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# Preconception Dietary Exposure to Calcium Carbide-Ripened Bananas Alters Fetal Cerebellar Histogenesis and Spatial Memory in Wistar Rats

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**Abstract:**

- ***Background:***

**Artificial ripening of bananas with calcium carbide ($CaC_2$) is widely practiced despite concerns regarding toxic impurities, particularly arsenic, which may adversely affect fetal neurodevelopment following maternal exposure. This study investigated the effects of maternal preconception consumption of calcium carbide-ripened bananas on fetal cerebellar histogenesis and spatial reference memory in Wistar rat offspring.**

- ***Methods:***

**Thirty-two adult Wistar rats (24 females and 8 males) were assigned to four groups. Females received diets containing naturally ripened banana (control), 1% $CaC_2$-ripened banana, 2% $CaC_2$-ripened banana, or commercially ripened banana for two weeks before mating. Fetal cerebella were processed for haematoxylin and eosin histology, while offspring spatial reference memory was assessed using the eight-arm radial maze. Data were analysed using independent-samples *t*-tests at $p < 0.05$.**

- ***Results:***

**Offspring from the 1% and 2% $CaC_2$-ripened banana groups completed the reference memory task in significantly shorter times than the positive control ($p = 0.026$ and $p = 0.014$, respectively), whereas the market sample group showed no significant difference. Histological examination revealed normal cerebellar layer differentiation in the control group, thinning of the developing granular layer in both $CaC_2$-treated groups, and poorly developed neuroblasts with poor staining affinity in the market sample group.**

- ***Conclusion:***

**Maternal preconception consumption of calcium carbide-ripened bananas was associated with altered fetal cerebellar histogenesis and differences in offspring spatial reference memory, suggesting potential developmental neurotoxicity of artificially ripened bananas.**

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## I. INTRODUCTION

Artificial fruit ripening using calcium carbide ($CaC_2$) is widely practiced in many developing countries because it accelerates fruit maturation and improves market availability (Okeke *et al*., 2022). However, commercial calcium carbide often contains toxic impurities, including arsenic and phosphorus compounds, which may contaminate ripened fruits and pose potential health risks following consumption (Vidhya *et al*., 2025; Vu *et al*., 2025). Maternal exposure to these toxicants is of particular concern because they can cross the placental barrier and interfere with critical stages of fetal neurodevelopment (Cardenas-Iniguez *et al*., 2022; Jiang *et al*., 2023). The cerebellum, which regulates motor coordination, balance, and contributes to cognitive processes such as learning and spatial memory, undergoes rapid growth during prenatal development and is especially vulnerable to toxic insults (Kim *et al*., 2023).

Previous studies have associated arsenic exposure with oxidative stress, impaired neurogenesis, neuronal apoptosis, and structural abnormalities in the developing brain (Thakur *et al*., 2021; Okeke *et al*., 2022; Tasci *et al*., 2024). Nevertheless, most investigations have focused on environmental or occupational arsenic exposure, while limited attention has been given to maternal dietary exposure through consumption of calcium carbide-ripened fruits. Consequently, the effects of preconception consumption of carbide-ripened bananas on fetal cerebellar development and subsequent cognitive function remain poorly understood (Parvez and Chen, 2023; Enyioma-Alozie and Ugbah, 2025).

This study investigated whether maternal dietary exposure to calcium carbide-ripened bananas before conception alters fetal cerebellar histogenesis and spatial memory in Wistar rat offspring. Cerebellar histomorphology was examined using haematoxylin and eosin staining, while offspring spatial reference memory was evaluated using the eight-arm radial maze. The findings provide experimental evidence on the potential developmental neurotoxicity associated with the consumption of artificially ripened bananas and contribute to food safety and maternal health research.

## II. MATERIALS AND METHODS

➢ *Study Design and Experimental Animals*

This experimental study utilized healthy adult Wistar rats maintained under standard laboratory conditions with free access to feed and water. Female rats were randomly assigned to four experimental groups and fed their respective diets for two weeks before mating.

➢ *Preparation of Experimental Diets*

Fresh unripe bananas (*Musa paradisiaca*) were obtain from International Market, Abakaliki. The obtained bananas were artificially ripened using 1% and 2% calcium carbide ($CaC_2$) solutions, while naturally ripened bananas served as the control. A market-purchased carbide-ripened banana sample was included to simulate common consumer exposure. Banana pulp was homogenized and incorporated into standard laboratory chow before administration.

