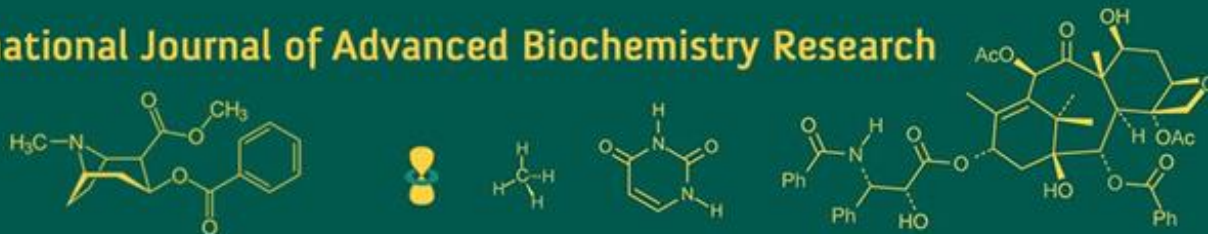


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Effect of Giloy (*Tinospora cordifolia*) on seminal parameters of boar spermatozoa during preservation

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Abstract

Artificial insemination (AI) is a widely adopted reproductive biotechnology in swine production; however, the prolonged storage of liquid semen is associated with progressive impairment of sperm functional competence, primarily through oxidative stress, lipid peroxidation and disruption of cellular antioxidant homeostasis. The present study was undertaken to investigate the potential protective effect of Giloy (*Tinospora cordifolia*) ethanolic extract on the semen quality characteristics and enzymatic profile of boar spermatozoa during liquid preservation. A total of 12 ejaculates were collected from clinically healthy breeding boars and subjected to preliminary quality assessment. Qualified ejaculates were divided into four experimental groups, with Group I serving as the untreated control and Groups II, III and IV supplemented with *T. cordifolia* extract at 100, 300 and 500 µg / 25×10⁶ spermatozoa, respectively. Semen was extended in Beltsville Thawing Solution (BTS), adjusted to a final concentration of 30×10⁶ spermatozoa/ml and preserved at 15% for 72 h. Semen quality attributes, including progressive motility, sperm viability, morphological integrity, acrosomal integrity and functional plasma membrane integrity, were evaluated at 24, 48 and 72 h of preservation. In parallel, seminal plasma was assessed for catalase (CAT), superoxide dismutase (SOD), glutamate oxaloacetate transaminase (GOT), lactate dehydrogenase (LDH), lipid peroxidation (MDA) and total antioxidant capacity (TAC). The experimental design enabled evaluation of the concentration-dependent and time-dependent effects of *T. cordifolia* supplementation on sperm functional characteristics and biochemical homeostasis during storage. The study provides a scientific framework for assessing *T. cordifolia* as a phytochemical antioxidant additive for mitigating preservation-associated oxidative injury and maintaining boar sperm quality. The findings may have practical implications for optimizing liquid semen preservation protocols and improving the efficacy of AI-based genetic dissemination in swine production.

Keywords: *Tinospora cordifolia*, giloy, boar semen, liquid semen preservation, artificial insemination, oxidative stress, antioxidant enzymes, sperm quality, lipid peroxidation, total antioxidant capacity

Introduction

Livestock husbandry plays a pivotal role in global agriculture by contributing substantially to food security, rural livelihoods and economic development. Among livestock species, the pig (*Sus scrofa domestica*) is considered one of the most efficient meat-producing animals due to its rapid growth, high reproductive potential and efficient feed conversion ratio (Thomas *et al.*, 2025) [1]. Pork is an important source of high-quality animal protein worldwide, and the expanding swine industry requires continuous genetic improvement and optimized reproductive management to achieve sustainable production (Thomas *et al.*, 2021) [2].

