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Original Research Article

Sildenafil Citrate Preserved the Antioxidant System and Testicular Function Markers Better Than Aqueous Extract of *Trigonella foenum-graecum* and Selected Alcoholic Bitters in Male Wistar Rats

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Abstract: Several pharmacological and non-pharmacological substances are widely consumed as aphrodisiacs due to their perceived benefits, often with little consideration for their potential adverse biological effects. This study evaluated and compared the effects of aqueous extract of *Trigonella foenum-graecum*, sildenafil citrate, and a selected alcoholic bitters on the antioxidant system and testicular function markers of adult male Wistar rats. An experimental randomized controlled design was adopted using forty (40) adult male Wistar rats assigned into eight groups (n = 5). Groups 1 and 2 received low and high doses of *Trigonella foenum-graecum* extract respectively; groups 3 and 4 received high and low doses of sildenafil citrate respectively; groups 5 and 6 received high and low doses of alcoholic bitters; group 7 received low doses of all test substances concurrently; while group 8 served as the control and received only rat feed and water. Treatments were administered orally for six weeks. At the end of the experimental period, the animals were sacrificed by cervical dislocation, and antioxidant enzymes and testicular function markers were analyzed using standard biochemical methods. Data were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's HSD post hoc test. The results showed a statistically significant reduction ($p < 0.05$) in most antioxidant parameters in the groups exposed to Trigonella foenum-graecum and alcoholic bitters when compared with the control, indicating compromised antioxidant defense. In contrast, the sildenafil citrate–treated groups showed relatively milder effects on antioxidant status, with no statistically significant differences in most parameters. However, a statistically significant increase ($p < 0.05$) in acid phosphatase activity was observed in several treated groups, suggesting varying degrees of testicular degeneration. Overall, the findings demonstrate that chronic administration of Trigonella foenum-graecum and alcoholic bitters exerts more pronounced adverse effects on the antioxidant system and testicular integrity compared to sildenafil citrate.

Keywords: Aphrodisiacs, Trigonella Foenum-Graecum, Sildenafil CitratE, Alcoholic Bitters, Antioxidant System, Testicular Function, Wistar Rats.

1. INTRODUCTION

Male sexual performance is a significant aspect of self-identity and social esteem, with erectile function serving as a critical determinant of sexual health (Peng *et al*., 2023). Historically, the search for aphrodisiacs has spanned all sexually active age groups, driven by the desire to enhance sexual desire, performance, and satisfaction. Aphrodisiacs—derived from the Greek goddess of love, Aphrodite—are substances that stimulate sexual desire (Greek-Aphrodisiakos-sexual). Among the most sought-after aphrodisiacs are sildenafil citrate, Trigonella foenum-graecum (fenugreek), and alcoholic herbal bitters, which are widely used to improve male sexual function. Sildenafil citrate, widely regarded as a pharmacological breakthrough, is a selective phosphodiesterase type 5 (PDE5) inhibitor with strong therapeutic effects on erectile dysfunction (Andersson, 2018). Introduced in the 1990s as the first pharmacologically approved treatment for erectile dysfunction, sildenafil prevents the breakdown of cyclic guanosine monophosphate (cGMP), thereby enhancing penile blood flow and sustaining erections sufficient for

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sexual intercourse. Despite the availability of effective conventional medications, plant-derived and herbomineral remedies remain popular alternatives, particularly among men seeking natural approaches to improve sexual performance (Moon *et al.,* 2017).

Trigonella foenum-graecum, commonly known as fenugreek, is a herb native to the Mediterranean, southern Europe, and western Asia (Baquer *et al.,* 2011; Belaïd-Nouira *et al*., 2012). While traditionally used to enhance lactation in women, research suggests that fenugreek may also improve libido and sexual performance in men (Lia *et al*., 2020). Similarly, alcoholic herbal preparations—referred to as bitters—have gained popularity as medicinal beverages due to their purported therapeutic properties, affordability, and availability. Bitters are produced through the alcoholic extraction of aromatic herbs, roots, barks, and fruits, yielding a bitter or bittersweet flavor, with anecdotal reports of efficacy in enhancing male sexual function.

Despite the widespread use of these aphrodisiacs, concerns remain regarding their safety, particularly when consumed in combination. Many men self-medicate with herbal or synthetic aphrodisiacs due to their accessibility, lower cost, and perceived minimal side effects, yet the long-term biological effects of chronic or combined use are largely unknown (Peng *et al*., 2023). Potential adverse effects may extend beyond reproductive function, highlighting the need to evaluate the impact of these agents on physiological systems.

This study seeks to investigate the effects of sildenafil citrate, aqueous extract of Trigonella foenum-graecum seeds, and alcoholic bitters on the antioxidant system and testicular function markers in male Wistar rats. By assessing both the potential benefits and risks of these substances, the study aims to provide evidence-based insights into their safety and biological plausibility. The findings will contribute to a better understanding of how these widely used aphrodisiacs influence male reproductive health and systemic antioxidant status.

2. MATERIALS AND METHODS

2.1 Study Area

The experimental study was conducted at the Animal House of the Faculty of Basic Medical Sciences, Rivers State University, Port Harcourt, Nigeria.

2.2 Experimental Animals

Forty healthy adults male Wistar rats weighing 100–150 g were obtained from the Animal House of the Department of Pharmacology, Rivers State University. Animals were housed in plastic cages under standard laboratory conditions (adequate ventilation, room temperature, and natural light/dark cycle) and provided with standard rat pellets and water ad libitum. The rats were allowed to acclimatize for three weeks prior to the commencement of treatment.

2.3 Study Design and Grouping

A randomized controlled experimental design was employed. After acclimatization, animals were randomly assigned into eight groups (n = 5) and treated once daily for six weeks as follows:

1. Fenugreek extract (50 mg/kg)
2. Fenugreek extract (100 mg/kg)
3. Sildenafil citrate (1 mg/kg)
4. Sildenafil citrate (0.5 mg/kg)
5. Alcoholic bitters (50 mg/kg)
6. Alcoholic bitters (25 mg/kg)
7. Fenugreek extract + sildenafil citrate + alcoholic bitters (all at low doses)
8. Control (distilled water)

All treatments were administered orally using an oral gavage.

2.4 Collection and Preparation of Test Substances

Trigonella foenum-graecum (Fenugreek)

Dried seeds of Trigonella foenum-graecum were purchased from a local market and ground into fine powder. The powder was stored in clean glass jars at room temperature. Aqueous extraction was carried out using the Soxhlet extraction method in accordance with the Association of Official Analytical Chemists (AOAC) method. Distilled water obtained from the Department of Chemistry, Rivers State University, was used as the extraction solvent. The resulting extract was stored in airtight containers at ≤10 °C until use.

Sildenafil Citrate (Viagra)

Sildenafil citrate tablets (100 mg each) were purchased from a registered pharmacy. Tablets were pulverized using a mortar and pestle and dissolved in distilled water to obtain the required concentrations for low and high doses.

