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Electrophoretic Analysis of Seminal LDH Isoenzyme Activity in Male Swiss Albino Mice Treated with Aqueous Seed Extract of *Foeniculum Vulgare*

Anand kumar[1], Roushni Parween[2]

[1,2] University Department of Zoology, T.M. Bhagalpur University, Bhagalpur, Bihar, India

Abstract

The present study was designed to evaluate the antifertility effect of aqueous seed extract of *Foeniculum vulgare* on seminal lactate dehydrogenase (LDH) isoenzyme activity in male Swiss albino mice using electrophoretic techniques. A total of 54 healthy male Swiss albino mice were divided into three groups consisting of control, low dose, and high dose treated animals. The aqueous seed extract was administered orally for 15, 30, and 45 days. Electrophoretic analysis revealed significant alterations in LDH isoenzyme patterns in treated groups. LDH-1, LDH-2, and LDH-3 activities gradually decreased with increasing duration and dose of treatment, whereas LDH-4 and LDH-5 activities increased markedly. A significant reduction in LDH-C4 activity was observed in both treatment groups, indicating impairment of sperm-specific metabolic activity. The findings suggest that aqueous seed extract of *Foeniculum vulgare* adversely affects seminal metabolism and may contribute to antifertility effects in male mice.

Keywords: *Foeniculum vulgare*, LDH isoenzymes, electrophoresis, antifertility, seminal plasma, Swiss albino mice.

1. Introduction

Male infertility is an important reproductive health problem associated with hormonal imbalance, oxidative stress, and altered testicular metabolism (Agarwal et al., 2014). Medicinal plants have long been investigated for their reproductive effects because of the presence of biologically active phytochemicals. Among these medicinal plants *Foeniculum vulgare* commonly known as fennel, has gained scientific attention due to its estrogenic, antioxidant, and reproductive modulatory properties (Rather et al., 2016). The seeds of *Foeniculum vulgare* contain flavonoids, alkaloids, phenolic compounds, and essential oils such as anethole and fenchone. These phytoconstituents are known to influence reproductive physiology and may alter spermatogenesis and seminal biochemical parameters (Badgujar et al., 2014). Several studies have suggested that fennel extracts can affect sperm count, sperm motility, testosterone secretion, and testicular histology in experimental animals (Mansouri et al., 2016). Lactate dehydrogenase (LDH) is an important glycolytic enzyme involved in anaerobic metabolism. It catalyzes the reversible conversion of pyruvate into lactate and provides energy necessary for sperm metabolism and motility (Bisht et al., 2017). LDH exists in different isoenzyme forms, namely LDH-1 to LDH-5, which can be separated electrophoretically according to their electrical charge and migration pattern. Among these isoenzymes,

LDH-C4 is highly specific to testicular tissue and mature spermatozoa and is considered an important biochemical marker of sperm viability and fertility potential (Goldberg, 1977). Electrophoretic analysis of LDH isoenzymes provides valuable information regarding sperm metabolism, testicular function, and reproductive efficiency. Alterations in LDH isoenzyme patterns may indicate testicular dysfunction, oxidative stress, or impaired spermatogenesis (Zhou et al., 2019).

Therefore, the present study was undertaken to evaluate the electrophoretic pattern of seminal LDH isoenzymes following administration of aqueous seed extract of *Foeniculum vulgare* in male Swiss albino mice.

2. Materials and Methods

2.1 Experimental Animals

Swiss albino male mice (*Mus musculus*) aged between 8–12 weeks and weighing 25–30 grams were used for the study. A total of 54 male mice were procured from the Department of Zoology, Tilka Manjhi Bhagalpur University, Bhagalpur. The animals were maintained under standard laboratory conditions with a 12-hour light/dark cycle at room temperature. Mice were housed in polypropylene cages containing sterile rice husk bedding and were acclimatized for one week prior to experimentation. Standard laboratory diet and water were provided ad libitum.

2.2 Plant Material and Extract Preparation

Dried seeds of *Foeniculum vulgare* were purchased from the local market of Bhagalpur district, Bihar, India. The seeds were washed thoroughly, air dried, powdered, and sieved through a 0.2 mm mesh sieve. The aqueous extract was prepared by reflux extraction. About 35 grams of seed powder was dissolved in 1000 ml distilled water and refluxed overnight. The extract was filtered through filter cloth and a Büchner funnel and stored at 4°C until use.

2.3 Experimental Design

The animals were randomly divided into three groups of 18 mice each:

Group I: Control

Received 0.1 ml glass distilled water orally.