Artificial insemination (AI) is a major reproductive biotechnology extensively employed in swine production for accelerating genetic gain through the dissemination of superior germplasm from genetically elite boars to a large number of females. It offers advantages over natural mating, including efficient utilization of superior sires, improved reproductive management and reduced risk of disease transmission (Foote, 2002; Lopez *et al.*, 2017) [3, 4]. However, the success of AI largely depends on the maintenance of sperm quality during semen preservation. Preservation-induced oxidative stress, resulting from excessive generation of reactive oxygen species (ROS) relative to antioxidant defense capacity, can adversely affect sperm membrane integrity, mitochondrial function, motility and fertilizing competence. Although physiological concentrations of ROS are involved in capacitation, hyperactivation and the acrosome reaction, excessive ROS induces lipid peroxidation and oxidative damage to proteins and nucleic acids (Aitken and Clarkson, 1987). Giloy (*Tinospora cordifolia*), a medicinal plant of the family Menispermaceae, contains diverse bioactive phytoconstituents, including alkaloids, diterpenoid lactones, steroids,

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glycosides, phenolics and polysaccharides, which possess documented antioxidant, anti inflammatory, immunomodulatory and cytoprotective properties (Saha and Ghosh, 2012; Gupta *et al.*, 2024) ^[5, 6]. Its antioxidant potential may therefore provide protection against preservation-associated oxidative injury in spermatozoa. However, the potential of *T. cordifolia* as a natural antioxidant supplement for maintaining boar semen quality and enzymatic homeostasis during preservation remains inadequately explored. Therefore, the present study was undertaken to evaluate the effect of *Giloy* (*T. cordifolia*) supplementation on the enzymatic profile and semen quality characteristics of boar spermatozoa during preservation, with particular emphasis on its potential protective role against preservation-induced oxidative stress.

3. Materials and Methods

3.1 Location and Experimental Animals

The study was conducted in the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Rewa, NDVSU, Jabalpur, Madhya Pradesh. Healthy breeding boars maintained at the Instructional Pig Unit of the Livestock Farm Complex were utilized.

3.2 Semen Collection and Experimental Design

A total of 12 ejaculates were collected using the double-gloved hand technique with a dummy. The gel fraction was removed using a gauze-filtered semen collection funnel. Ejaculates were transported to the laboratory at approximately 37 °C. Only samples exhibiting $\geq 70\%$ progressive motility and sperm concentration $> 100 \times 10^6$ spermatozoa/ml were included. Each ejaculate was divided into four equal aliquots. Group I served as control and received BTS extender without *Giloy* (*Tinospora cordifolia*) extract. Groups II, III and IV were supplemented with *T. cordifolia* extract at 100, 300 and 500 μg / 25×10^6 spermatozoa, respectively. Following equilibration at 22 °C for 2 h, semen was diluted with BTS to a final concentration of 30×10^6 spermatozoa/ml and preserved at 15% for 72 h. Assessments were performed at 24, 48 and 72 h of preservation.

3.3 Preparation of Giloy Extract

Authenticated *T. cordifolia* stem material was dried at 40 °C, powdered and subjected to Soxhlet extraction using 70% ethanol. The solvent was removed under reduced pressure using a rotary evaporator, and the concentrated extract was stored at 4 °C until use.

3.4 Semen Quality Evaluation

Semen samples were evaluated for progressive motility, sperm concentration, viability, morphological abnormalities, acrosomal integrity and functional plasma membrane integrity using CASA, eosin-nigrosin staining, Giemsa staining and hypo-osmotic swelling test (HOST), respectively.

3.5 Biochemical and Antioxidant Assessment

Seminal plasma was analyzed for catalase (CAT), superoxide dismutase (SOD), glutamate oxaloacetate transaminase (GOT), lactate dehydrogenase (LDH), lipid peroxidation (MDA) and total antioxidant capacity (TAC) using established spectrophotometric and ELISA-based methods.

3.6 Statistical Analysis

Data were analyzed using two-way analysis of variance (ANOVA) in IBM SPSS Statistics version 22.0 to determine the effects of treatment, preservation period and their interaction. Statistical procedures were performed according to Snedecor and Cochran (1994) ^[7].