Alcoholic Bitters

The selected alcoholic bitters were purchased commercially and administered at the manufacturer's concentration. Low and high doses were prepared based on body weight.

2.5 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Distilled water was obtained from the Department of Chemistry, Rivers State University.

2.6 Sample Collection

At the end of the six-week treatment period, the rats were sacrificed by cervical dislocation. Blood

samples were collected into plain sample bottles, and the testes were carefully excised for subsequent biochemical analyses.

2.7 Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine differences in antioxidant enzyme activities and testicular function indices among the experimental groups. Tukey's HSD post-hoc test was applied for multiple comparisons. Results were considered statistically significant at $p < 0.05$, with indicating intra-variable significant differences at $p < 0.05$.

3. RESULTS

Effect of Extracts on Reduced Glutathione (GSH) Level

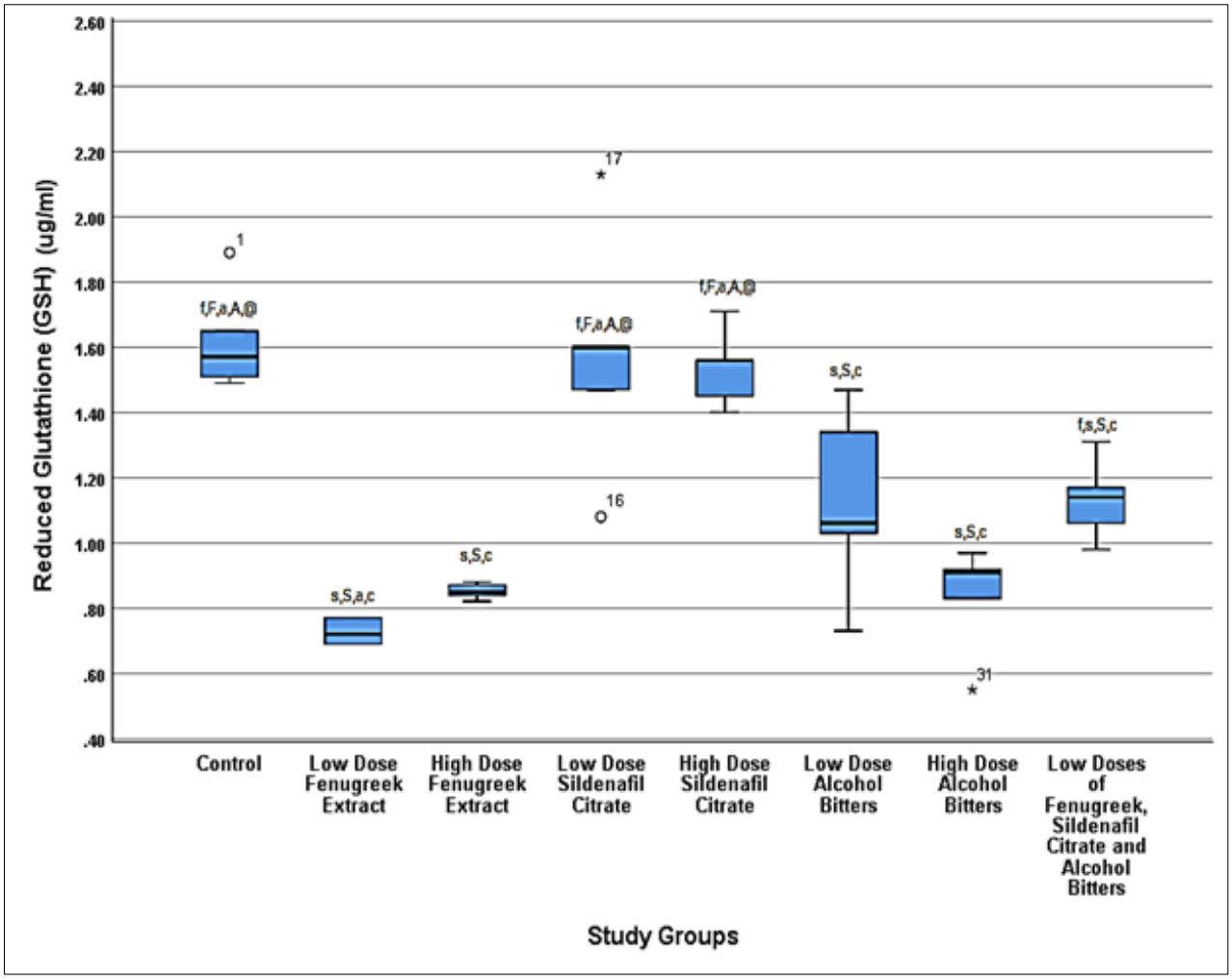

Fig. 3.1: A box plot showing Levels of Reduced Glutathione across the Study Groups

Figure 3.1 illustrates the effect of the various extracts and treatments on reduced glutathione (GSH) levels across the experimental groups. One-way ANOVA revealed a statistically significant difference in GSH levels among the groups ($F(7,32) = 16.785$, $p = 0.000$), indicating that treatment significantly influenced antioxidant status. Post hoc analysis using Tukey's HSD test showed significant differences ($p < 0.05$) across multiple group pairings. A marked reduction in reduced glutathione levels was observed in groups 1, 2, 5, 6, and 7, suggesting compromised antioxidant defense in these groups. Significant differences were noted among the low-dose fenugreek (f), high-dose fenugreek (F), low-dose sildenafil citrate (s), high-dose sildenafil citrate (S), low-dose alcohol bitters (a), high-dose alcohol bitters (A), low-dose combination of all substances, and the control (c) groups, as depicted in the figure. These findings demonstrate that the treatments exert differential effects on GSH levels, reflecting varying impacts on oxidative balance across the study groups.