➢ *Experimental Animals and Grouping*

Thirty-two healthy adult Wistar rats, comprising 24 females and 8 fertile males (female:male ratio, 3:1), were used in this study. The animals were obtained from the Animal House of the Department of Anatomy and were acclimatized for two weeks under standard laboratory conditions (temperature 22–25°C, 12 h light/12 h dark cycle) with free access to standard laboratory chow and clean drinking water *ad libitum*. Following acclimatization, the female rats were randomly allocated into four experimental groups (n = 6 females per group), while two fertile males were assigned to each group for mating. Group A (positive control) received standard laboratory chow supplemented with naturally ripened banana. Group B received chow prepared with 1% calcium carbide ($CaC_2$)-ripened banana, Group C received chow prepared with 2% $CaC_2$-ripened banana, and Group D received chow prepared with commercially ripened banana. The experimental diets were administered daily for two weeks before mating (preconception period), after which females were paired with fertile males at a ratio of one male to three females.

➢ *Mating Procedure*

After the two-week preconception dietary exposure, females were paired with fertile males at a 3:1 female-to-male ratio overnight. Successful mating was confirmed by the presence of a vaginal plug or spermatozoa in vaginal smears, which was designated as gestational day 0 (GD0).

➢ *Spatial Memory Assessment*

Spatial reference memory was assessed in the offspring following postnatal maturation using the eight-arm radial maze, as described by Shimizu et al. (2025). The total time required for each animal to correctly visit all baited arms without repetition was recorded and used as the primary index of spatial reference memory performance.

➢ *Tissue Collection and Histological Analysis*

Pregnant dams were sacrificed at the predetermined gestational stage, and fetal brains were harvested. Cerebellar tissues were fixed in 10% neutral-buffered formalin, processed routinely, embedded in paraffin wax, sectioned at 3–5 µm, and stained with haematoxylin and eosin for histomorphological evaluation under light microscopy.

➢ *Statistical Analysis*

Data were expressed as mean ± standard error of the mean (SEM). Comparisons between the positive control and treatment groups were performed using independent-samples *t*-tests. Statistical significance was set at $p < 0.05$.

## III. RESULTS

➢ *Reference Memory Assessment*

Table 2 shows the comparison of the total time taken to visit all arms during the reference memory assessment. Offspring in the positive control group required 223.33 ± 80.54 s to complete the task, while the negative control

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recorded 218.33 ± 81.43 s, with no significant difference between the groups (p = 0.091). In contrast, offspring from Group B (180.78 ± 64.03 s) and Group C (200.03 ± 72.63 s) completed the task in significantly shorter times than the positive control (p = 0.026 and p = 0.014, respectively). The market sample group (220.67 ± 81.01 s) showed a completion time comparable to the positive control, with no statistically significant difference (p = 0.120).

Table 1 Reference Memory Assessment: Comparison of Total Time to Visit All Arms among Experimental Groups

| Comparison | Positive Control (2A) Mean ± SEM (s) | Comparison Group Mean ± SEM (s) | *p*-value |
|---|---|---|---|
| A vs Negative Control | 223.33 ± 80.54 | 218.33 ± 81.43 | 0.091 |
| A vs Group B | 223.33 ± 80.54 | 180.78 ± 64.03 | 0.026* |
| A vs Group C | 223.33 ± 80.54 | 200.03 ± 72.63 | 0.014* |
| A vs Market Sample (D) | 223.33 ± 80.54 | 220.67 ± 81.01 | 0.120 |

Values were expressed as mean ± standard error of the mean (SEM). Pairwise comparisons between the positive control (2A) and the negative control, treatment groups, and market sample were performed using an independent samples *t*-test. *Statistical significance was accepted at* $p < 0.05$.

➢ *Histological Observation*

Figure 1 shows the cerebellar section of fetuses from the positive control group, demonstrating neuroblasts differentiating into distinct histological layers, including the granular layer, with an apparently normal pattern of development. The developing cerebellar architecture appeared well organized without obvious structural abnormalities. Figure 2 shows the cerebellar section of fetuses from Group B, revealing thinning of the developing neuroblasts, particularly within the granular layer. The reduction in the thickness of this layer was evident when compared with the positive control. Figure 3 shows the cerebellar section of fetuses from Group C, also demonstrating thinning of the developing neuroblasts, especially within the granular layer, with the general cerebellar organization remaining identifiable. Figure 4 shows the cerebellar section of fetuses from the market sample group, characterized by poor affinity for haematoxylin and eosin staining and the presence of poorly developed neuroblasts. These observations indicate differences in cerebellar histological appearance among the experimental groups without inferring changes beyond those directly observed.

