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RESEARCH ARTICLE

EVALUATION OF MUTAGENICITY INDUCED BY IRON TABLETS IN MOUSE SPERM CELLS AND ITS AMELIORATION BY *AEGLE MARMELOS* LEAVES

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ABSTRACT

Iron supplementation is commonly prescribed to prevent or treat iron deficiency anaemia. However, its potential mutagenic effects on reproductive cells have been a topic of increasing concern. This study aims to evaluate the mutagenic effect of iron tablets on mouse sperm cells and the productive role of *Aegle marmelos* leaves, a medicinal plant. In India it's common name is bel patra. We conducted a series of experiment involving the administration of iron tablets to male mice and assessed the induced mutagenic effect in sperm cells, by analysing the sperm morphology. Additionally, we explored the ameliorative potential of *Aegle marmelos* leaf extract, which has been previously recognised for its antioxidant and protective properties. Results showed that iron supplementation significantly increased the frequency of sperm abnormalities and DNA damage. However treatment with *Aegle marmelos* possesess potential protective properties against the mutagenic impact of iron supplementation on male reproductive health.

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INTRODUCTION

Iron is an essential mineral needed for many physiological processes, such as cellular respiration and oxygen transport (Anderson *et al*., 2017). Iron deficiency anaemia is commonly treated with iron supplements, especially in high-risk groups like pregnant women, newborns, and people with chronic blood loss (Zimmermann *et al*., 2007). Despite its advantages, consuming too much iron can harm different organ systems. Specifically, oxidative stress, cellular damage, and mutagenicity in several tissue, including reproductive cells, have been linked to iron overload (Fenton *et al*., 2003). Sperm cells are particularly vulnerable to genetic damage from environmental or chemical sources because of their weak antioxidant defenses, which make them extremely vulnerable to oxidative stress. According to current research, sperm abnormalities may result from high iron supplementation. decreased fertility and DNA fragmentation(Agarwal *et al*., 2014). Furthermore, it is yet unknown whether iron has any mutagenic impact on male reproductive health, which calls for greater research. The medicinal plant *Aegle marmelos* has long been utilized in ayurveda medicine due to its many therapeutic qualities, such as its anti-inflammatory and antioxidant activities, which help to reduce oxidative cell damage (Singh *et al*., (2010).

The carcinogenic effect that iron supplementation has on sperm cells may be mitigated by *aeglemarmelos*, a natural remedy. By connecting the present health risks of oxidative stress with excessive iron intake. The goal of the study is to present a thorough understanding of the problems caused by iron tablets and investigate possible mitigation strategies using natural plant-based therapies. The findings of this study improved our knowledge of *Aegle marmelos's* function in reducing iron-induced cytotoxicity and may help develop safer usage guidelines for iron supplements. Since, sperm morphology is one of the convenient and widely used techniques in genetic toxicology and has potential in identifying chemical that induces spermatogenic dysfunction and perhaps heritable mutations (wyrobek *et al*., 1983).

MATERIALS AND METHODS

Experimental Animal: A 4-6 weeks old Swiss Albino (male mouse) with an average body weight of 25 grams where used as test animal. Mice were divided into 4 experimental groups, 6 mice ineach group as shown in table-1 and was subjected for treatment duration of 60 days. Animals were obtained from animal house colony, University department of zoology, TMBU.

Treatment Groups

- Control group(no treatment)
- Iron tablet treatment group
- Bael leaves treated group
- Iron+Bael leaves

Animals were caged in 4 different groups and were provided with normal laboratory as well as nutritional condition throughout the period of the experiment to treatment iron tablets of 200 mg/kg bodyweight (Jain, et.al, 2022) were used in the powdered form and bael leaves were shed dried and powdered (250mg/kg bodyweight).

Iron Supplementation: Iron tablets were given orally to experimental group of mice for 60 days. The dosage was based on previous study indicating its potential to induced oxidative stress and DNA damage.

Aegle marmelos leaf extract: The Aegle marmelos leaf were, dried and powdered. An aquousextract was prepared by boiling the powdered leaves in distilled water at controlled temperature in water bath. The extract was then filtered and concentrated

Dosage

Sperm collection and analysis: After 60 days of treatment, sperms were collected from the Cauda Epididymis of each mouse. Sperm concentration, motility and morphology were assesed using microscope.

RESULTS

Sperm Morphology: Mice treated with iron tablets showed a significant & increase in sperm abnormalities including coiled tail, hookless, dumble shaped, fused head, hammer head, banana shaped, pin headed etc. The result from the experiment done shows that value of control where no treatment was done shows the value of (3% with standard error value of 2.93. The mice treated with iron tablet for 35 days shows abnormality of 56.06%. which was much higher compared to control. similarly, the groups treated with Bel leaves show that percentage value of 2.28% which was upto the control level and no significant value was observed. the group treated with iron and bel leaves shows the percentage abnormality value of 3.64% which was upto control level and very much similar to the volume of 2.28%.

STATISTICAL ANALYSIS: For statistical analysis t-test were used for the evaluation of data.

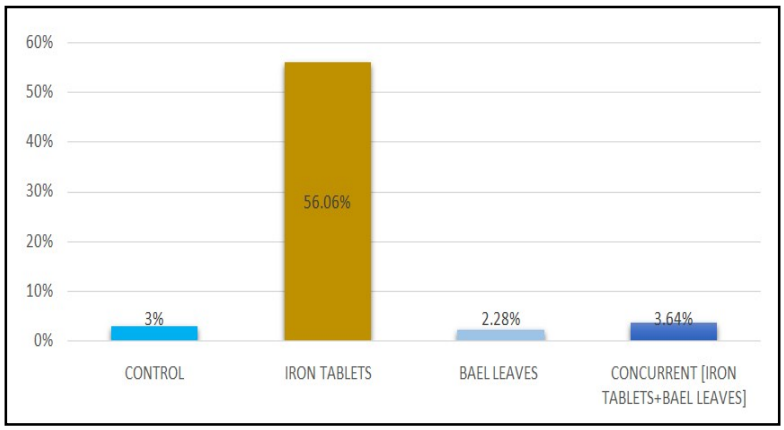

Figure 1. Graph showing the percentage of four experimental variant group

Figure 2. Abnormalities examined under microscope in sperms cells of iron tablets treated group

Treatment varient	Symbols	No.of sperms studied	No.of abnormal sperms	%	% ± s.e.
Control	C	2500	75	3%	3 ± 2.93
Iron tablets	I	2208	1238	56.06%	56.06* ± 1.055
Bael leaves	B	1922	44	2.28%	2.28 ± 0.221
Concurrent [iron Tablets+bael leaves]	I+B	1810	66	3.64%	3.640.225

**indicates the significant difference with that of control.*

DISCUSSION

- This experiment shows various sperm abnormalities like hookless, hammer headed, dumble headed were observed most abruptly in a iron treated group.
- Total sperm abnormality in iron treated group were significantly (56.06%) higher than control (3%).
- When Aegle marmelos leaf expert with iron shows value.3.64% the value was significantly lower than iron treated and almost equivalent upto the control value.
- Iron treatment with the concurrent treatment of Bael leaf extract significantly minimized the mutagenic and genotoxic effect of iron tablet and thus produced a suitable ameliorating effect against iron tablet induced mutagenicity.

CONCLUSION

From the obtained result it is concluded that iron tablets produce various morphological deformities or disrupted structural morphology in the treated sperm cells. Baelleave extract can be potent ameliorating agent. The objective of the experiment is to make people aware regarding the health hazards caused by iron supplements and to promote the usage of Bael leave extract for its antioxidant rich property.

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