuronal vacuolation and focal gliosis represent a critical early disruption of cellular homeostasis. The significant correlation between these histological scores and prolonged neurobehavioral latencies suggests that even during early embryonic patterning, L-APM metabolites initiate a cascade of oxidative damage that destabilizes the structural foundation necessary for subsequent functional and sensorimotor integration in the offspring.

The relatively subtler phenotypic alterations observed during early gestation may be explained by the developmental stage of the embryos. At this period, organ systems are not yet fully differentiated, and some tissues may possess compensatory mechanisms that temporarily mitigate functional disruption.

Nonetheless, measurable oxidative stress, genotoxicity, metabolic disturbances, and inflammatory activation indicate that even early exposure to L-APM can initiate pathways leading to later developmental consequences.

Collectively, these findings indicate that early gestation represents a sensitive window for maternal exposure to L-APM. Exposure was associated with dose-dependent increases in oxidative stress, DNA damage, maternal metabolic disruption, reproductive disturbances in dams, and growth, inflammatory, and early neurobehavioral alterations in offspring. Effects were more pronounced at 300 mg/kg than at 240 mg/kg, underscoring the vulnerability of early embryonic development to xenobiotic-induced toxicity. Limitations of the study include the focus on early postnatal outcomes and the inference of mechanistic pathways from biochemical, behavioral, and histopathological markers rather than direct molecular analyses, highlighting the importance of further studies to assess potential long-term neurodevelopmental and metabolic consequences. Further studies are warranted to evaluate long-term developmental consequences and clarify underlying mechanisms.

CONCLUSIONS

Early gestation is a highly sensitive window for maternal exposure to L-APM. Exposure during this period induced dose-dependent oxidative stress, DNA damage, and reproductive disturbances in dams, along with growth retardation, inflammatory activation, and early neurobehavioral alterations in offspring. Effects were more pronounced at 300 mg/kg than at 240 mg/

kg, demonstrating a clear dose-response relationship. Limitations include the focus on early postnatal outcomes and inference of mechanistic pathways from biochemical, behavioral, and histopathological markers. Further studies are needed to evaluate long-term developmental consequences and clarify underlying mechanisms.

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NOVELTY STATEMENT

This study provides original experimental data on the dose-dependent developmental effects of L-aspartyl-L-phenylalanine methyl ester (240 and 300 mg/kg) during early pregnancy in female rats. The study applies a structured histopathological scoring system to evaluate tissue damage and developmental alterations during this critical gestational stage, contributing to a more detailed assessment of potential risks associated with L-APM exposure.

AUTHORS' CONTRIBUTION

ANH and FMK designed the study. ANH conducted the experimental work and collected the samples. FMK performed the statistical analysis and interpretation of data. ANH wrote the manuscript. All authors read and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors confirm that no generative AI or AI-assisted technologies were used in the writing, data collection, analysis, or interpretation of this manuscript.

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Conflict of interest

The authors have declared no conflict of interest.

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