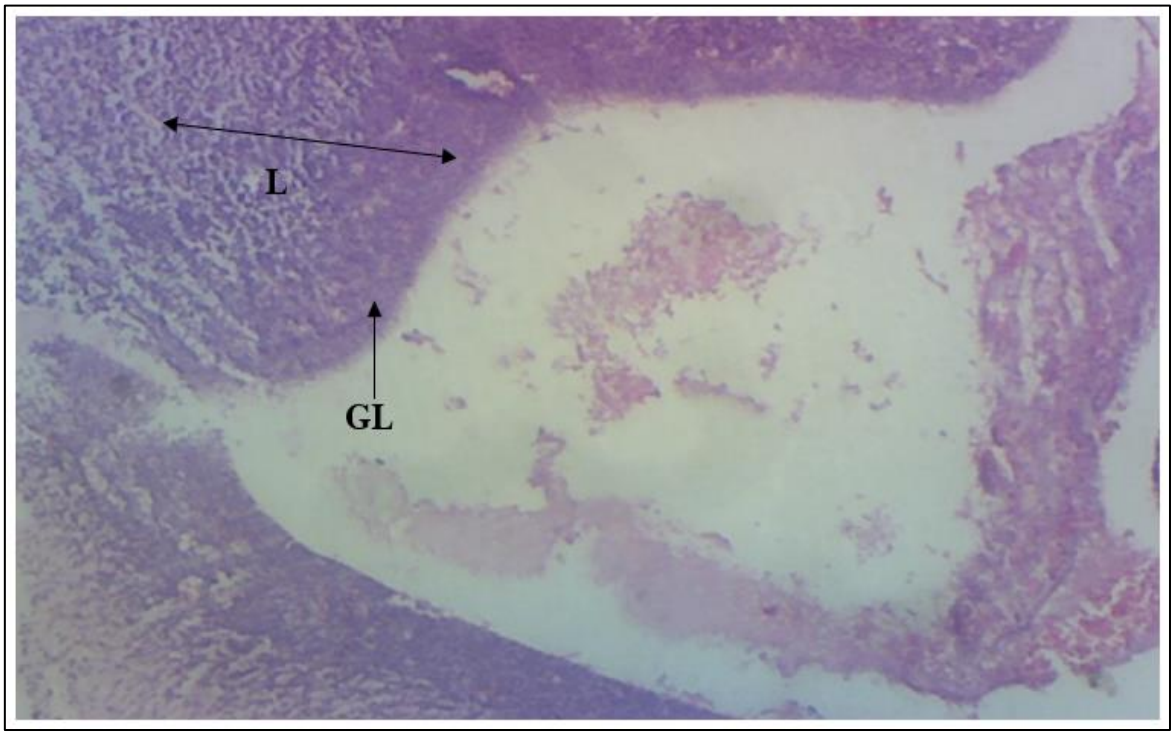


Fig 1 Cerebellar Section of Fetus whose Mother Ate Food Prepared with 0% Carbide Ripened Banana before Pregnancy Showing Neuroblast Differemtiating into their Histological Layers (L, GL) Appearing to be in a Normal Progression. H and E; X60.

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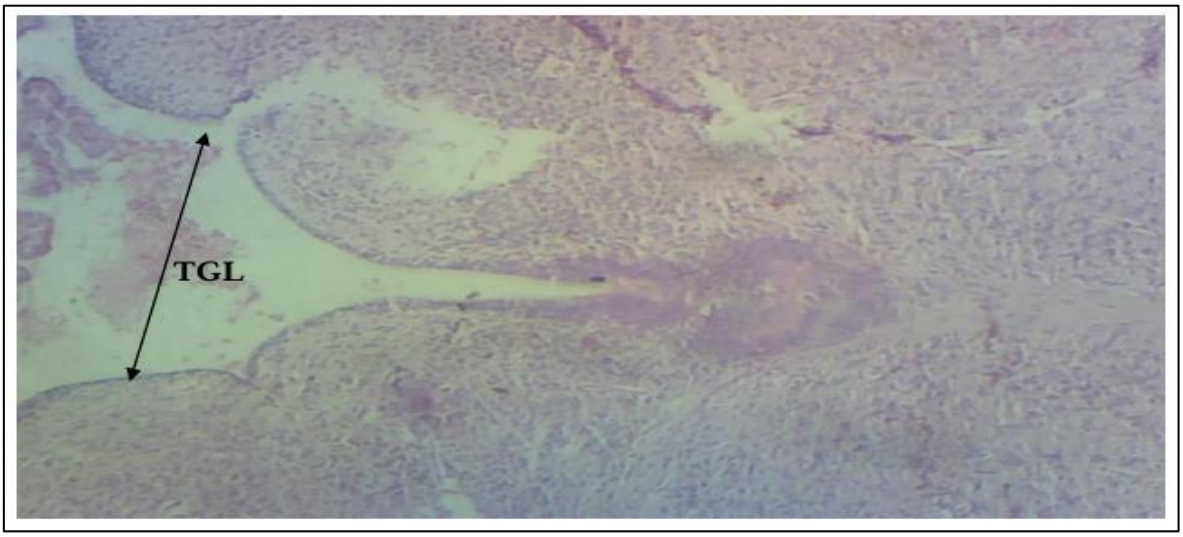


