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RESEARCH ARTICLE

IRON INDUCED MICRONUCLEUS FORMATION AND ITS AMELIORATION BY *TACHYSPERMUM AMMI* IN *MUS MUSCULUS*

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ABSTRACT

Iron (Fe) is a heavy metal critical in trace amounts for the cellular functions of humans, plants, and animals, playing an important role in oxygen transport through hemoglobin protein. Nevertheless, excessive Fe intake can lead to toxicity in organisms. In this study, the mitigating effect of *Tachyspermum ammi* seeds against genotoxic damage induced by Iron on PCEs and NCEs in mice bone marrow cells was assessed using the Micronucleus test. The results revealed a significant increase in micronucleus formation in the $FeSO_4$-treated group compared to the control. However, concurrent treatment with *T. ammi* leaf extract and $FeSO_4$ resulted in significantly lower micronucleus formation compared to the $FeSO_4$-treated group, nearly reaching levels equivalent to the control. Hence, while Fe is essential in trace amounts for maintaining metabolic activities in organisms, it is observed that excessive Fe exposure can induce toxicity, potentially affecting genetic integrity.

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INTRODUCTION

Metals represent a significant category of toxic substances come across in everyday life, both in occupational settings and the environment. The effect of these harmful agents on human health is currently a topic of great interest, due to their extensive exposure resulting from increased industrial and everyday usage of several metals. (1,2,3) Heavy metals are significant environmental contaminants and their toxicity poses a major problem for ecological, evolutionary, nutritional and environmental balances. (4,5) Iron is a vital metal present in every cell of the body, where it plays a crucial role in fulfilling numerous physiological and cellular functions. Various diseases are linked to iron metabolism, affecting a large number of individuals. Iron deficiency was recognized by the WHO as the most prevalent nutritional deficiency, affecting approximately 30% of world population.(6) Additionally, there are several diseases that are linked to iron overload and toxicity. The existence of excess iron serves as an adverse prognostic indicator for all diseases. Mainly in individuals heavily burdened with iron, iron-induced toxicity resulting in organ damage and fatalities. In some regions, a significant portion of the population has been exposed to higher levels of iron through drinking water, surpassing the permissible limits set for water quality. (7) Pediatricians have long been concerned about iron poisoning in children due to their heightened susceptibility, given their frequent exposure to iron-containing products. (8) Workers exposed to asbestos, which typically contains approximately thirty percent iron, face an elevated risk of developing asbestosis, the second leading cause of lung cancer. Free radicals may be the significant factor for lung cancer, as asbestos associated cancer have been linked to free radicals. (9) A progressive elevation in body iron levels resulting from persistent increases in dietary iron absorption is noted as a contributing factor to iron overload toxicity in the liver and other organs. (10)The existence of excess iron deposition or unstable forms of iron in any body area poses a potential risk and is ongoing source of toxicity (11,12). Several studies have confirmed the important role of herbs, fruits, and vegetables in ameliorating the toxicity of chemicals and drugs. In this context, *Tachyspermum ammi* revealed to possess antiseptic, stimulant, carminative, diuretic, antiulcer, anesthetic, antimicrobial, antiviral, antiplatelet and antioxidant as well as hepatoprotective effect. (13,14) It contains many chemical constituents, Fiber (11.9%), carbohydrates (24.6%), protein (17.1%), tannins, glycosides, fat (21.1%), moisture (8.9%), saponins, flavones and other components (7.1%) involving calcium, cobalt, phosphorous, iron, copper, iodine, manganese, thiamine, riboflavin and nicotinic acid. (15,16,17,18). Therefore, the current study aims to explore the genotoxic effects of Iron on bone marrow cells of mice using the Micronucleus test. This investigation seeks to assess the incidence of micronuclei formation in polychromatic erythrocytes and normochromatic erythrocytes induced by Iron toxicity, as well as to evaluate the potential mitigating effects of *T. ammi* seeds.

MATERIALS AND METHODS

Swiss albino mice, aged 4-6 weeks with an average weight of 25-30gram was used as an experimental animal. They were separated into 4 groups; each group comprises of 6 mice. This study was carried out in full compliance with the Guide for the Care and Use of Laboratory Animals. Commercially available Iron in the form of Ferrous sulphate was purchased from the local market, Bhagalpur, Bihar in powdered form and was used as toxicant. Freshly collected, shed dried and powdered *T. ammi* (ajwain) seeds in form of extract was used as ameliorating agent.