Group II: Lower Dose

Received aqueous seed extract of *F. vulgare* at a concentration of 1.75 mg/0.1 ml corresponding to 70 mg/kg body weight.

Group III: Higher Dose

Received aqueous seed extract of *F. vulgare* at a concentration of 3.5 mg/0.1 ml corresponding to 140 mg/kg body weight.

Each group was further subdivided into 15-, 30-, and 45-day treatment durations with six mice in each subgroup.

Group	Treatment	Dose Administered	Route of Administration	Treatment Duration	Number of Animals
Group I (Control)	Glass distilled water	0.1 ml	Oral	15, 30, and 45 days	6 mice per duration subgroup
Group II (Lower Dose)	Aqueous seed extract of	1.75 mg/0.1 ml	Oral	15, 30, and 45 days	6 mice per duration subgroup

	Foeniculum vulgare				
Group III (Higher Dose)	Aqueous seed extract of *Foeniculum vulgare*	3.5 mg/0.1 ml	Oral	15, 30, and 45 days	6 mice per duration subgroup

2.4 Electrophoretic Analysis of LDH Isoenzymes

After completion of treatment, seminal plasma samples were collected from experimental animals. Electrophoretic separation of LDH isoenzymes was carried out using polyacrylamide gel electrophoresis (PAGE). Following electrophoresis, the gels were stained using specific staining solutions for LDH activity visualization. The relative activity of LDH isoenzymes was determined by observing electrophoretic band intensity and migration pattern.

Sample Preparation:

Seminal plasma was separated from sperm suspensions by centrifuging at 3000 rpm for 15 minutes. The supernatant containing soluble proteins was carefully collected and used as the protein sample for subsequent analysis.

LDH isozymes were separated and visualized using native polyacrylamide gel electrophoresis (PAGE), which was performed under non-denaturing conditions to preserve the enzymatic activity of the LDH isozymes.

Seminal plasma samples were used to ensure that the native conformation and enzymatic activity of the enzymes remained intact. Polyacrylamide gels with an 8.5% concentration were prepared to achieve optimal resolution of the LDH isoforms.

Electrophoresis was conducted at a low temperature (approximately 4°C) to maintain enzyme stability, and a constant voltage of approximately 100 V was applied for 2–3 hours (Manchenko, 2002).

Following electrophoresis, the gels were incubated in a staining solution containing NAD^+, L-lactate, phenazine methosulfate (PMS), and nitro blue tetrazolium (NBT). The enzymatic reaction between LDH and its substrates led to the reduction of NBT, which resulted in the formation of purple-coloured bands at the positions where LDH was active. These bands indicated the presence and activity of the LDH isoenzymes in seminal plasma (Dietz and Lubrano, 1967).

The protein banding patterns were analysed for differences in mobility and intensity. Variations in these parameters indicated qualitative differences in the protein composition between control and experimental groups.

3. Results

Electrophoretic profiling of seminal LDH isoenzymes demonstrated significant alterations in enzyme activity following treatment with aqueous seed extract of *Foeniculum vulgare*. LDH-C4 activity was evaluated separately from other LDH isoenzymes because it is a sperm-specific isoenzyme and was analyzed independently to assess spermatogenic activity.

In the low dose group, LDH-1 activity decreased gradually from 12.5% in control animals to 6.1% after 45 days of treatment. LDH-2 and LDH-3 also showed progressive reduction over the treatment duration. (Table.1 and Figure1)

In contrast, LDH-4 and LDH-5 activities increased continuously with prolonged exposure. LDH-5 activity increased from 30.4% in control animals to 41.2% after 45 days. A marked reduction in LDH-C4 activity was also observed, declining from 55.2% in control mice to 31.5% at the end of treatment.

The high dose group exhibited more pronounced changes in LDH isoenzyme activity. (Table.2 and Figure.2). LDH-1 activity decreased sharply from 12.5% in control animals to 2.4% after 45 days. Similar reductions were observed in LDH-2 and LDH-3 activities.

On the other hand, LDH-4 and LDH-5 activities increased significantly, with LDH-5 reaching 48.1% after prolonged treatment. LDH-C4 activity showed severe suppression, decreasing from 55.2% in control animals to 18.6% after 45 days.