Fig 2 Cerebellar Section of Fetus whose Mother Ate Food Prepared with 1% Carbide Ripened Banana before Pregnancy Showing the Presence of Thinning of Developing Neuroblast Especially the Granular Layer (TGL). H and E; X60.

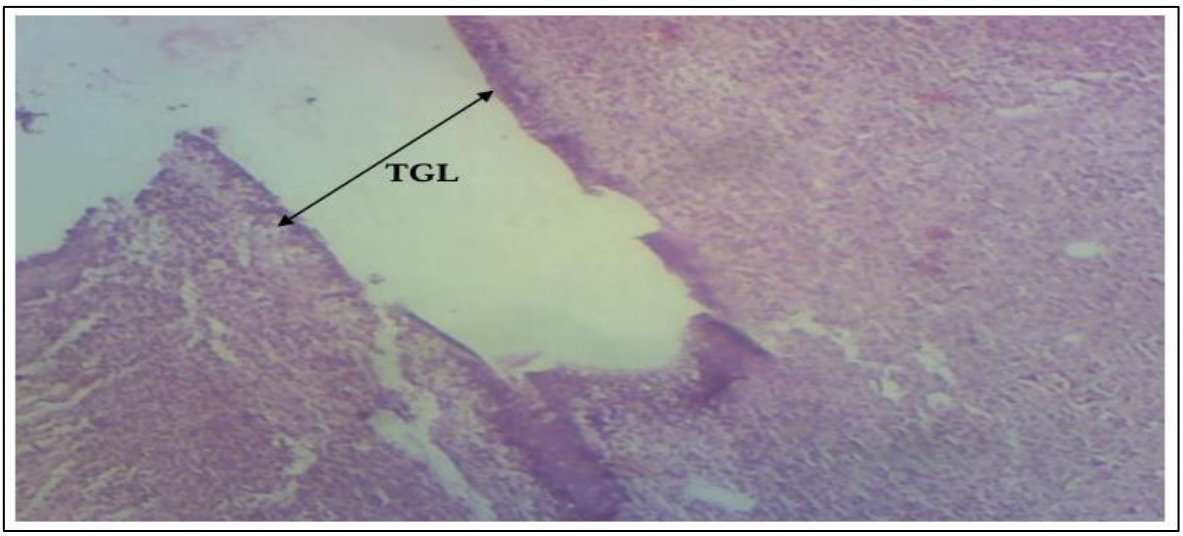


Fig 3 Cerebellar Section of Fetus whose Mother Ate Food Prepared with 2% Carbide Ripened Banana before Pregnancy Showing the Presence of Thinning of Developing Neuroblast Especially the Granular Layer. H and E; X60.