Effect of Extracts on Glutathione Peroxidase (GPx) Levels

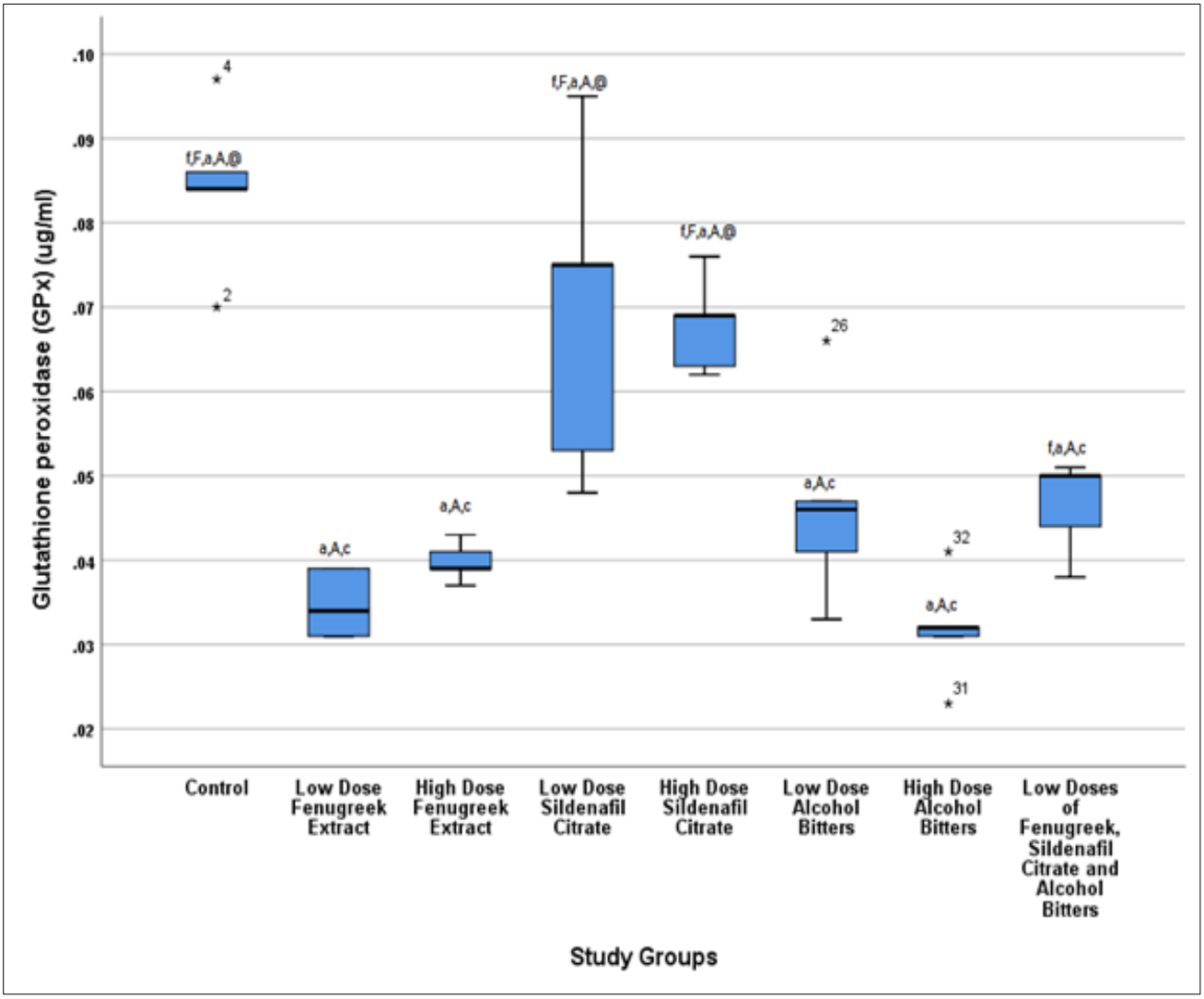

Fig. 3.2: A box plot showing Levels of Glutathione Peroxidase across the Study Groups

Figure 3.2 presents the effect of the various extracts and treatments on glutathione peroxidase (GPx) activity across the experimental groups. One-way ANOVA showed a statistically significant difference in GPx levels among the groups ($F(7,32) = 19.594$, $p = 0.000$), indicating that the treatments significantly influenced enzymatic antioxidant activity. Tukey's HSD post hoc test for multiple comparisons revealed statistically significant differences ($p < 0.05$) across several group pairings, as illustrated in the figure. A significant decrease in glutathione peroxidase levels was observed in groups 1, 2, 5, 6, and 7, reflecting reduced antioxidant enzyme activity in these groups. Significant differences were also evident among the low-dose fenugreek (f), high-dose fenugreek (F), low-dose sildenafil citrate (s), high-dose sildenafil citrate (S), low-dose alcohol bitters (a), high-dose alcohol bitters (A), low-dose combination of all substances, and the control (c) groups. Overall, the findings indicate differential effects of the treatments on GPx activity, underscoring variations in antioxidant defense across the study groups.

