**Research Article**

# Hematological Parameters and Splenic Histopathology in Offspring of Wistar Rats Exposed to Prenatal Stress and *Moringa oleifera*

**Odochi O. Chukwu [a, b, c], Anthony C. U. Ezimah[b], Nwaeze G. Konyefom[b], Grace Ottah-Umahi[a], Sylvester N. Ibekailo[b],**

*a. Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, David Umahi Federal University of Health Sciences, Ebonyi State, Nigeria.*

*b. Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike. Abakaliki, Ebonyi, State, Nigeria.*

*c. Physiology Unit, College of Health Sciences, Evangel University, Akaeze, Nigeria.*

**Corresponding Author:** Dr. Odochi Ogbu Chukwu
**Postal Address:** Physiology Unit, College of Health Sciences, Evangel University, Akaeze, Nigeria.



**Running Head:** Effect of *Moringa oleifera* on Prenatal Stress-Induced Hematotoxicity

**Acknowledgements**
The authors thank the Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, AE-FUNAI, Ebonyi State, for supporting this study.

**Competing Interests:**
The authors declare that there is no conflict of interest.

**Abstract**

This study investigates the effects of prenatal exposure to Moringa oleifera leaf extract (MoLE) and chronic unpredictable stress (CUS) on the immune development of offspring in a rat model. Twenty-five pregnant Albino-Wistar rats were divided into five groups: a control group, two MoLE-treated groups (5 mg/kg and 10 mg/kg), and two CUS-exposed MoLE-treated groups (5 mg/kg and 10 mg/kg). MoLE was administered via gavage from gestation day 8 to 21. Hematological parameters and splenic histopathology were assessed in offspring at puberty. Results showed that MoLE exposure affected blood cell counts and splenic histology. Specifically, MoLE treatment resulted in decreased monocyte and granulocyte counts, while lymphocyte levels remained unchanged. The high-dose MoLE group exhibited increased mean platelet volume (MPV) and platelet distribution width (PDW). Severe histopathological changes, including fibrosis and hemorrhage, were observed in the CUS-exposed groups. These findings suggest that prenatal MoLE and CUS exposure influence immune development, warranting further research into their impact on immune function and blood clotting.

**Keywords:** Prenatal Stress, Hematotoxicity, Immunity, Blood, Moringa oleifera

**Abbreviations:**

CUS = Chronic Unpredictable Stress

MoLE = Moringa oleifera Leaf Extract

HPA = Hypothalamic-Pituitary-Adrenal

**Introduction**

Chronic unpredictable stress (CUS) during pregnancy is a significant public health concern due to its potential to disrupt fetal development, impacting both physiological and behavioral outcomes in offspring [1]. Prenatal stress, especially in the form of CUS, has been associated with alterations in hematological profiles, such as changes in white blood cell (WBC) counts and platelet function, which could have lasting effects on immune development and blood clotting processes [1-3]. These hematological alterations may increase offspring susceptibility to infections and other health complications [4]. Thus, identifying potential interventions to mitigate these adverse effects is crucial for promoting healthy offspring development.

*Moringa oleifera*, commonly known as the “drumstick tree,” is renowned for its health-promoting properties. This drought-resistant tree is cultivated in tropical and subtropical regions and is particularly valued for its leaves, which are rich in essential nutrients, vitamins,

minerals, and bioactive compounds, including flavonoids, glucosinolates, and phenolic acids [5, 6]. These compounds are believed to contribute to the plant's antioxidant, anti-inflammatory, and immunomodulatory effects [7].

Despite the increasing body of research on MoLE, there remains a gap in understanding its effects during critical developmental windows, particularly during pregnancy. The combined influence of MoLE and CUS exposure on offspring development, especially regarding hematological parameters and splenic health, has not been fully elucidated. This study aims to bridge this gap by examining the effects of MoLE on hematological markers and splenic histopathology in offspring exposed to prenatal CUS, thus contributing valuable insights into maternal health and offspring development.