4. Results and Discussion

The present research work entitled “the effect of *Giloy* (*Tinospora cordifolia*) on seminal parameters of boar spermatozoa during preservation” was carried out in the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Rewa (NDVSU, Jabalpur), Madhya Pradesh. In the present study, breeding boar having age between 1.0 to 2.0 years and weighing around 80-100 kg were used for the collection of semen. Semen was collected twice a week by double glove hand method and was evaluated for the various seminal attributes *e.g.* volume (ml), seminal pH, colour, consistency, motility, sperm concentration (millions/ml). The extended semen was evaluated at different groups of preservation for initial progressive motility, spermatozoa viability and abnormality, acrosomal integrity, HOST, enzymatic profiles (LDH, GOT, Catalase, SOD), LPO and TAC. Results obtained in the study are written under following sub headings:

4.1. Physico-morphological evaluation of fresh boar semen

The physico-morphological profiles of boar ejaculates were evaluated immediately postcollection to confirm physiological normalcy. The mean values for the key physicomorphological parameters are detailed in Table 2.0.

Table 2: Mean values of physico-morphological parameters of boar semen.

Physico-morphological parameters	Mean $\pm$ S.E.
Volume (ml)	219.17 $\pm$ 6.92
Colour	Milky White
pH	7.45 $\pm$ 0.05
Consistency	Thin milky
Sperm concentration	285.83 $\pm$ 7.68 million/ml

The average boar semen ejaculate volume was quantified at 219.17 $\pm$ 6.92, exhibiting a typical milky white coloration and a thin milky consistency. The mean seminal pH was slightly alkaline at 7.45 $\pm$ 0.05. Microscopic assessment revealed a mean spermatozoa concentration of 285.83 $\pm$ 7.68 million/ml, establishing that the boar semen ejaculate quality was within the optimal physiological range. In the present investigation, ejaculate volume was recorded as 219.17 $\pm$ 6.92 ml in boars which was in agreement reported by Ravimurugan *et al.* (2008) ^[8] and Saini *et al.* (2016) ^[9].

4.2. Spermatozoal functional microscopic characteristics

4.2.1. Initial progressive motility of boar spermatozoa

The initial progressive motility (%) at 0, 24, 48 and 72 hours in control (Group 1) as well as *Giloy* (*Tinospora cordifolia*) treated groups (Group 2, 3 and 4) were analyzed and results indicated that at 0 hours, initial progressive motility (%) did not differ significantly ($p > 0.05$) among the experimental groups, maintaining a baseline range of 85.57 $\pm$ 1.91 to 86.65 $\pm$ 2.96 as detailed in Table 3.0 and Figure 1.0. Temporal preservation induced a significant, linear decline ($p < 0.05$) in initial progressive motility across all groups from 24 to 72 hours.

Table 3: Initial progressive motility (%) at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	86.65±2.96 ^{Aa}	85.57±1.91 ^{Aa}	86.52±1.44 ^{Aa}	86.17±2.11 ^{Aa}
24	68.75±1.71 ^{Bc}	76.67±2.30 ^{Ba}	77.57±1.46 ^{Ba}	73.71±1.61 ^{Bb}
48	56.55±2.04 ^{Cc}	71.77±1.47 ^{Ca}	72.59±2.15 ^{Ca}	68.75±2.60 ^{Cb}
72	48.85±1.09 ^{Dc}	56.87±2.14 ^{Db}	62.50±1.56 ^{Da}	58.79±1.69 ^{Db}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

However, addition of Giloy (*Tinospora cordifolia*) extract significantly mitigated this decline. By 72 hours post-preservation, Group 3 demonstrated the highest retention of progressive motility (62.50±1.56), which was significantly superior ($p < 0.05$) to the untreated control Group 1 (48.85±1.09), as well as Group 2 (56.87±2.14) and Group 4 (58.79±1.69), respectively. The higher percent of initial progressive motility of preserved semen was reported as 67.60±0.47 in crossbred boars (Matharoo, 2015); 49.7±2.5 in boar (Mughal *et al.*, 2013); 48.33±1.06 in boar (Wafa *et al.*, 2021) and it was found that the similar results are seen. Our

results are in concurrence with these reported values and are considered to be in normal range.