Table 1. LDH Isoenzyme Activity in Low Dose Group

LDH Isoenzymes	Control (%)	15 Days (%)	30 Days (%)	45 Days (%)
LDH-1	12.5	10.2	8.4	6.1
LDH-2	15.4	13.7	11.5	9.3
LDH-3	18.2	16.8	15.0	12.7
LDH-4	23.5	25.6	28.1	30.7
LDH-5	30.4	33.7	37.0	41.2
LDH-C4	55.2	48.3	39.7	31.5

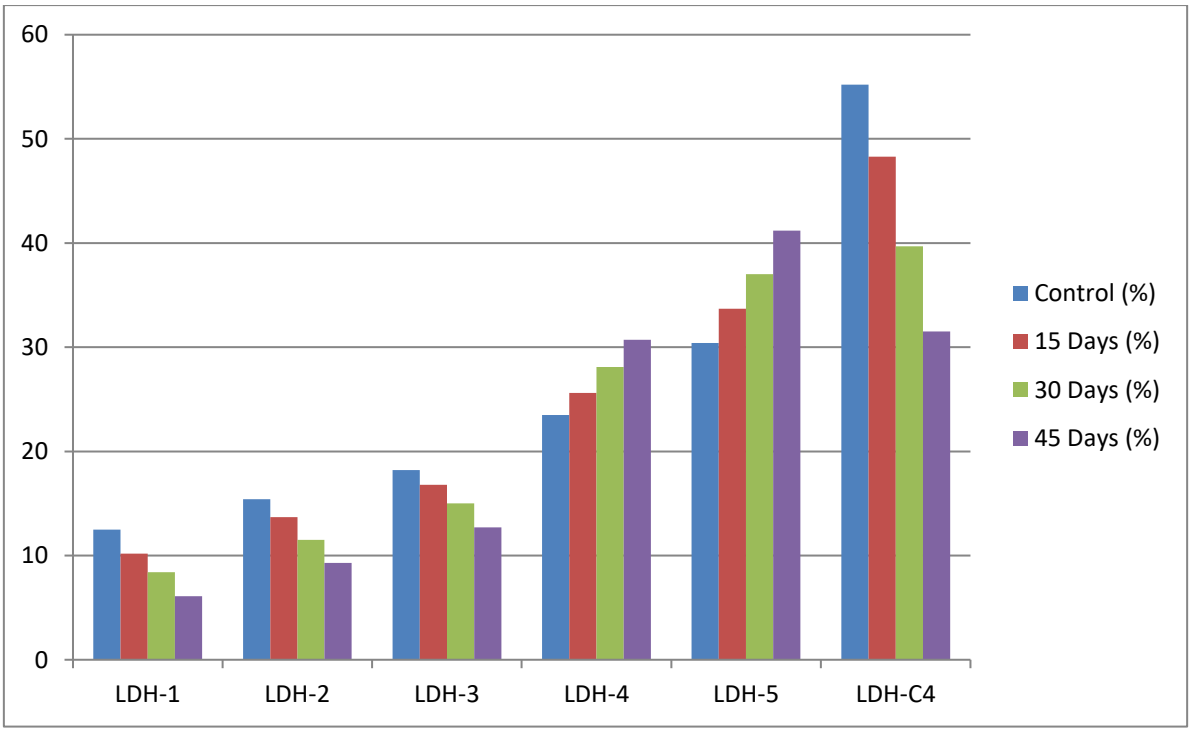

Figure- 1: Area percentage of the Antifertility Effect of Aqueous Seed Extract of *Foeniculum vulgare* on Seminal LDH isoenzymes activity in Male Swiss Albino Mice at Different Durations

Table 2. LDH Isoenzyme Activity in High Dose Group

LDH Isoenzymes	Control (%)	15 Days (%)	30 Days (%)	45 Days (%)
LDH-1	12.5	8.1	5.3	2.4

LDH-2	15.4	11.2	8.4	5.7
LDH-3	18.2	15.0	12.0	9.1
LDH-4	23.5	28.4	31.8	34.7
LDH-5	30.4	37.3	42.5	48.1
LDH-C4	55.2	42.5	29.3	18.6