## Results

### White Blood Cell and Differential Counts

Prenatal exposure to *Moringa oleifera* leaf extract (MoLE) and/or chronic unpredictable stress (CUS) significantly altered the leukocyte profiles of offspring (Table 2). Total white blood cell (WBC) counts were markedly elevated in the High-dose MoLE group (17.42±1.78 $\times 10^9$/L) and the CUS + High-dose MoLE group (15.32±3.39 $\times 10^9$/L) compared to Control (5.40±0.22 $\times 10^9$/L, $p<0.05$). Lymphocyte counts followed a similar trend, showing significant increases in High-dose MoLE and CUS + High-dose MoLE groups ($p<0.05$).

Granulocyte counts were significantly higher in the High-dose MoLE (3.45±0.15 $\times 10^9$/L) and CUS + High-dose MoLE groups (2.83±0.72 $\times 10^9$/L) relative to Control (0.46±0.15 $\times 10^9$/L, $p<0.05$). No significant changes were observed in monocyte counts across groups ($p>0.05$). These findings suggest a stress-induced inflammatory response that was partially modulated by MoLE supplementation in a dose-dependent manner.

### Platelet Counts and Indices

Platelet profiles were also significantly affected by prenatal treatments (Table 3). Offspring from High-dose MoLE and both CUS-exposed groups exhibited a sharp reduction in platelet counts compared to Control ($p<0.05$), with values dropping by over 70% in High-dose MoLE and CUS + High-dose MoLE groups. In contrast, CUS + Low-dose MoLE resulted in a significant increase in platelet count (1445.0±101.9 $\times 10^9$/L, $p<0.05$) compared to Control.

Among platelet indices, mean platelet volume (MPV) and platelet distribution width (PDW-SD and PDW-CV) were significantly elevated only in the High-dose MoLE group ($p<0.05$). No significant differences were observed in MPV or PDW for the CUS + Low-dose MoLE group compared to Control, indicating preserved platelet morphology under low-dose supplementation.

**Histopathological Findings**

Representative photomicrographs of spleen sections are shown in Figure 1 (Panels A–E). Control animals exhibited normal splenic architecture with clearly defined white and red pulp regions (Figure 1A). Low-dose MoLE-treated offspring displayed mild tissue degeneration with moderate inflammatory infiltration and focal ground-glass changes (Figure 1B). High-dose MoLE offspring showed mild to moderate fibrosis and occasional hemorrhage (Figure 1C).

The most severe lesions were observed in the CUS + Low-dose MoLE group, characterized by marked tissue degeneration, extensive fibrosis, and hemorrhagic red pulp (Figure 1D). The CUS + High-dose MoLE group demonstrated moderate degeneration with focal fibrosis but less severe damage than Group IV (Figure 2E), suggesting a partial protective effect of higher MoLE dosing under stress exposure.

Lesion scores summarized in Table 4 confirm these observations: fibrosis and hemorrhage were significantly more severe in the CUS + Low-dose MoLE group compared to Control ($p<0.05$), whereas High-dose MoLE co-treatment attenuated lesion severity relative to low-dose supplementation under CUS conditions ($p<0.05$).

**Figure 1.** Representative photomicrographs of spleen sections (H&E staining) showing:
**(A) Control:** Normal architecture with intact white pulp (WP) and red pulp (RP);
**(B) Low-dose MoLE:** Mild degeneration with inflammatory infiltration;
**(C) High-dose MoLE:** Mild fibrosis and hemorrhage;
**(D) CUS + Low-dose MoLE:** Severe degeneration, fibrosis, and hemorrhagic red pulp;
**(E) CUS + High-dose MoLE:** Moderate degeneration with focal fibrosis.