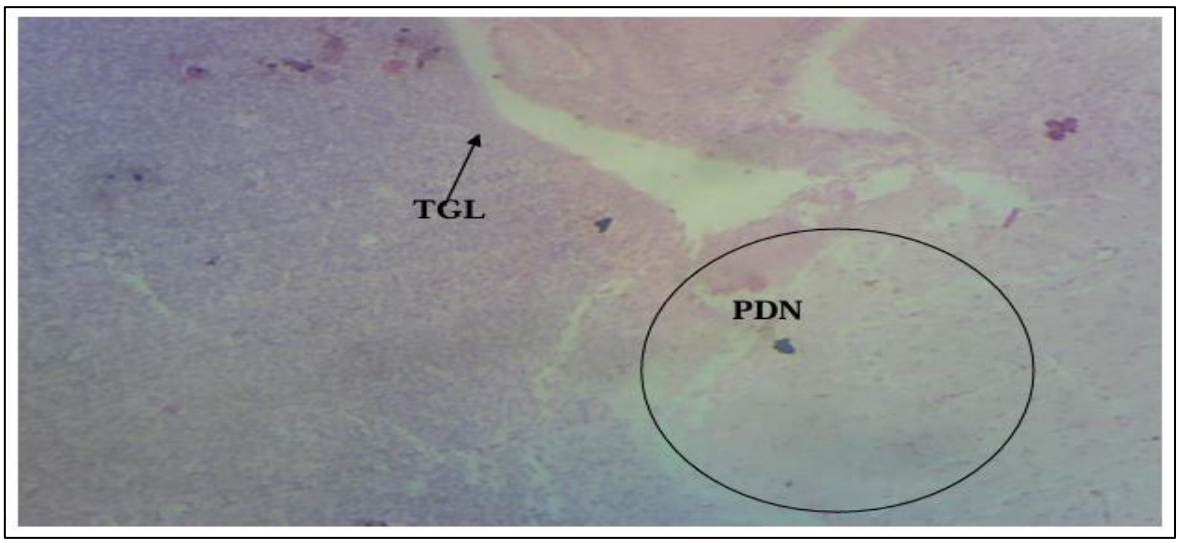


Fig 4 Cerebellar Section of Fetus whose Mother Ate Food Prepared with Commercially Ripened Banana with Carbide before Pregnancy Showing the Presence of Poor Adhesion to Staining which is Always as a Result Poor Developing Neuroblast (PDN). H and E; X60.

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## IV. DISCUSSION

➢ *Discussion*

The present study investigated the effects of maternal preconception consumption of calcium carbide-ripened bananas on fetal cerebellar histogenesis and offspring spatial reference memory in Wistar rats. Histological examination revealed differences in cerebellar morphology between the control and treatment groups. Fetuses from the positive control group exhibited normal differentiation of neuroblasts into distinct cerebellar layers, including the granular layer, indicating an apparently normal developmental pattern. In contrast, fetuses from the 1% and 2% calcium carbide-ripened banana groups demonstrated thinning of the developing neuroblasts, particularly within the granular layer. Similarly, the market sample group showed poor affinity for haematoxylin and eosin staining accompanied by poorly developed neuroblasts. These observations suggest that maternal dietary exposure to calcium carbide-ripened bananas before conception was associated with alterations in the histological appearance of the developing cerebellum.

The reference memory assessment also demonstrated differences among the experimental groups. Offspring from the 1% and 2% calcium carbide-ripened banana groups completed the radial arm maze in significantly shorter times than the positive control group, whereas the negative control and market sample groups showed no statistically significant differences from the positive control. These findings indicate that preconception dietary exposure influenced performance during the spatial reference memory assessment, although the observed reduction in task completion time should not be interpreted as a direct measure of improved cognitive function without consideration of other behavioural indices (Fan *et al*., 2013; Clouard *et al*., 2016; Martin *et al*., 2021).

The cerebellum is an important component of the central nervous system and contributes to motor coordination, balance, and aspects of learning and memory (Rudolph *et al*., 2023; Zhang *et al*., 2023). Consequently, alterations in the developing granular layer observed in the treatment groups may reflect changes in cerebellar histogenesis during fetal development. The histological findings and behavioural observations should therefore be interpreted together, as structural changes do not necessarily correspond directly with behavioural outcomes.

Overall, the present findings demonstrate that maternal preconception consumption of calcium carbide-ripened bananas was associated with observable alterations in fetal cerebellar histology and differences in offspring performance during spatial reference memory assessment. These results provide experimental evidence that maternal dietary exposure before pregnancy may influence early neurodevelopment and support continued investigation into the developmental effects of artificially ripened fruits.

## V. CONCLUSION

Maternal preconception consumption of calcium carbide-ripened bananas was associated with alterations in fetal cerebellar histogenesis, characterized by thinning of the developing granular layer and poorly developed neuroblasts, alongside differences in offspring spatial reference memory performance. These findings suggest that dietary exposure to artificially ripened bananas before conception may influence fetal neurodevelopment and support the need for improved food safety practices and further mechanistic studies.