4.2.2. Livability profiles

Spermatozoal livability patterns (%) were observed (Table 4.0 and Figure 2.0) and results indicate no significant inter-group variations ($p > 0.05$) at 0 hours. Storage duration caused a progressive reduction in the proportion of viable cells across all treatments ($p < 0.05$)

Table 4: Livability (%) at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	87.89±0.75 ^{Aa}	88.25±1.60 ^{Aa}	89.25±1.92 ^{Aa}	87.93±1.15 ^{Aa}
24	76.66±2.10 ^{Bc}	82.17±2.98 ^{Bb}	84.78±2.10 ^{Ba}	81.19±2.23 ^{Bb}
48	65.69±1.46 ^{Cc}	74.13±1.89 ^{Cb}	81.06±1.40 ^{Ca}	75.23±1.56 ^{Cb}
72	58.61±0.95 ^{Dc}	67.19±2.47 ^{Db}	72.17±2.28 ^{Da}	65.08±2.24 ^{Db}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

At 24, 48 and 72-hour intervals, Group 3 consistently maintained significantly higher livability percentages ($p < 0.05$) than all other experimental groups. At the final 72 hours mark, livability in the control group dropped sharply to 58.61±0.95%, whereas Group 3 exhibited a highly preserved viable population of 72.17±2.28%. The percent livability spermatozoa in diluted semen were reported as 82.79±0.29 in boar (Saurabh *et al.*, 2021); 82.53±0.70 in boar (Varghese *et al.*, 2015); 65.12±1.44 in boar (Hegazy *et al.*, 2020)

4.2.3. Morphological abnormalities

The incidence of morphological abnormalities (%) in boar spermatozoa was observed and results indicate that in boar spermatozoa morphological abnormalities increased over time across all preservation setups ($p < 0.05$, Table 5.0 and Figure 3.0). At 0 hours, aberrant morphology remained uniform across groups, ranging from 11.89±0.29% to 12.47±0.29%.

Table 5: Abnormality (%) at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	12.47±0.29 ^{Ca}	12.33±0.59 ^{Da}	11.89±0.29 ^{Da}	12.21±0.59 ^{Ca}
24	14.83±0.27 ^{Ba}	13.83±0.45 ^{Cb}	12.92±0.19 ^{Cb}	14.08±0.27 ^{Ba}
48	15.58±0.23 ^{Aa}	14.75±0.98 ^{Bb}	13.59±0.62 ^{Bb}	15.08±0.45 ^{Aa}
72	15.83±0.24 ^{Aa}	15.33±0.78 ^{Aa}	14.83±0.53 ^{Aa}	15.42±0.86 ^{Aa}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

During intermediate storage phases (24 and 48 hours), Giloy (*Tinospora cordifolia*) extract supplementation in Group 2 and Group 3 significantly suppressed ($p < 0.05$) the rate of morphological degradation compared to the control group. However, by 72 hours of preservation, the protective threshold was surpassed, resulting in statistically equivalent structural abnormality rates ($p > 0.05$) across all four treatment groups, maximizing between 14.83±0.53% to 15.83±0.24%. In the present study, the percent of abnormal spermatozoa was ranging from 10.83±0.30 to 15.50±0.50 and this was in accordance with the findings of Chaudhary *et al.*, 2017 and Singh *et al.*, (2020).

4.2.4. Acrosomal Integrity

The maintenance of an intact acrosome is crucial for fertilization and these metrics was observed and results are summarized in the Table 6.0 and Figure 4.0. No baseline disparities were observed at hours ($p > 0.05$). Storage-induced stress caused significant time-dependent acrosomal degradation ($p < 0.05$) in all groups. At 24 and 48 hours, all Giloy (*Tinospora cordifolia*)-treated groups (Group 2, 3 and 4) exhibited significantly higher acrosomal stability ($p < 0.05$) compared to control *i.e.* Group 1. By 72 hours, Group 2 and 3 displayed the highest acrosomal retention (64.58±2.87% and 64.83±2.12%, respectively), which was significantly greater ($p < 0.05$) than Group 4 (62.03±1.93%) and the control group (52.83±2.92%).