Experimental Protocol: The group of mice were subjected to specific treatment outlined in table-I with treatment duration of 15 days. Following the completion of the treatment period, the mice were euthanized via cervical dislocation. Slides were prepared using the method proposed by Schmid (1976), (19) with minor adaptations as suggested by Das and Kar (1986) (20) and Salamone and Heddle (1983). (21) The slides were examined, with micronuclei in polychromatic erythrocytes (PCEs) stained blue and normochromatic erythrocytes (NCEs)stained pink. Approximately 1500PCEs and NCEs were counted per animal within each experimental group. Statistical analysis was conducted using the T-test.

RESULTS

To assess the occurrence of polychromatic and normochromatic erythrocytes, the micronucleus test was conducted across all four experimental groups. In the control group, a total of 17 (0.53 ± 0.12) micronuclei were observed, with 9(0.57 ± 0.18) found in PCEs and 8 (0.50 ± 0.17) in NCEs.

These findings indicate a notably low frequency of micronucleated erythrocytes in the control group. In another group of mice, only iron ($FeSO_4$)was administered, resulting in a total of 491micronuclei (15.91 ± 0.65), with 264 (17.46±0.97) observed in PCEs and 227(14.42 ± 0.88) in NCEs. This outcome exhibited a significantly higher value compared to the control group, suggesting that paracetamol induces cellular damage and leads to the highest frequency of micronuclei formation. In a separate group, ajwain seed extract was administered to the animals, yielding a total of 15 micronuclei (0.48 ± 0.12), with 9 (0.56 ± 0.18) found in PCEs and 6 (0.39 ± 0.15) in NCEs.

These values were lower than those of the control group, indicating a slight reduction in micronucleus formation compared to the control, although this decrease was not statistically significant. When *T.ammi* leaf extract was administered concurrently with iron, a total of 46 micronuclei (1.47 ± 0.21) were observed, with 27 (1.74 ± 0.33) found in PCEs and 19 (1.21 ± 0.27) in NCEs. The results from this group suggest that the leaf extract of *T.ammi* was highly effective in reducing the number of micronuclei induced by iron. It is noteworthy that the occurrence of micronuclei was relatively higher in PCEs across all four groups.

DISCUSSION

Based on the aforementioned findings, it can be inferred that Iron serves as a potent genotoxic agent, contributing to the escalation of micronucleus formation in both PCE and NCE. The excessive buildup of heavy metals in organisms initiates the generation of reactive nitrogen and oxygen species (22).

Acting as a transition metal, iron (Fe) amplifies the production of hydroxyl radicals through Fenton and Haber-Weiss reactions. Furthermore, Fe has the potential to interact with DNA and proteins, leading to their functional deterioration (23). Research conducted in in vivo model systems indicates that oxidative harm is a significant contributor to Fe toxicity (24). Moreover, it has been documented that overconsumption of dietary Fe heightens the susceptibility to various forms of cancer (24).

Table I. Experimental protocol

S. No.	Experimental Variant	Symbol	Dose
1.	Control	C	No treatment of IR and TA
2.	Iron ($FeSO_4$) powder	IR	400 mg/kg.b.wt.
3.	*T. ammi*seed powder	TA	250 mg/kg.b.wt.
4.	Both Iron and Ajwain powder concurrently	IR+TA	As 2 & 3

Table-II. The occurrence of micronucleated PCEs and NCEs, assessed in the bone marrow cells of mice treated with Iron (IR) and *T. ammi*extract (TA) over a 15-day period

Treatment	PCEs			NCEs			PCEs + NCEs		
	Score	Mn	% ± S.E.	Score	Mn	% ± S.E.	Score	Mn	% ± S.E.
C	1575	9	0.57±0.18	1587	8	0.50±0.17	3162	17	0.53 ± 0.12
IR	1512	264	17.46±0.97[a]	1574	227	14.42 ± 0.88[a]	3086	491	15.91 ± 0.65[a]
TA	1583	9	0.56 ± 0.18[b]	1525	6	0.39 ± 0.15[b]	3108	15	0.48 ± 0.12[b]
IR +TA	1548	27	1.74±0.33[abc]	1563	19	1.21 ± 0.27[abc]	3111	46	1.47 ±0.21[abc]

a, b and c indicate significant difference with corresponding value in the control (C), iron (IR) and *Tachyspermumammi* (TA) respectively

CONCLUSION

While iron (Fe) is crucial for normal biological functions, excessive intake can lead to toxicity. The current study revealed the genotoxic impact of Fe, evident through the formation of micronuclei (MN) in bone marrow cells of M. musculus following administration of $FeSO_4$. *T. ammi* seeds, rich in antioxidants, were found to alleviate this toxicity. Consequently, it is recommended to incorporate antioxidant-rich herbs into our diet to mitigate the toxicity of various chemicals or heavy metals.