Effect of Extracts on Catalase (CAT) Levels

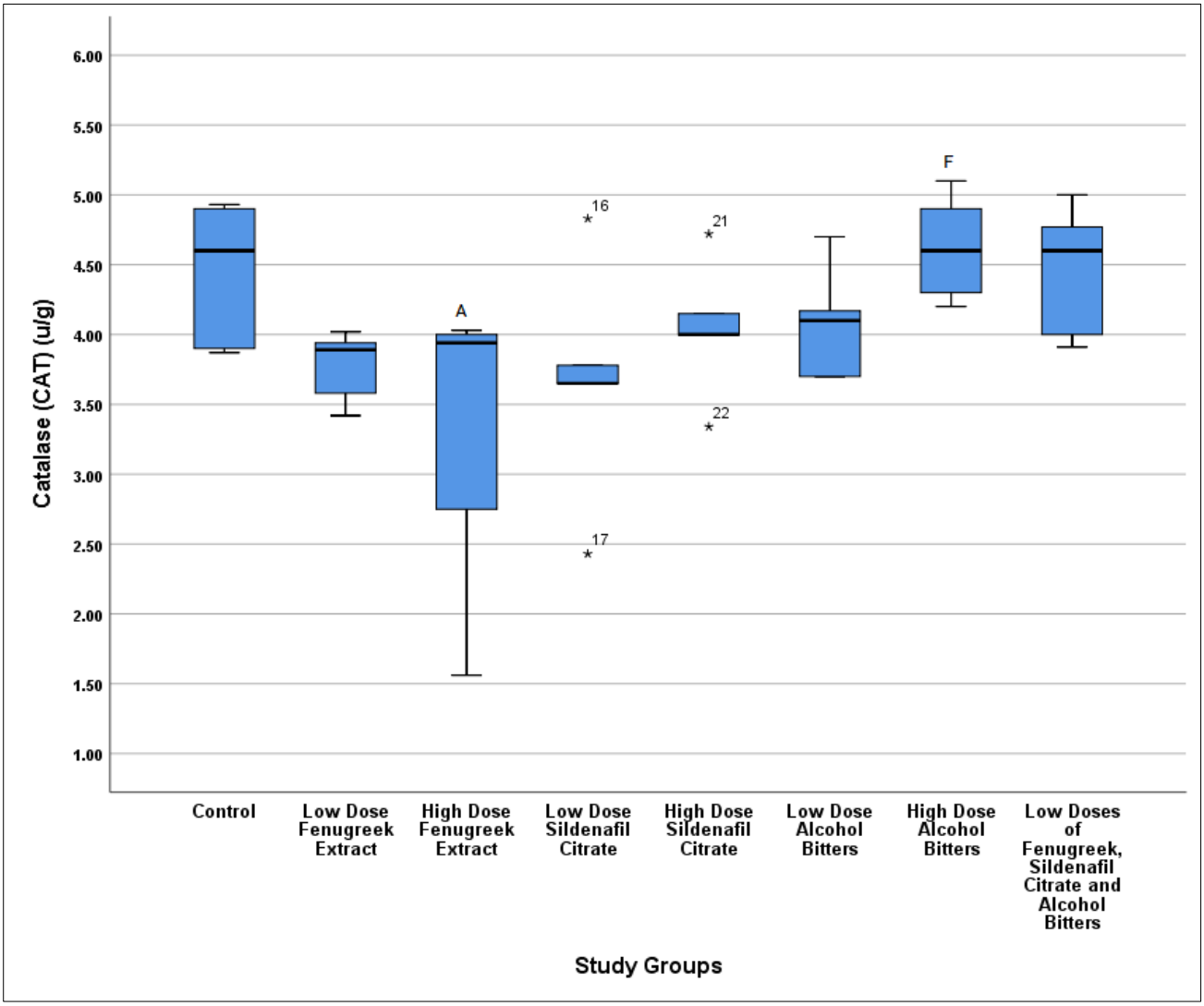

Fig. 3.3: A Box Plot showing Levels of Catalase across the Study Groups

Figure 3.3 illustrates the effect of the various extracts and treatments on catalase (CAT) activity across the experimental groups. One-way ANOVA revealed a statistically significant difference in catalase levels among the groups ($F(7,32) = 2.812$, $p = 0.021$), indicating that treatment influenced catalase activity. Tukey's HSD post hoc test showed a statistically significant difference between Group 2 and Group 6 ($p = 0.027$), while comparisons involving other group pairings were not statistically significant ($p > 0.05$). Overall, catalase levels did not differ significantly across most of the groups, as reflected in the figure. However, a significant difference was observed between the high-dose fenugreek (F) and high-dose alcohol bitters (A) groups, suggesting a differential effect of these treatments on catalase activity.

Effect of Extracts on Superoxide Dismutase (SOD) Levels

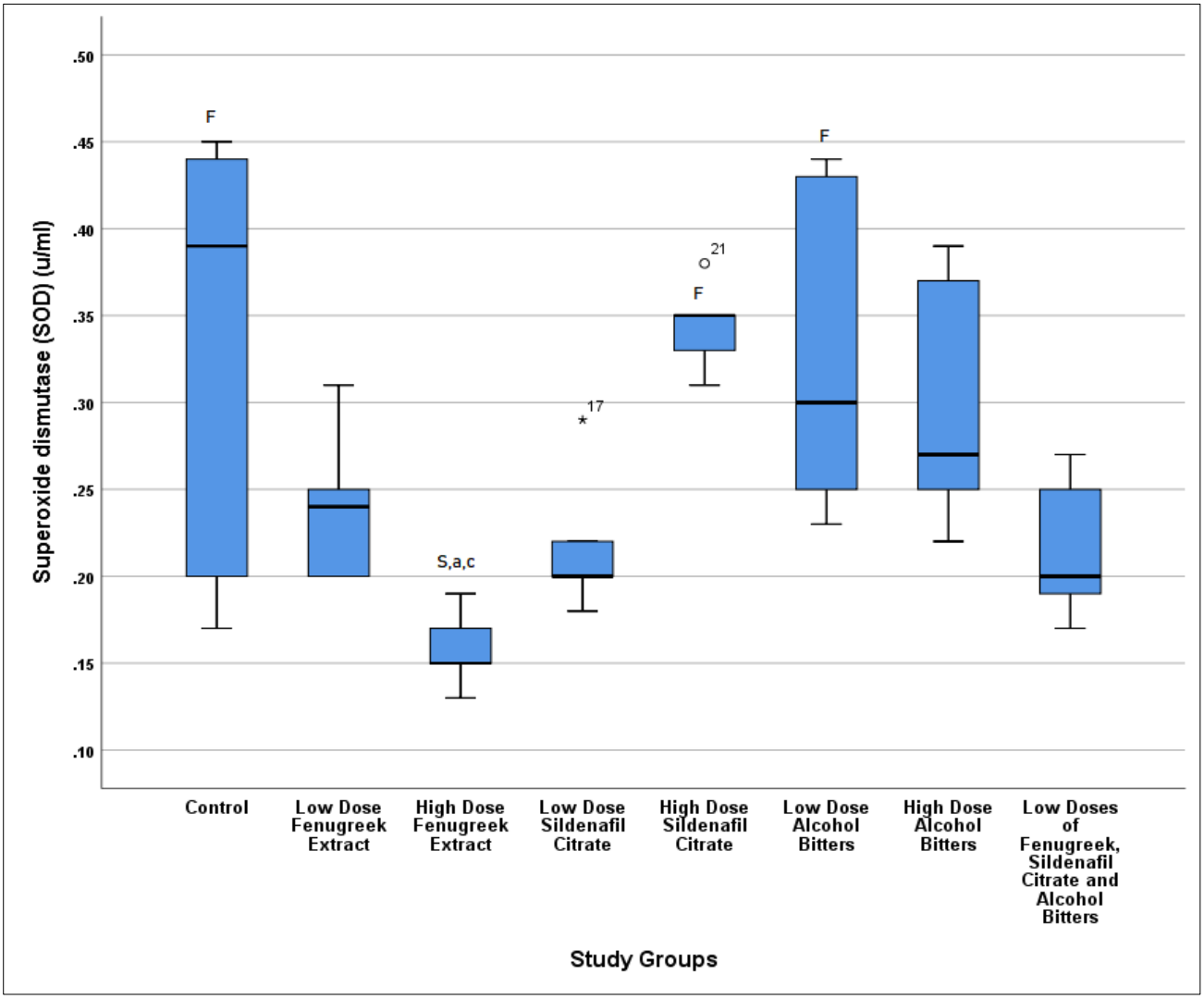

Fig. 3.4: A Box Plot showing Levels of Superoxide Dismutase across the Study Groups

Figure 3.4 depicts the effect of the various extracts and treatments on superoxide dismutase (SOD) activity across the experimental groups. One-way ANOVA demonstrated a statistically significant difference in SOD levels among the groups ($F(7,32) = 4.589$, $p = 0.001$), indicating that the treatments exerted a significant effect on SOD activity. Tukey's HSD post hoc test revealed statistically significant differences when Group 2 was compared with Groups 4, 5, and 8 ($p = 0.005$, $p = 0.012$, and $p = 0.012$, respectively), while other group comparisons showed no statistically significant differences ($p > 0.05$). A significant reduction in superoxide dismutase levels was observed in the fenugreek-treated group, suggesting a diminished antioxidant enzyme response. Significant differences were also noted among the high-dose fenugreek (F), high-dose sildenafil citrate (S), low-dose alcohol bitters (a), and control (c) groups, as illustrated in the figure. These results indicate that the extracts differentially modulated SOD activity across the study groups.