Table 6: Acrosomal integrity (%) at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	87.58±1.10 ^{Aa}	86.75±1.43 ^{Aa}	87.67±1.97 ^{Aa}	86.09±1.87 ^{Aa}
24	71.03±1.82 ^{Bb}	78.75±2.07 ^{Ba}	79.67±2.89 ^{Ba}	77.17±2.87 ^{Ba}
48	64.07±2.67 ^{Cc}	73.75±1.19 ^{Cb}	74.67±1.30 ^{Ca}	72.08±2.61 ^{Cb}
72	52.83±2.92 ^{Dc}	64.58±2.87 ^{Da}	64.83±2.12 ^{Da}	62.03±1.93 ^{Db}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

In our study the percent acrosomal integrity in preserved semen was in agreement with the finding of Mital *et al.* (2014).

4.2.5. Hypo-Osmotic Swelling Test (HOST) responsiveness

The functional integrity of the tail plasma membrane was assessed via HOST and results are shown in the Table 7 and Figure 5. Initial responsiveness was statistically uniform

($p > 0.05$) across the groups. Extended preservation caused a notable decline ($p < 0.05$) in membrane responsiveness over 72 hours.

Table 7: HOST (%) at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment.

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	79.91±0.67 ^{Aa}	81.75±0.88 ^{Aa}	81.67±1.97 ^{Aa}	80.19±1.32 ^{Aa}
24	73.16±1.70 ^{Bc}	76.92±1.07 ^{Ba}	77.92±2.83 ^{Ba}	75.16±2.13 ^{Bb}
48	68.16±0.72 ^{Cc}	70.42±1.55 ^{Cb}	72.92±1.13 ^{Ca}	70.31±1.43 ^{Cb}
72	51.04±0.74 ^{Dc}	59.92±1.53 ^{Db}	62.92±2.47 ^{Da}	60.23±2.31 ^{Db}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

Throughout the temporal evaluation (24, 48 and 72 hours), Group 3 consistently yielded significantly higher HOST-positive percentages ($p < 0.05$) than the remaining groups. At 72 hours, membrane responsiveness was optimally preserved in Group 3 (62.92±2.47%), followed by Groups 4 (60.23±2.31%) and 2 (59.92±1.53%), while the control group reached a minimum value of 51.04±0.74%. The percent HOST positive sperm in preserved semen reported as 60.56±2.93 in crossbred boar (Singh *et al.*, 2017) [10]; 56.83±0.90 in boar (Wafa *et al.*, 2021).

4.3. Seminal Enzymatic and Oxidative Biomarkers

4.3.1. Catalase and Superoxide Dismutase an endogenous antioxidant enzyme kinetics

The activities of enzyme Catalase and Superoxide Dismutase (SOD) were observed and results are exhibited in the Tables 8.0 and Figures 6.0 and 7.0, respectively. For both enzymes, Group 1 (control) demonstrated a significant, time-dependent surge ($p < 0.05$) in raw activity levels, peaking at 72 hours (Catalase: 3.62±0.04 U/ml; SOD: 5.11±0.14U/ml).

Table 8: Catalase (U/ml) activity at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	1.11±0.02 ^{Da}	1.45±0.03 ^{Aa}	1.36±0.03 ^{Aa}	1.29±0.03 ^{Aa}
24	1.90±0.02 ^{Ca}	1.16±0.05 ^{Ab}	1.05±0.04 ^{Ab}	0.98±0.03 ^{Bb}
48	2.52±0.03 ^{Ba}	0.99±0.12 ^{Bb}	0.68±0.12 ^{Bb}	0.88±0.11 ^{Bb}
72	3.62±0.04 ^{Aa}	0.86±0.13 ^{Bb}	0.58±0.12 ^{Bb}	0.63±0.03 ^{Bb}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$)

Table 9: Superoxide Dismutase (U/ml) activity at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	2.23±0.39 ^{Da}	2.16±0.44 ^{Aa}	2.12±0.42 ^{Aa}	2.15±0.19 ^{Aa}
24	3.23±0.41 ^{Ca}	2.16±0.65 ^{Ab}	2.02±0.45 ^{Ab}	2.05±0.44 ^{Ab}
48	4.23±0.02 ^{Ba}	3.16±0.66 ^{Ab}	3.12±0.42 ^{Ab}	3.52±0.85 ^{Ab}
72	5.11±0.14 ^{Aa}	3.67±0.22 ^{Ab}	3.15±0.34 ^{Ab}	3.28±0.04 ^{Ab}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$)