**Figures**

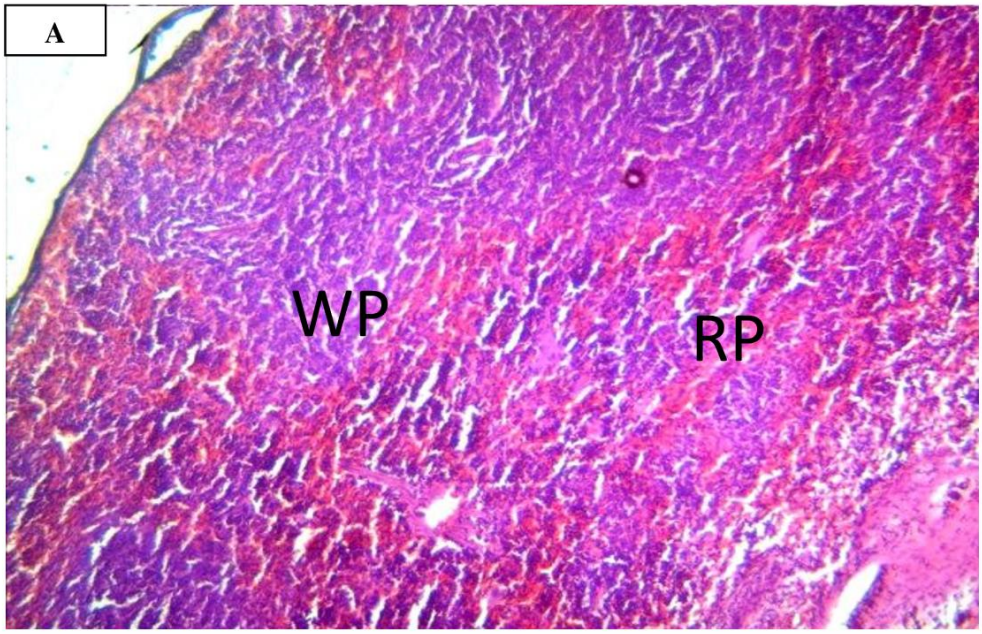


Figure 1A: **Photomicrograph of Control section of the spleen (x150) (H/E) shows normal spleen architecture with Red Pulp (RP) and White Pulp (WP), with Central Spleenic Artery.**



## Discussion

This study investigated the modulatory effects of *Moringa oleifera* leaf extract (MoLE) on hematological parameters and splenic histopathology in offspring exposed to prenatal chronic unpredictable stress (CUS). Our findings demonstrate that prenatal stress markedly disrupted leukocyte and platelet profiles and caused significant structural alterations in the spleen. MoLE supplementation exerted partial protective effects, with outcomes varying by dose and stress exposure context.

Exposure to CUS during gestation significantly elevated total white blood cell (WBC) counts and granulocyte levels in offspring, suggesting an activated inflammatory state. Prenatal stress has previously been shown to dysregulate fetal immune development through maternal HPA axis activation, increased glucocorticoid release, and heightened pro-inflammatory cytokine signaling [2, 3, 19, 20]. Epidemiological evidence supports these findings, linking prenatal stress to greater susceptibility to infections and allergic diseases in children [1, 3, 4]. Experimental studies further demonstrate that maternal stress during late gestation can have

long-lasting effects on immune system programming and leukocyte differentiation [12, 21]. Our data align with this body of evidence, indicating that prenatal stress can prime offspring for altered immune responses, reflected here in leukocytosis and neutrophil predominance.

MoLE supplementation partially modulated stress-induced changes in WBC counts and lymphocyte distribution. At high doses, MoLE increased lymphocyte counts compared to controls, possibly enhancing adaptive immunity. The leaves of *Moringa oleifera* contain flavonoids, phenolic acids, and essential micronutrients known to support hematopoietic function and modulate immune activity [5-7, 8, 21]. Previous research has demonstrated MoLE's ability to mitigate stress-induced developmental defects and neuroinflammatory processes in prenatally stressed rats [11, 22], suggesting that its bioactive compounds may reduce oxidative and inflammatory stress signals that disrupt immune cell maturation.