Effect of Extracts on Malondialdehyde (MDA) Levels

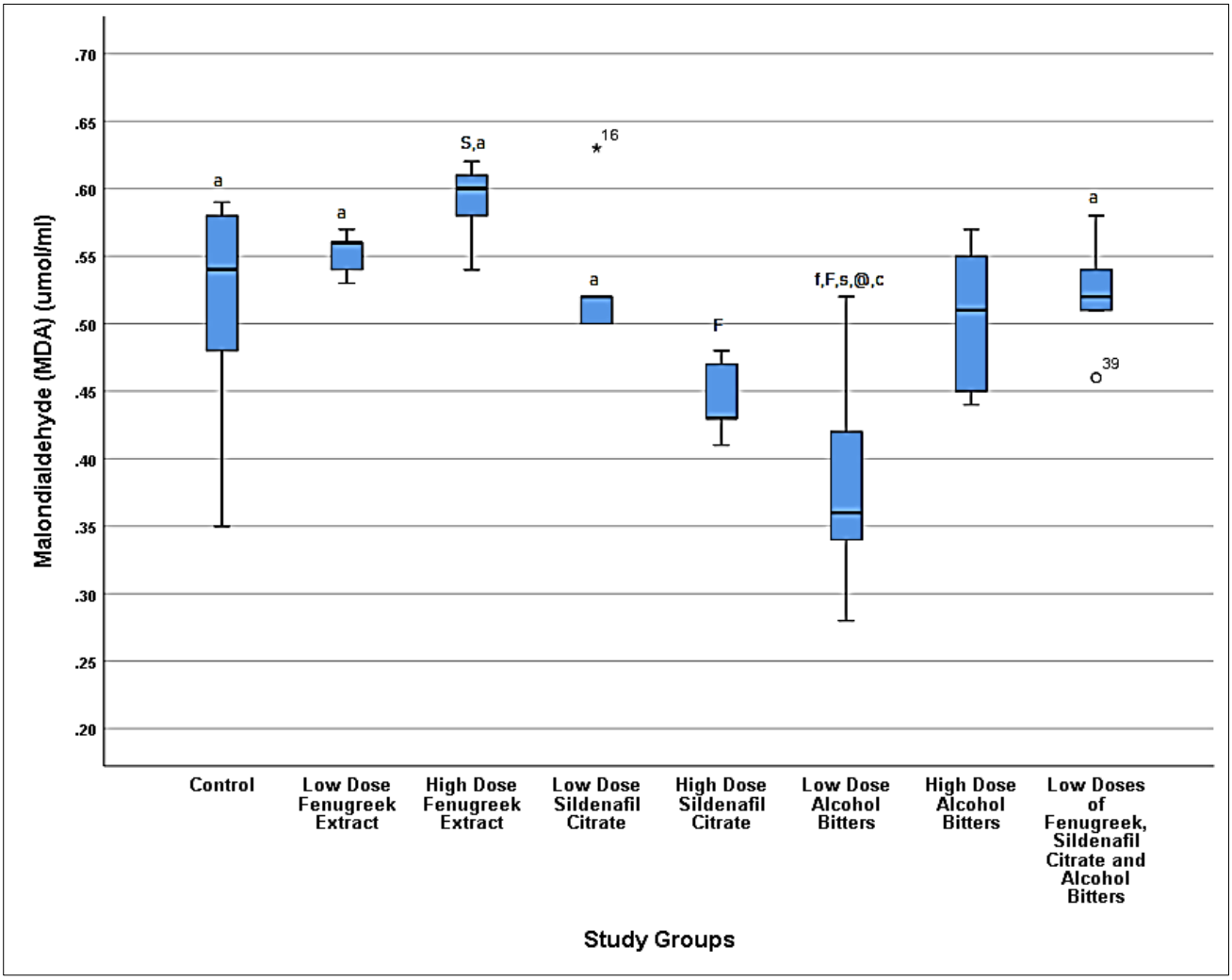

Fig. 3.5: A Box Plot showing Levels of Malondialdehyde across the Study Groups

Figure 3.5 shows the effect of the various extracts and treatments on malondialdehyde (MDA) levels across the experimental groups. One-way ANOVA revealed a statistically significant difference in MDA levels among the groups ($F(7,32) = 5.826$, $p = 0.000$), indicating significant variation in lipid peroxidation across treatments. Tukey's HSD post hoc analysis demonstrated a statistically significant difference between Group 2 and Group 4 ($p = 0.010$), as well as significant differences when Group 5 was compared with Groups 1, 2, 3, 7, and 8 ($p = 0.002$, $p = 0.000$, $p = 0.008$, $p = 0.018$, and $p = 0.044$, respectively). Other group comparisons did not show statistically significant differences ($p > 0.05$). A significant reduction in malondialdehyde levels was observed in Group 5, suggesting reduced lipid peroxidation in this group. Significant differences were also noted among the low-dose fenugreek (f), high-dose fenugreek (F), low-dose sildenafil citrate (s), high-dose sildenafil citrate (S), low-dose alcohol bitters (a), low-dose combination of all substances, and the control (c) groups, as depicted in the figure. These findings indicate that the treatments differentially influenced oxidative stress, as reflected by variations in MDA levels across the study groups.