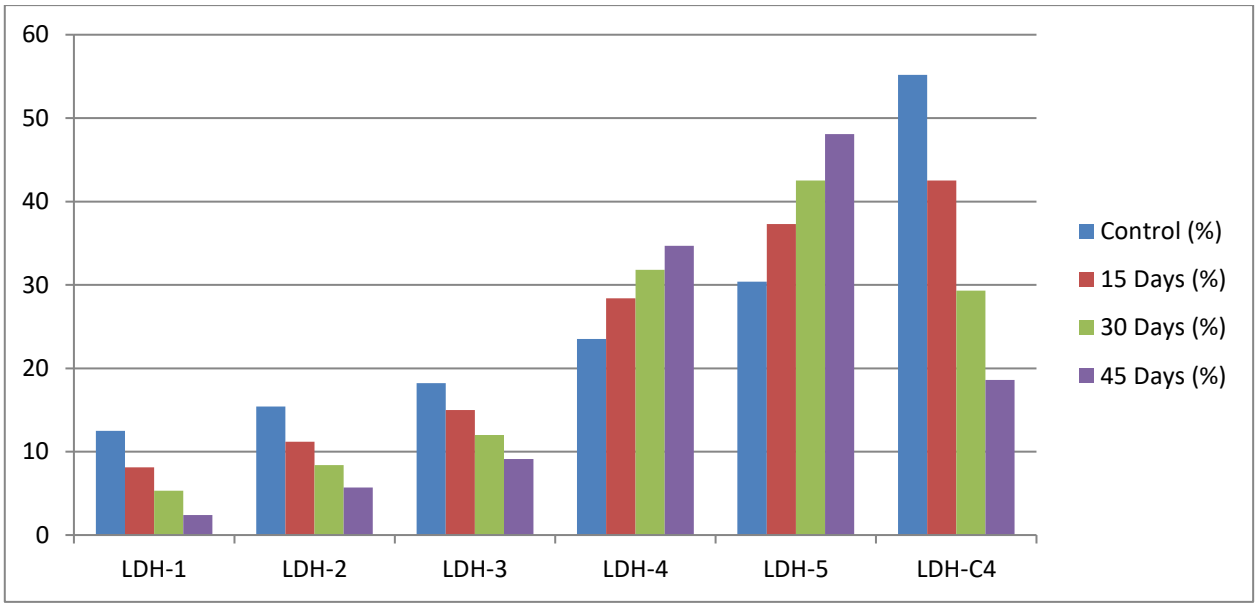

Figure-2: Area percentage of the Antifertility Effect of Aqueous Seed Extract of *Foeniculum vulgare* on Seminal LDH isoenzymes activity in Male Swiss Albino Mice at Different Durations.

4. Discussion

The present investigation demonstrated that aqueous seed extract of *Foeniculum vulgare* induced significant changes in seminal LDH isoenzyme activity in male Swiss albino mice. The progressive decline in LDH-1, LDH-2, and LDH-3 activities observed in treated groups suggests suppression of normal seminal metabolic processes and impaired sperm energy metabolism. (Bisht et al., 2017).

In contrast, increased activities of LDH-4 and LDH-5 may indicate tissue stress and altered anaerobic metabolism in seminal plasma. The most important finding of the study was the marked reduction in LDH-C4 activity in treated groups. LDH-C4 is a sperm-specific isoenzyme closely associated with spermatogenesis, sperm maturation, and motility (Goldberg, 1977). Reduction in LDH-C4 activity therefore indicates impaired sperm metabolism and possible reproductive dysfunction (Zhou et al., 2019).

The alterations observed in LDH isoenzyme patterns may be attributed to the phytoestrogenic compounds present in *Foeniculum vulgare*. Constituents such as anethole and flavonoids may interfere with hormonal regulation and testicular metabolism, leading to impaired spermatogenic activity (Badgujar et al., 2014). The dose-dependent decline in LDH-C4 activity strongly supports the antifertility potential of fennel seed extract (Mansouri et al., 2016).

Electrophoretic analysis of LDH isoenzymes proved to be an effective biochemical approach for evaluating reproductive toxicity and metabolic disturbances in seminal plasma. The findings of the present study are consistent with earlier reports indicating that medicinal plant extracts may adversely affect sperm metabolism and fertility parameters (Patel et al., 2021).

5. Conclusion

The present electrophoretic study revealed that aqueous seed extract of *Foeniculum vulgare* significantly altered seminal LDH isoenzyme patterns in male Swiss albino mice. A dose-dependent reduction in LDH-C4 activity along with changes in other LDH isoenzymes suggests impairment of sperm metabolism and reproductive function. These findings support the antifertility potential of *Foeniculum vulgare* and indicate that its mechanism of action may involve disruption of testicular and seminal energy metabolism.