Prenatal stress and high-dose MoLE treatment both significantly reduced platelet counts compared to controls, while CUS combined with low-dose MoLE increased platelet numbers. Platelet reduction under stress exposure may be linked to altered megakaryocyte activity or increased platelet consumption during inflammatory activation [15, 16]. The contrasting increase in platelet count observed with low-dose MoLE suggests that moderate supplementation could support thrombopoiesis, potentially through antioxidant protection of bone marrow stem cells [5, 6]. However, platelet morphology indices (MPV and PDW) showed significant alterations only in the high-dose MoLE group, indicating that excessive supplementation might perturb platelet size distribution, consistent with previous observations of dose-dependent variability in hematological outcomes [15, 16].

The spleen plays a pivotal role in immune cell maturation, erythrocyte clearance, and platelet storage [23]. In this study, prenatal CUS exposure led to severe splenic tissue degeneration, marked fibrosis, and hemorrhage, as reflected in high lesion scores for Group IV. Similar findings have been reported in experimental models where environmental stressors disrupted postnatal immune cell development and splenic architecture [24].

MoLE supplementation partially ameliorated splenic lesions, particularly in the high-dose CUS group, where degeneration and fibrosis were less severe compared to low-dose CUS-exposed offspring. These results support earlier evidence that *Moringa oleifera* possesses antioxidant and anti-inflammatory properties that can protect developing tissues against stress-induced injury [5, 6, 8]. The observed dose-dependent protection of splenic structure suggests that MoLE may help preserve immune organ integrity during prenatal stress exposure, potentially improving immune competence in offspring.

This study has several limitations. First, the absence of a CUS-only control group restricted the ability to isolate stress-induced changes from MoLE-related effects. Second, functional immune assays (e.g., cytokine profiling and platelet aggregation tests) were not performed, limiting conclusions on immune competence and clotting activity. Third, despite random selection of pups and treating each dam as the experimental unit, sampling multiple pups per litter could not be completely avoided due to litter size constraints. Future studies should include larger sample sizes, direct stress biomarkers (e.g., corticosterone), and functional immune tests to further clarify the protective role of MoLE under prenatal stress conditions.

**Conclusion**

Overall, our findings indicate that prenatal CUS disrupts hematological balance and damages splenic architecture in rat offspring. MoLE supplementation during gestation provides partial protection, particularly at moderate doses, by modulating leukocyte profiles, supporting platelet production, and preserving splenic tissue integrity. These results suggest that *Moringa oleifera* may serve as a beneficial maternal dietary supplement for mitigating adverse immune effects of prenatal stress, though optimal dosing and mechanistic pathways warrant further investigation.

**Acknowledgments**

The authors express their gratitude to the Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, AE-FUNAI, Ebonyi State, for their support during this study.

**Materials and Methods**

**Ethical Approval**

All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and approved by the Faculty of Basic Medical Sciences Research Ethics Committee, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria (Approval Code: FBMS/EC/AE/1983).

**Plant Collection, Identification, and Extraction**

Fresh Moringa oleifera leaves were collected early in the morning from a cultivated garden in Abakaliki, Ebonyi State. Botanical identification was confirmed at the Herbarium Unit, Department of Biology, AE-FUNAI. Leaves were thoroughly washed, air-dried at room temperature for seven days, and milled into a coarse powder using an electric blender (Model MS-233, China). Extraction was performed using methanol according to standardized

procedures [5, 6]. The filtrate was concentrated at 40°C under reduced pressure to yield a dark-green paste, which was stored at 4°C until use.

The phytochemical profile of the extract was characterized by gas chromatography–mass spectrometry (GC–MS) as previously described by Chukwu et al. [8]. Briefly, analysis was performed using an Agilent GC–MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm). The oven temperature was programmed from 60°C (2 min hold) to 280°C at 10°C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Major compounds identified are known for antioxidant and anti-inflammatory activity [8] (table 1)

**Experimental Animals and Housing Conditions**

Twenty-five mature, nulliparous, virgin female Albino-Wistar rats (weighing 150–180 g) were obtained from the Animal House, AE-FUNAI. Animals were housed in well-ventilated polypropylene cages under standard laboratory conditions (12h light/dark cycle, temperature 23±2°C, humidity 50-60%) with ad libitum access to standard rat chow (Vital feed®, Nigeria) and tap water. Animals were acclimatized for two weeks before mating.