Effect of Extracts on acid Phosphatase (ACP) Levels

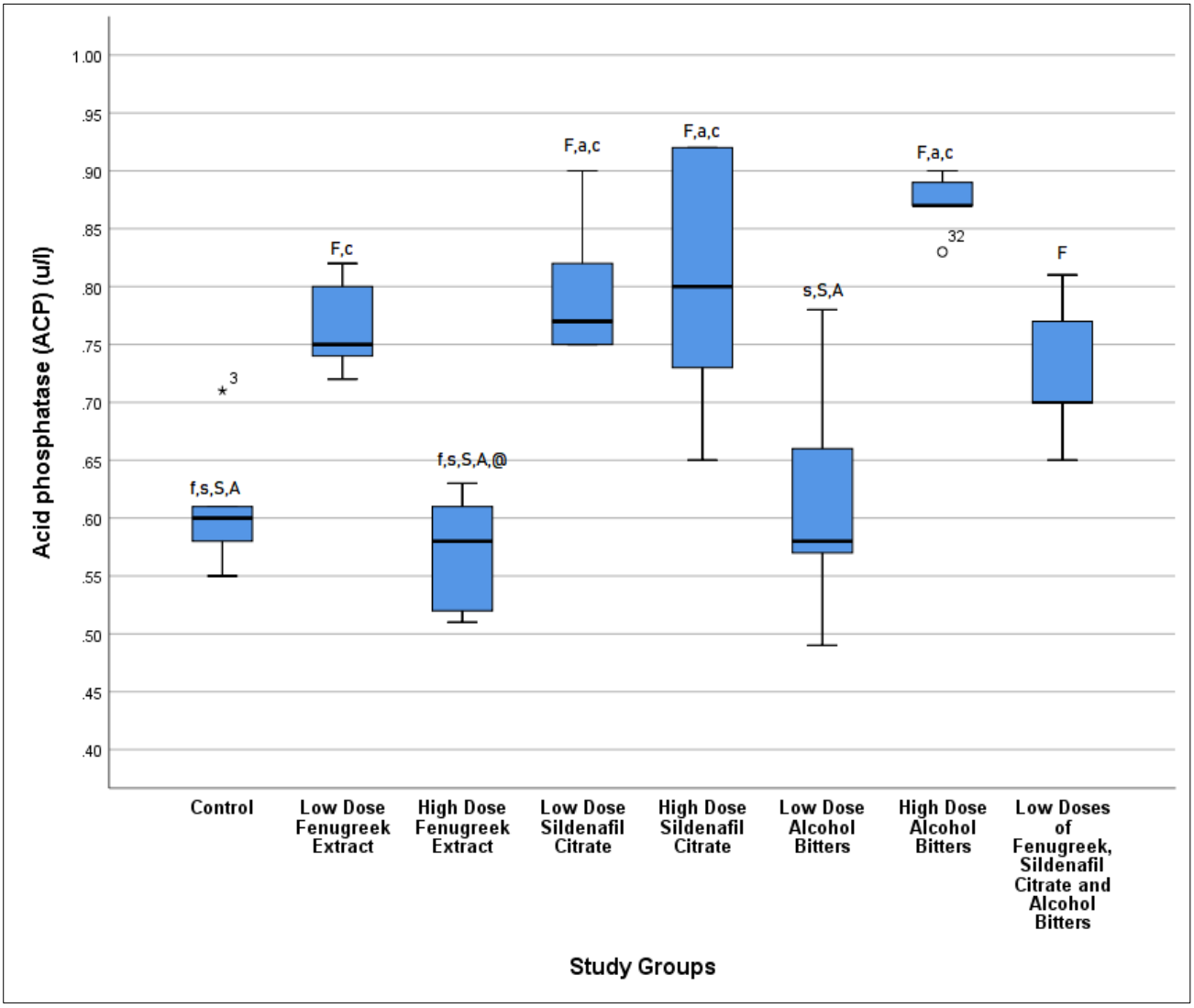

Fig. 3.6: A Box Plot showing Levels of Acid Phosphatase across the Study Groups

Figure 3.6 illustrates the effect of the various extracts and treatments on acid phosphatase (ACP) levels across the experimental groups. One-way ANOVA revealed a statistically significant difference in ACP levels among the groups ($F(7,32) = 11.096$, $p = 0.000$), indicating that the treatments significantly influenced ACP activity. Tukey's HSD post hoc test showed statistically significant differences when Group 2 was compared with Groups 1, 3, 4, 6, and 7 ($p = 0.004$, $p = 0.001$, $p = 0.000$, $p = 0.000$, and $p = 0.038$, respectively). Significant differences were also observed when Group 5 was compared with Groups 3, 4, and 6 ($p = 0.009$, $p = 0.007$, and $p = 0.000$, respectively), as well as when Group 8 was compared with Groups 1, 3, 4, and 6 ($p = 0.038$, $p = 0.007$, $p = 0.005$, and $p = 0.000$, respectively). Other group comparisons showed no statistically significant differences ($p > 0.05$). A significant increase in acid phosphatase levels was observed in Groups 1, 3, 4, and 6, suggesting altered phosphatase activity in these groups. Significant differences were also evident among the low-dose fenugreek (f), high-dose fenugreek (F), low-dose sildenafil citrate (s), high-dose sildenafil citrate (S), low-dose alcohol bitters (a), high-dose alcohol bitters (A), low-dose combination of all substances (@), and the control groups, as illustrated in the figure. These results demonstrate that the extracts exerted differential effects on ACP levels across the study groups.

Effect of Extracts on Alkaline Phosphatase (ALP) Level

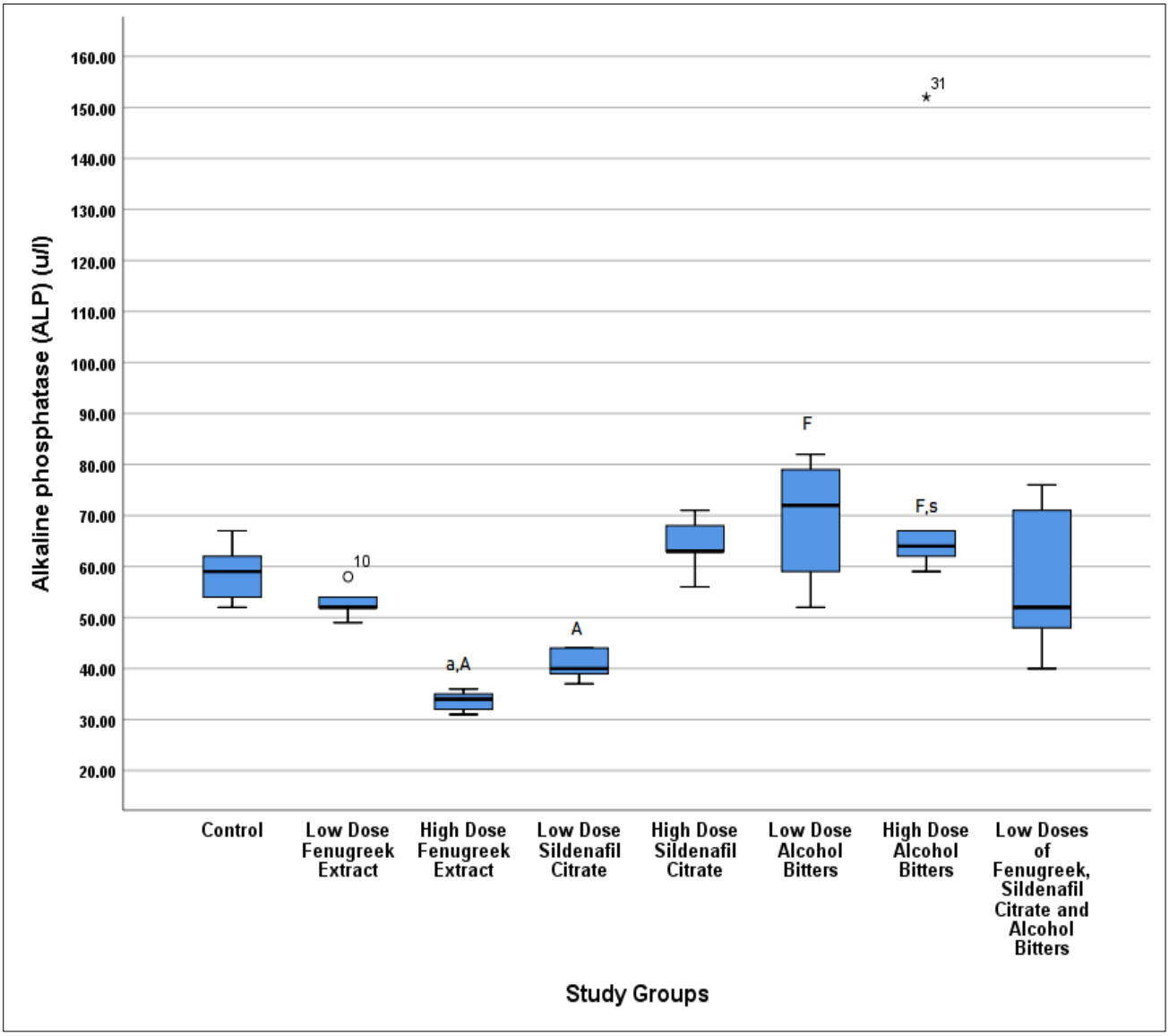

Fig. 3.7: A Box Plot showing Levels of Alkaline Phosphatase across the Study Groups