Conversely, the addition of Giloy (*Tinospora cordifolia*) extract successfully checked this compensatory enzyme up regulation. In Groups 2, 3 and 4, both Catalase and SOD activities remained suppressed and statistically equivalent ($p > 0.05$) across the 24-, 48- and 72-hour intervals. This indicates a reduced requirement for endogenous enzyme synthesis due to the exogenous antioxidant protection provided by the Giloy extract. In our study, the SOD enzyme activity of spermatozoa at a fresh and preserved stage was ranging from 29.64±0.48 to 22.63±0.42. Our findings are accordance with the findings of Kadirvel *et al.*, (2014). In our study the catalase activity of spermatozoa at a

preserved semen was ranging from 0.77±0.10 to 0.41±0.11. It was seen that the lower finding is found with accordance Gebreselassie, 2009 (1.10±0.09); and higher finding are seen with the accordance Lone *et al.*, 2018 (0.001±0.00nM / mg)

4.3.2. Glutamic-Oxaloacetic transaminase and Lactate Dehydrogenase enzyme activity

The activity of Glutamic-Oxaloacetic Transaminase (GOT) and Lactate Dehydrogenase (LDH) into the extracellular medium was observed and are depicted in the Tables 10.0 and 11.0, Figure 8.0 and 9.0, respectively.

Table 10: Glutamic-Oxaloacetic transaminase (U/L) activity at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	1076.56±11.10 ^{Da}	1052.07±12.36 ^{Ca}	991.26±16.41 ^{Ca}	1043.47±13.32 ^{Ca}
24	1147.94±11.97 ^{Ca}	738.37±12.91 ^{Bd}	832.26±9.62 ^{Bc}	956.26±12.19 ^{Bb}
48	1228.04±9.15 ^{Ba}	797.64±14.56 ^{Ad}	899.33±12.01 ^{Ac}	1017.75±13.15 ^{Ab}
72	1258.49±9.59 ^{Aa}	694.40±14.28 ^{Ad}	895.01±11.88 ^{Ac}	1029.15±12.32 ^{Ab}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

Extracellular GOT levels rose significantly over time in the control group ($p < 0.05$) reaching a peak 1258.49±9.59 U/L at 72 hours. Groups treated with Giloy (*Tinospora cordifolia*) showed significantly reduced activity ($p < 0.05$). Group showed the lowest GOT efflux (694.40±14.28 U/L) at 72 hours. For LDH, efflux remained steady up to 48 hours ($p > 0.05$) across all

groups, followed by a sharp, significant escalation ($p < 0.05$) at 72 hours. Although Group 1 had the highest absolute activity at 72 hours (358.66±7.99 U/L), no statistically significant variations ($p > 0.05$) were established among the rows at any specific time point, indicating that treatment effects on LDH kinetics were elusive.

Table 11: Lactate Dehydrogenase (U/L) activity at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	260.11±6.40 ^{Ba}	237.83±2.15 ^{Ba}	243.12±7.60 ^{Ba}	253.18±7.74 ^{Ba}
24	268.58±6.57 ^{Ba}	246.33±1.6 ^{Ba}	250.50±3.50 ^{Ba}	260.08±5.02 ^{Ba}
48	279.91±6.09 ^{Ba}	259.50±1.32 ^{Ba}	260.51±4.35 ^{Ba}	270.42±4.88 ^{Ba}
72	358.66±7.99 ^{Aa}	332.75±2.16 ^{Aa}	334.75±8.26 ^{Aa}	343.83±7.59 ^{Aa}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

In our study, the GOT enzyme activity in diluted semen at a preserved stage was ranging from 1419.98±10.76 to 516.38±15.09. Our findings are similar to the finding of Chaudhary *et al.*, (2017). In our study, the LDH activity in preserved semen was ranging from the 365.83±12.41 to 270.66±6.03, which was lower as compared to the values reported by Gebreselassie, (2009) and higher as compared with the values of Patel *et al.*, (2016).