**Estrous Cycle Monitoring and Mating**

Estrous cycle monitoring was performed by vaginal cytology using light microscopy following established protocols [9]. Females with two consecutive regular four-day cycles were considered for mating. During the proestrus phase, identified by the predominance of nucleated epithelial cells and absence of cornified cells, females were co-housed overnight with proven male breeders (1:2 ratio). The presence of spermatozoa in morning smears confirmed successful mating and marked gestational day (GD) 1 [10].

**Experimental Design and MoLE Administration**

Pregnant rats were randomly assigned (n=5 per group) to the following treatment groups:

- Control: Standard diet and water ad libitum.
- Low-dose MoLE: MoLE 5 mg/kg/day via oral gavage from GD 8–21.
- High-dose MoLE: MoLE 10 mg/kg/day via oral gavage from GD 8–21.
- CUS + Low-dose MoLE: CUS exposure plus 5 mg/kg/day MoLE from GD 8–21.
- CUS + High-dose MoLE: CUS exposure plus 10 mg/kg/day MoLE from GD 8–21.

Doses were freshly prepared in distilled water daily and administered in volumes not exceeding 1 mL/100 g body weight to avoid gastric discomfort [8]. The gestational window GD 8-21 was selected to reflect the late second to third trimester in human pregnancy, a critical period for immune and hematopoietic development [11, 12].

**Chronic Unpredictable Stress (CUS) Protocol**

Animals in CUS groups were subjected to a validated CUS protocol [13] consisting of the following randomly applied stressors:

- Wet bedding (300 mL water mixed with sawdust).
- Cage tilting at 45° for 6 hours.
- Overnight food deprivation.
- Psychological stress by exposure to a caged cat.
- Sleep deprivation using a pedestal in shallow water.
- Restraint stress in 50 mL plastic tubes for 2-hour intervals.
- Continuous overnight light exposure.
- Social isolation for 6-12 hours.

Stressors were rotated daily in an unpredictable order from GD 8–21 to mimic human psychosocial stress [13. 14].

**Sample Collection and Litter Effect Consideration**

At the onset of puberty (postnatal day 21 onward), five offspring per treatment group were sampled for hematological analysis, with at least one pup selected from each dam (total 5 dams per group). To minimize litter effect bias and maintain balanced sex representation, pups were randomly selected across litters, ensuring that no more than one male and one female pup per dam contributed to the dataset. This approach ensured equal representation of each dam while avoiding over-representation of littermates.

Although individual pup data were recorded, the dam (litter) was treated as the experimental unit (n = 5 dams per group) for all statistical analyses, in accordance with internationally accepted developmental toxicology guidelines [12, 16], thereby preventing pseudo-replication. Blood samples (~2 mL) were collected via retro-orbital venous puncture under light isoflurane anesthesia into EDTA-coated tubes and processed within 3 hours at 4°C to preserve cell morphology and ensure accurate hematological profiling.

**Hematological Analysis**

A calibrated automated hematology analyzer (Mindray BC-2800, Shenzhen, China) was used to determine:

- Total WBC counts ($\times 10^9$/L).
- Differential counts: Lymphocytes (Lym), monocytes (MID), and granulocytes (Gran), reported as absolute counts and percentages.

- Platelet parameters: Platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW-CV, PDW-SD), plateletcrit (PCT), and platelet-large cell ratio (P-LCR) [16, 17].

**Histological Studies**

Following blood collection, pups were humanely euthanized with sodium pentobarbital (150 mg/kg, i.p.). Spleens were excised, trimmed of fat, and fixed in 10% neutral-buffered formalin for 24-48 hours. Standard tissue processing, paraffin embedding, and hematoxylin-eosin (H&E) staining were performed [18]. Sections were examined under a light microscope (×100–150 magnification). Histopathological changes were semi-quantitatively graded (fibrosis, hemorrhage, inflammatory infiltration, ground-glass appearance; scores 0-3) independently by two blinded observers, with discrepancies resolved by consensus [19].