Figure 3.7 presents the effect of the various extracts and treatments on alkaline phosphatase (ALP) levels across the experimental groups. One-way ANOVA revealed a statistically significant difference in ALP levels among the groups ($F(7,32) = 4.335$, $p = 0.002$), indicating that the treatments significantly affected ALP activity. Tukey's HSD post hoc test showed statistically significant differences when Group 2 was compared with Groups 5 and 6 ($p = 0.031$ and $p = 0.001$, respectively), and when Group 3 was compared with Group 6 ($p = 0.009$). Other group comparisons did not show statistically significant differences ($p > 0.05$). Significant differences were observed among the high-dose fenugreek (F), low-dose sildenafil citrate (s), low-dose alcohol bitters (a), and high-dose alcohol bitters (A) groups, as illustrated in the figure. These findings indicate that the extracts exerted differential effects on alkaline phosphatase activity across the study groups.

Effect of Extracts on Lactate Dehydrogenase (LDH) Level

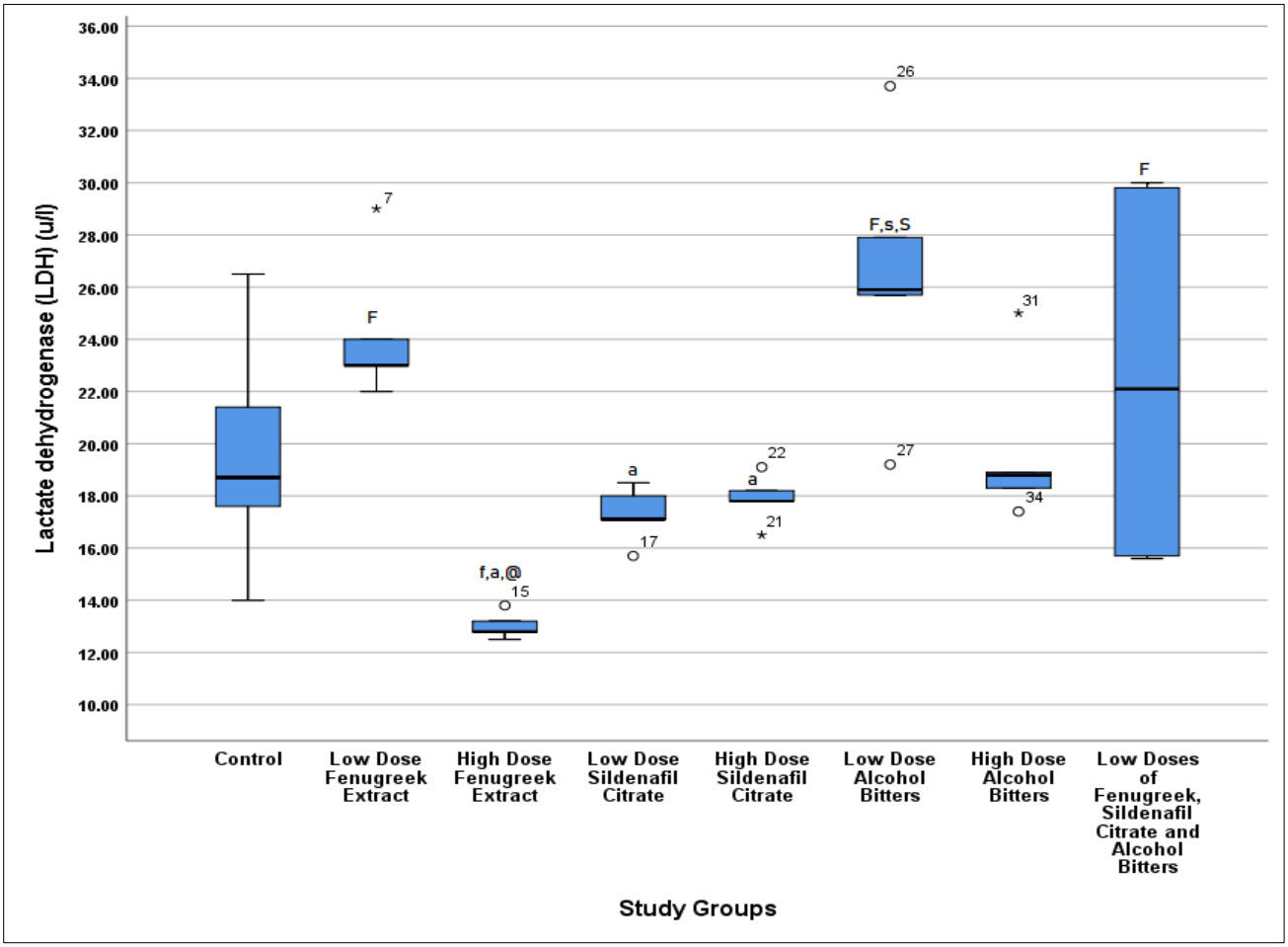

Fig. 3.8: A Box Plot showing Levels of Lactate Dehydrogenase across the Study Groups

Figure 3.8 illustrates the effect of the various extracts and treatments on lactate dehydrogenase (LDH) levels across the experimental groups. One-way ANOVA revealed a statistically significant difference in LDH levels among the groups ($F(7,32) = 6.126$, $p = 0.000$), indicating that the treatments significantly influenced LDH activity. Tukey's HSD post hoc analysis showed statistically significant differences when Group 2 was compared with Groups 1, 5, and 7 ($p = 0.002$, $p = 0.000$, and $p = 0.009$, respectively), and when Group 5 was compared with Groups 3 and 4 ($p = 0.013$ and $p = 0.025$, respectively). Other group comparisons did not show statistically significant differences ($p > 0.05$). Significant differences were observed among the low-dose fenugreek (f), high-dose fenugreek (F), low-dose sildenafil citrate (s), high-dose sildenafil citrate (S), low-dose alcohol bitters (a), and low-dose combination of all substances groups, as depicted in the figure. These results indicate that the extracts exerted differential effects on LDH levels across the study groups.