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7. References

1. Agarwal, A., et al. (2014). Effect of oxidative stress on male reproduction. *World Journal of Men's Health, 32*(1), 1–17.
2. Dietz, A. A., & Lubrano, T. (1967). Separation and quantitation of lactic dehydrogenase isoenzymes by disc electrophoresis. *Analytical Biochemistry, 20*(2), 246–257.
3. Ebrahim Mansouri, et al. (2016). Effects of fennel extract on reproductive parameters in male mice. *International Journal of Reproductive BioMedicine, 14*(9), 561–568.
4. Goldberg, E. (1977). Lactate dehydrogenase-X from mouse testes and spermatozoa. *Methods in Enzymology, 41*, 318–325.
5. Hameed, Z. R., Zabbon, A., & Al-Bairuty, G. (2025). The effects of fenugreek seeds on the albino rat male reproductive system, MDA and SOD levels, and CD16 responses to Al2O3 NPs administration. *Acta Scientiarum. Animal Sciences, 47*, e71295.
6. Hind, B., Zineb, M., Elbachir, H., Najat, E. A., Siham, A., & Driss, R. (2017). Evaluation of potential effects of the aqueous extract of fenugreek seeds on fertility in male rats. *Journal of Ayurvedic and Herbal Medicine, 3*(4), 210–215.
7. Kassem, A., Al-Aghbari, A., Molham, A. H., & Al-Mamary, M. (2006). Evaluation of the potential antifertility effect of fenugreek seeds in male and female rabbits. *Contraception, 73*(3), 301–306.
8. Luaibi, N. M. (2018). *Physiological, hormonal and histological effects of fennel seeds (Foeniculum vulgare) in thyroid and testes of male rats* [Master's thesis].
9. Manchenko, G. P. (2002). *Handbook of detection of enzymes on electrophoretic gels*. CRC Press.
10. Mansour, A. B., Abou Elghait, A., Abo-youssef, A., Abdelwahab, N. S., & Helaly, H. (2021). A comparative study on the effects of fenugreek seeds powder and its aqueous and oil extracts on the male reproductive system in albino rats. *Bulletin of Pharmaceutical Sciences Assiut University, 44*(2), 623–635.
11. Mohammed, M. M. A. (2010). *The possible protective role of Foeniculum vulgare Mill. against radiation-induced certain biochemical changes in albino rats* (No. INIS-EG--246) [Doctoral dissertation, Faculty of Science, Beni-Suef University].
12. Yakubu, M. A., et al. (2007). Effects of medicinal plants on male reproductive functions. *Journal of Ethnopharmacology, 114*(1), 1–14.
13. Patel, R., et al. (2021). Electrophoretic analysis of seminal LDH isoenzymes in reproductive studies.

14. Pragya, S., Birla, S., Singh, V., & Singh, S. (2023). Fertility regulation of male mice by selective and directional influence of aqueous leaf extract of *Ocimum sanctum* L. on anodic electrophoretic proteins and M-isozymes of LDH in the semen of mice. *Annals of Plant and Soil Research, 25*(4), 626–629.
15. Patel, R., et al. (2021). Electrophoretic analysis of seminal LDH isoenzymes in reproductive studies. *Journal of Reproductive Biochemistry, 8*(2), 45–53.
16. Badgujar, S. B., et al. (2014). *Foeniculum vulgare* Mill: A review of its botany, phytochemistry, pharmacology, and toxicology. *BioMed Research International, 2014*, 842674.
17. Sasi, S. M., Alghoul, N. M., Prastiya, R. A., & Salem, K. (2025). Effects of boiled fenugreek seed extract on testicular histology and reproductive parameters in adult male mice. *Open Veterinary Journal, 15*(10), 5041.
18. Shayan, A., et al. (2019). Preparation and pharmacological evaluation of aqueous fennel seed extract.
19. Singh, M., & Verma, G. N. (2021). Investigating the effect of ethanolic extract of *Trigonella foenum-graecum* L. seeds on reproductive system of male albino rats. *Highlights on Medicine and Medical Research, 7*, 77–85.
20. Bisht, S., et al. (2017). Role of lactate dehydrogenase in sperm metabolism and fertility. *Andrologia, 49*(10), e12715.
21. Zhou, X., et al. (2019). LDH-C4 as a biomarker for sperm quality and reproductive function. *Reproductive Biology and Endocrinology, 17*(1), 109.
22. Zeweil, H. S., Zahran, S. M., Abd El-Rahman, M. H., El-Gindy, Y., & Embark, J. (2015). Effect of fenugreek and anise seeds as natural growth promoter on the performance, carcass, blood constituents and antioxidant status of growing rabbits. *Egyptian Poultry Science Journal, 35*(4).