**Statistical Analysis**

All data are presented as mean ± standard error of the mean (SEM). Each dam was considered the experimental unit (n = 5 per group), as recommended for developmental studies to avoid pseudo-replication. When more than one pup per dam was sampled, their values were averaged to generate a single representative data point per dam. Hematological parameters were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Non-parametric data from semi-quantitative splenic lesion scoring were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism (Version 9.0, GraphPad Software, San Diego, CA, USA).

**References**


1. Nielsen NM, Hansen AV, Simonsen J, Hviid A. Prenatal stress and risk of infectious diseases in offspring. Am J Epidemiol. 2011;173(7):990-7. DOI: https://doi.org/10.1093/aje/kwq492
2. Brunton PJ, Russell JA, Douglas AJ. Adaptive Responses of the Maternal Hypothalamic-Pituitary-Adrenal Axis during Pregnancy and Lactation. J Neuroendocrinol. 2008;20(10):764-76. DOI: https://doi.org/10.1111/j.1365-2826.2008.01735.x
3. Lim R, Fedulov AV, Kobzik L. Maternal stress during pregnancy increases neonatal allergy susceptibility: role of glucocorticoids. Am J Physiol Lung Cell Mol Physiol. 2014;307(1):L141-8. DOI: https://doi.org/10.1152/ajplung.00250.2013

4. Liu X, Olsen J, Agerbo E, Yuan W, Sigsgaard T, Li J. Prenatal stress and childhood asthma in the offspring: role of age at onset. Eur J Public Health. 2015;25(6):1042-6. DOI: https://doi.org/10.1093/eurpub/ckv129
5. Ali A, Yusof A, Chin L, Ibrahim MN, Muneer S. Development and standardization of Moringa oleifera leaves as a natural dietary supplement. J Diet Suppl. 2019;16(1):66-85. DOI: https://doi.org/10.1080/19390211.2018.1429517
6. Adedapo A, Falayi O, Oyagbemi A. Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed Moringa oleifera in some laboratory animals. J Basic Clin Physiol Pharmacol. 2015; 26(5):491-9. DOI: https://doi.org/10.1515/jbcpp-2014-0105
7. Bhargave A, Pandey I, Nama KS, Pandey M. Moringa oleifera Lam. Sanjana (horseradish tree) a miracle food plant with multipurpose uses in Rajasthan-India-an overview. Int J Pure Appl Biosci. 2015; 3:237-8. DOI: http://dx.doi.org/10.18782/2320-7051.2169
8. Chukwu OO, Iyare CO, Emelike CU, Ezimah ACU, Asogwa NT, Konyefom NG. GC-MS analysis of Moringa oleifera leaf extract and effects of administration on histology of reproductive organs and liver of female rats exposed to chronic unpredictable stress. Food Chem Adv. 2024;4:100661. DOI: https://doi.org/10.1016/j.focha.2024.100661
9. Goldman JM, Murr AS, Cooper RL. The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. Birth Defects Res B Dev Reprod Toxicol. 2007 80(2):84-97. doi: 10.1002/bdrb.20106.
10. Chukwu OO, Emelike CU, Konyefom NG, Ibekailo SN, Ekakitie OO, Ghasi S, Iyare EE. Histological Studies of the Heart and Biochemical Changes Due to the Perinatal Consumption of *Hibiscus sabdariffa* (Flavonoid-rich Extract) to Feed-restricted Rats on Offspring. Iran J Vet Med. 2022;17(1):37-46. DOI: 10.22059/IJVM.17.1.1005272.
11. Chukwu OO, Iyare C, Ezimah ACU, Okorocha AE, Konyefom NG, Asogwa NT et al. Moringa oleifera Docking to Estrogen Receptor α Ameliorates Placental and Brain Damage in Stressed Rats. Avicenna J Med Biotech 2025; 17(1):14-23. DOI https://doi.org/10.18502/ajmb.v17i1.17673
12. Couret D, Jamin A, Kuntz-Simon G, Prunier A, Merlot E. Maternal stress during late gestation has moderate but long-lasting effects on the immune system of the piglets. Vet Immunol Immunopathol. 2009;131(1-2):17-24. DOI: https://doi.org/10.1016/j.vetimm.2009.03.003.