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CONFLICT OF INTEREST: No conflict of interest

REFERENCES

Guan, D. S., Chen, Y. J., & Ruan, G. D. (2001). Study on heavy metal concentrations and the impact of human activity on them in urban and suburb soils of Guangzhou. *Acta Scientiarum Naturalium Unversitatis Sunyatseni*, *40*(4), 93-6.

Yan, J., Ye, Z. X., Yan, Y., & Huang, X. P. (2008). Study on heavy metals distribution in atmospheric particulate matter on both sides of the Cheng-Ya Expressway. *Sichuan environment*, *2*(2), 19-21.

Christoforidis, A., & Stamatis, N. (2009). Heavy metal contamination in street dust and roadside soil along the major national road in Kavala's region, Greece. *Geoderma*, *151*(3-4), 257-263.

Ra, G. O. Y. (1991). Toxic effects of metals. *Casarett and Doull's toxicology*.

Wang, S., & Shi, X. (2001). Molecular mechanisms of metal toxicity and carcinogenesis. *Molecular and cellular biochemistry*, *222*, 3-9.

Murphy, J. F. (2002). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and mineral nutrition information system. Geneva: World Health Organization; 2011.

Demeke, A., & Tassew, A. (2016). A review on water quality and its impact on fish health. *International journal of fauna and biological studies*, *3*(1), 21-31.

Hershko, C. (2007). Mechanism of iron toxicity. *Food and nutrition bulletin*, *28*(4_suppl4), S500-S509.

Ghosh, L., Adhikari, S., & Ayyappan, S. (2007). Assessment of toxic interactions of heavy metals and their effects on accumulation in tissues of freshwater fish.

Adams, P. C. (2015). Epidemiology and diagnostic testing for hemochromatosis and iron overload. *International journal of laboratory hematology*, *37*, 25-30.

Iancu, T. C. (1983). Iron overload. *Molecular aspects of medicine*, *6*(1), 1-100.

Zurlo, M., De Stefano, P., Borgna-Pignatti, C., Di Palma, A., Melevendi, C., Piga, A., ... & Terzoli, S. (1989). Survival and causes of death in thalassaemia major. *The Lancet*, *334*(8653), 27-30.

Goyal, S., Chaturvedi, V., Dhingra, G., Tanwar, S., Sharma, K., & Singh, S. (2022). Trachyspermum ammi: A review on traditional and modern pharmacological aspects. *Biological Sciences*, *2*(4), 324-337.

Vharamble, N. R., Patil, S. B., Naik, O. S., & Shah, R. (2023). Overview of Trachyspermum ammi and its Medicinal applications. *Research Journal of Pharmacognosy and Phytochemistry*, *15*(4), 319-323.

Duke, J. A. (1992). Database of phytochemical constituents of GRAS herbs and other economic plants.

Qureshi, A. A., & Kumar, K. E. (2010). Phytochemical constituents and pharmacological activities of Trachyspermum ammi.

Ranjan B, Manmohan S, Singh SR, Singh RB. Medicinal uses of *Trachyspermum ammi*: a review. Pharmacogn Rev. 2012; 6(11): 56–60.

Bhadra, P. (2020). An Overview of Ajwain (Trachyspermum ammi). *Indian Journal of Natural Sciences*, *10*(59), 18466-182474.

Schmid, W. (1976). The micronucleus test for cytogenetic analysis. *Chemical mutagens: principles and methods for their detection*, 31-53.

Das, R. K., & Kar, R. N. (1980). Sodium citrate as a substitute for fetal calf serum in the micronucleus test. *Stain Technology*, *55*(1), 43-45.

Salamone, M. F., & Heddle, J. A. (1983). The bone marrow micronucleus assay: rationale for a revised protocol. In *Chemical Mutagens: Principles and Methods for Their Detection Volume 8* (pp. 111-149). Boston, MA: Springer US.

Kim, J. J., Kim, Y. S., & Kumar, V. (2019). Heavy metal toxicity: An update of chelating therapeutic strategies. *Journal of Trace elements in Medicine and Biology*, *54*, 226-231.

Valko, M., Jomova, K., Rhodes, C. J., & Kuča, K. and non-redox-metal-induced formation of free radicals and their role in human disease., 2016, 90. 1-37.

Dunkel, V. C., San, R. H., Seifried, H. E., & Whittaker, P. (1999). Genotoxicity of iron compounds in Salmonella typhimurium and L5178Y mouse lymphoma cells. *Environmental and molecular mutagenesis*, *33*(1), 28-41.

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