4. DISCUSSION

This study evaluated the chronic effects of a pharmacological aphrodisiac (sildenafil citrate), a plant-derived aphrodisiac (Trigonella foenum-graecum), and a selected alcoholic bitters on the antioxidant defense system and testicular function indices of male Wistar rats. Erectile dysfunction is projected to affect over 300 million men globally by 2025, driving increased reliance on both orthodox and nonorthodox aphrodisiacs (Banihashem Ahmad *et al*., 2025). While these agents are widely perceived as beneficial for sexual performance, their long-term biochemical consequences, particularly on reproductive oxidative balance, remain insufficiently characterized. The present findings provide evidence that chronic exposure to these substances can significantly alter testicular antioxidant status and enzymatic markers of testicular function.

Oxidative stress plays a central role in male reproductive health, with physiological levels of reactive oxygen species (ROS) required for normal sperm maturation and capacitation. However, excessive ROS generation overwhelms endogenous antioxidant

defenses, leading to lipid peroxidation, protein oxidation, and cellular dysfunction. The testis is particularly vulnerable due to its high content of polyunsaturated fatty acids and active mitochondrial metabolism. In this study, significant reductions in reduced glutathione (GSH/GR), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) were observed in several treatment groups compared with controls, indicating disruption of the intrinsic antioxidant defense system. These enzymes function cooperatively to detoxify superoxide anions and hydrogen peroxide, thereby maintaining redox homeostasis within testicular tissue (Mirończuk-Chodakowska *et al*., 2018).

The marked depletion of GSH and GPx observed following administration of Trigonella foenum-graecum and alcoholic bitters suggests impaired glutathione-dependent detoxification pathways. Reduced glutathione serves as a primary non-enzymatic antioxidant and a substrate for GPx, and its depletion renders cells susceptible to oxidative injury. These findings are consistent with the report of Suresh, (2019), who demonstrated significant reductions in GSH and GPx following fenugreek seed administration. Similarly, Ujowundu *et al*., (2022) reported suppression of glutathione peroxidase activity following exposure to alcoholic bitters, reinforcing the possibility that chronic consumption of such preparations may compromise antioxidant capacity.

Sildenafil citrate–treated groups exhibited particularly low levels of reduced glutathione activity in the testes, suggesting heightened oxidative stress. Although sildenafil is therapeutically effective in improving erectile function through phosphodiesterase-5 inhibition, its chronic influence on testicular redox balance may be less favorable. Prolonged modulation of nitric oxide signaling and cyclic GMP pathways may enhance ROS production, thereby placing additional oxidative burden on testicular cells when antioxidant defenses are insufficient.

Catalase activity showed no statistically significant difference across treatment groups, indicating that hydrogen peroxide detoxification via catalase may be relatively preserved under the experimental conditions. This may reflect compensatory mechanisms or lower sensitivity of CAT to the specific oxidative challenges induced by these substances. Similarly, superoxide dismutase activity remained largely unchanged in most groups, with the exception of a significant reduction observed in rats treated with high-dose Trigonella foenum-graecum. This selective reduction suggests a dose-dependent inhibitory effect on superoxide scavenging, which may predispose testicular tissue to superoxide-mediated damage.

Lipid peroxidation, assessed using malondialdehyde (MDA), did not differ significantly across experimental groups. Interestingly, alcoholic bitters administration was associated with a reduction in MDA levels relative to controls, suggesting a possible attenuation of membrane lipid peroxidation. MDA is a terminal product of polyunsaturated fatty acid oxidation and serves as a reliable indicator of oxidative membrane damage. The observed reduction aligns with the findings of Alabi *et al*., (2013), who reported decreased MDA concentrations following alcoholic bitters administration, implying the presence of constituents with lipid-stabilizing or radical-scavenging properties. This paradoxical effect underscores the complex and sometimes opposing biochemical actions of multicomponent herbal and alcoholic preparations.

Beyond oxidative stress markers, this study also assessed testicular function indices, including acid phosphatase (ACP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). A significant increase in ACP activity was observed in rats treated with low-dose Trigonella foenum-graecum, both doses of sildenafil citrate, and high-dose alcoholic bitters. Acid phosphatase is a lysosomal enzyme involved in intracellular digestion and tissue remodeling, and its elevation in testicular tissue may reflect lysosomal membrane destabilization, altered spermatogenic activity, or early degenerative changes. Elevated ACP has been associated with impaired testicular integrity and disrupted germ cell maturation.

In contrast, ALP and LDH activities did not show significant variations across groups, suggesting that overall energy metabolism and membrane-associated phosphatase activity may not have been severely compromised under the study conditions. The absence of significant changes in LDH, a key enzyme in anaerobic glycolysis, indicates relative preservation of cellular metabolic capacity despite oxidative perturbations. These findings differ from those of (El-Bahr *et al*., 2021), who reported no significant alterations in ACP following fenugreek administration, highlighting the influence of dose, duration, and experimental context on testicular enzymatic responses.

Collectively, the findings of this study suggest that chronic consumption of sildenafil citrate, Trigonella foenum-graecum, and selected alcoholic bitters can disrupt testicular antioxidant defenses and modify key enzymatic markers of testicular function. While some preparations may exhibit partial protective effects against lipid peroxidation, the overall reduction in antioxidant enzyme activities indicates a state of redox imbalance that may predispose testicular tissue to oxidative damage over time. These results underscore the need for caution in the prolonged use of both pharmacological and non-pharmacological aphrodisiacs, particularly in the absence of medical supervision.

Further studies incorporating histopathological evaluation, hormonal profiling, and molecular analysis of oxidative stress and apoptotic pathways are recommended to elucidate the long-term reproductive consequences of these agents. Such investigations would provide deeper insight into the mechanisms underlying aphrodisiac-induced testicular alterations and inform safer therapeutic and lifestyle practices.

5. CONCLUSION

5.1 Conclusion

This study evaluated the effects of commonly used aphrodisiacs—Sildenafil citrate, Trigonella foenum-graecum, and alcoholic bitters—on the antioxidant system and testicular function markers of male Wistar rats. The findings demonstrate that chronic exposure to Trigonella foenum-graecum and alcoholic bitters significantly compromised the testicular antioxidant defense system, indicating increased oxidative stress. In contrast, the effects observed in the Sildenafil citrate–treated groups were comparatively milder, suggesting a relatively lower impact on antioxidant balance.

Notably, concurrent administration of Sildenafil citrate, Trigonella foenum-graecum, and alcoholic bitters produced marked alterations in the antioxidant system of the testes, highlighting the potential risks associated with combined use of these substances. Although Sildenafil citrate showed less pronounced effects on antioxidant enzymes, it was associated with increased acid phosphatase (ACP) activity, an indicator of possible testicular degeneration. This finding underscores that even pharmacologically approved aphrodisiacs may exert adverse effects on testicular integrity when used chronically.

Overall, the study emphasizes that consumers' preference for readily available and fast-acting aphrodisiacs often overlooks potential long-term biological consequences. The results underscore the need for caution in the chronic and combined use of aphrodisiacs, as well as the importance of evaluating their safety beyond perceived sexual performance benefits. These findings contribute to a better understanding of the oxidative and testicular implications of aphrodisiac use and highlight the need for increased awareness and further research into their long-term reproductive health effects.

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