13. Chukwu O O, Iyare C O, Okorocha A E, Konyefom N G, Emelike C U, Onyeji G N, et al . *Moringa oleifera* Lam. is a potential mitigator of neurodevelopmental defects caused by prenatal stress in Wistar rats. J. Med. Plants 2025; 24 (93) :79-101 URL: http://jmp.ir/article-1-3709-en.html.
14. Brenes JC, Fornaguera J. Effects of environmental enrichment and social isolation on sucrose consumption and preference: Associations with depressive-like behaviour and ventral striatum dopamine. Neurosci Lett. 2008;436(2):278-82. DOI: 10.1007/s11055-022-01297-1
15. Latger-Cannard V, Fenneteau O, Salignac S, Lecompte TP, Schlegel N. Platelet morphology analysis. Methods Mol Biol. 2013;992:207-25. DOI: 10.1007/978-1-62703-339-8_16
16. Ali HEA, Alarabi AB, Karim ZA, Rodriguez V, Hernandez KR, Lozano PA, El-Halawany MS, Alshbool FZ, Khasawneh FT. In utero thirdhand smoke exposure modulates platelet function in a sex-dependent manner. Haematologica. 2022;107(1):312-5. DOI: 10.3324/haematol.2021.279388.
17. Bancroft JD, Layton C. The hematoxylins and eosin. In: Bancroft JD, Layton C, Suvarna KS, editors. Theory and practice of histological techniques. 7th ed. Philadelphia: Elsevier; 2012. p. 172-214.
18. Liu, T., Fu, Y., Shi, J. Noninvasive ultrasound stimulation to treat myocarditis through splenic neuro-immune regulation. *J Neuroinflammation*, 2023; 20, 94. DOI: https://doi.org/10.1186/s12974-023-02773-2.
19. Coussons-Read ME, Okun ML, Nettles CD. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain Behav Immun. 2007;21(3):343-50. DOI: https://doi.org/10.1016/j.bbi.2006.08.006
20. O'Connor TG, Winter MA, Hunn J, Carnahan J, Pressman EK, Glover V. Prenatal maternal anxiety predicts reduced adaptive immunity in infants. Brain Behav Immun. 2013;32(1):21-8. DOI: https://doi.org/10.1016/j.bbi.2013.02.002
21. Sinkora M, Butler JE, Holtmeier W, Sinkorova J. Lymphocyte development in foetal piglets: facts and surprises. Vet Immunol Immunopathol. 2005;108(3-4):177-84. DOI: https://doi.org/10.1016/j.vetimm.2005.08.013
22. Chukwu OO, Iyare CO, Emelike CU, Konyefom NG, Okorocha AE, Ekakitie OO, Ibekailo SN, Ezimah ACU. Impact of gestational stress and administration of *Moringa oleifera* leaves on post-natal reproductive development of male offspring of Wistar rats. Trop J Nat Prod Res. 2025 Jan 1;9(1). DOI: 10.26538/tjnpr/v9i1.43

23. Liu, T., Fu, Y., Shi, J. Noninvasive ultrasound stimulation to treat myocarditis through splenic neuro-immune regulation. *J Neuroinflammation*, 2023; 20, 94. DOI: https://doi.org/10.1186/s12974-023-02773-2.
24. Chen L, Bennett E, Wheeler AJ, Lyons AB, Woods GM, Johnston F, et al. Maternal exposure to particulate matter alters early post-natal lung function and immune cell development. Environ Res. 2018;164:625-35. doi: 10.1016/j.envres.2018.03.029.