## REFERENCES

[1]. Fan, Y., Ding, S., Ye, X., Manyande, A., He, D., Zhao, N., Yang, H., Jin, X., Liu, J., Tian, C. and Xu, S. (2013). Does preconception paternal exposure to a physiologically relevant level of bisphenol A alter spatial memory in an adult rat?. *Hormones and behavior*, *64*(4), 598-604.

[2]. Clouard, C., Kemp, B., Val-Laillet, D., Gerrits, W. J., Bartels, A. C., and Bolhuis, J. E. (2016). Prenatal, but not early postnatal, exposure to a Western diet improves spatial memory of pigs later in life and is paired with changes in maternal prepartum blood lipid levels. *The FASEB Journal*, *30*(7), 2466-2475.

[3]. Grandjean, P., and Landrigan, P. J. (2022). Neurodevelopmental toxicity of industrial chemicals: a global concern. *The Lancet Public Health*, 7(3), e236–e247.

[4]. Jiang, Xiaodong, Du, Juan, and Chen, Michael. (2023). Mechanisms of regulated neurodevelopment and cellular migration. *Annual Review of Neuroscience*, 46, 121–145.

[5]. Kim, M., Jun, S., Park, H., Tanaka-Yamamoto, K., and Yamamoto, Y. (2023). Regulation of cerebellar network development by granule cells and their molecules. *Frontiers in Molecular Neuroscience*, 16, 1236015.

[6]. Enyioma-Alozie, S., and Ugbah, V. (2025). Embryofetal toxicity of artificially ripened fruits: Effects of calcium carbide ripened mango fruits on fetal development in Wistar rats. *Innovation in Science and Technology*, 4(4), 13–25.

[7]. Martin, D., Papageorgiou, M., Colgan, H., Bandelow, S., Greeves, J.P., Tang, J.C., Fraser, W.D., Cooper, S.B., Sale, C. and Elliott-Sale, K.J. (2021). The effects of short-term low energy availability, achieved through diet or exercise, on cognitive function in oral contraceptive users and eumenorrheic women. *Applied Physiology, Nutrition, and Metabolism*, *46*(7), 781-789.

[8]. Okeke, E. S., Okagu, I. U., Okoye, C. O., and Ezeorba, T. P. C. (2022). Calcium carbide in fruit ripening: Mechanisms of toxicity. *Toxicology*, 468, 153112.

[9]. Parvez, S., and Chen, Y. (2023). Environmental arsenic exposure, microRNA dysregulation, and neurodevelopmental outcomes. *Toxicological Sciences*, 156(1), 74–87.

[10]. Rudolph, S., Badura, A., Lutzu, S., Pathak, S.S., Thieme, A., Verpeut, J.L., Wagner, M.J., Yang, Y.M. and Fioravante, D. (2023). Cognitive-affective functions of the cerebellum. *Journal of Neuroscience*, *43*(45), 7554-7564.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul708

[11]. Shimizu, T., Nayar, S. G., Ransom, B. R., and Richardson, W. D. (2025). An Appetitive Spatial Working Memory Task for Mice in a Semi-Automated 8-Arm Radial Maze, Reducing Fearful Memory Association in the Maze. *J. Vis. Exp*, *221*, e66456.
[12]. Tasci, T., Orta-Yilmaz, B., Aydin, Y., and Caliskan, M. (2024). N-acetylcysteine attenuates sodium arsenite-induced oxidative stress and apoptosis in embryonic fibroblast cells. *Toxicological Research (Cambridge)*, 13(4), tfae128.
[13]. Thakur, M., Rachamalla, M., Niyogi, S., Datusalia, A. K., and Flora, S. J. S. (2021). Molecular mechanism of arsenic-induced neurotoxicity including neuronal dysfunctions. International Journal of Molecular Sciences, 22(18), 10077.
[14]. Vidhya, D., Mahanti, N.K., Barun, Dakho, J., Kumar, A., Chaubey, S. and Chhetri, K.B. (2025). Calcium carbide ripening in fruits: Health risks and regulation. *Comprehensive Reviews in Food Science and Food Safety*, 24, e70140.
[15]. Vu, N. D., Hang, N. T. T. N., Mi, K. D. Q., Anh, N. H. T., and Pham, B. A. (2025). Nutritional composition and biological characteristics of bananas. *Food Chemistry X*, 28, 102594.
[16]. Zhang, P., Duan, L., Ou, Y., Ling, Q., Cao, L., Qian, H., Zhang, J., Wang, J. and Yuan, X. (2023). The cerebellum and cognitive neural networks. *Frontiers in human neuroscience*, *17*, 1197459.