Malondialdehyde generation measured via LPO, escalated over time across all groups ($p < 0.05$) the control group exhibited severe oxidative stress, with LPO rising to 6.52±0.03 µmol / L at 72 hours. Giloy (*Tinospora cordifolia*) extract administration significantly suppressed ($p < 0.05$) peroxide accumulation. At 72 hours, Groups 2, and effectively restricted LPO levels (µmol/L) to a range of 3.79±0.02, 3.68±0.35 and 3.58±0.45 respectively, were showing no significant differences among themselves ($p > 0.05$).

4.3.3. Lipid Peroxidation (LPO) and Total Antioxidant Capacity (TAC) estimation

The biochemical markers for oxidative stress were analyzed and are given in the Tables 12.0 and 13.0 and Figures 10.0 and 11.0.

Table 12: Lipid Peroxidation (µmol/L) at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	1.18±0.15 ^{Da}	1.46±0.15 ^{Ba}	1.34±0.15 ^{Ba}	1.24±0.15 ^{Ba}
24	2.52±0.03 ^{Ca}	1.79±0.02 ^{Bb}	1.68±0.19 ^{Bb}	1.58±0.14 ^{Bb}
48	4.73±0.09 ^{Ba}	2.32±0.09 ^{Ac}	2.55±0.12 ^{Ac}	3.64±0.11 ^{Ab}
72	6.52±0.03 ^{Aa}	3.79±0.02 ^{Ab}	3.68±0.35 ^{Ab}	3.58±0.45 ^{Ab}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

Table 13: Total antioxidant capacity (µmol/L) at 0, 24, 48 and 72 hrs of boar spermatozoa (Mean±SE) after Giloy (*Tinospora cordifolia*) treatment.

Duration (hrs)	Group 1	Group 2	Group 3	Group 4
0	773.50±33.54 ^{Aa}	797.09±35.39 ^{Aa}	787.25±26.32 ^{Aa}	782.08±27.88 ^{Aa}
24	673.50±33.19 ^{Ba}	597.15±28.24 ^{Bb}	587.25±37.20 ^{Bb}	582.18±21.89 ^{Bb}
48	523.14±23.27 ^{Ca}	433.33±20.53 ^{Cb}	439.33±19.50 ^{Cb}	433.67±20.07 ^{Cb}
72	454.33±37.04 ^{Da}	366.67±36.64 ^{Db}	372.67±36.58 ^{Db}	367.15±38.46 ^{Db}

Means within a column (A, B, C, D) and row (a, b, c) with different superscripts differ significantly ($p < 0.05$).

Concurrently, Total Antioxidant Capacity (TAC) degraded progressively over time across all experimental groups ($p < 0.05$, Table 12). Interestingly, at all post-baseline intervals (24, 48 and 72 hours), the untreated control group (Group 1) maintained significantly higher TAC levels ($p < 0.05$) in the supernatant fluid compared to all the *T. cordifolia*-supplemented groups. By

72 hours, the control group held a TAC value of 454.33±37.04 µmol/L, whereas the Giloy extract-treated groups dropped uniformly ($p > 0.05$) to a lower range *i.e.* 366.67±36.64 µmol/L (Group 2), 372.67±36.58 µmol/L (Group 3) and 367.15±38.46 µmol/L (Group 4) respectively.

Lipid peroxidation's effects on the spermatozoa are numerous such as membrane damage, irreversible loss of motility,

inactivation of enzymes and a high rate of leakage of intracellular sperm constituents like adenine, pyridine and enzymes, protein and DNA damage (Kadirvel *et al.*, 2014). In the present study, levels of TAC in the preserved boar semen were 1.13 ± 0.05 nmol/ 10^8 and 1.22 ± 0.05 nmol/ 10^8 spermatozoa, respectively. The TAC values obtained for preserved semen were 1.6 ± 0.04 nmol/ 10^8 spermatozoa and 1.69 ± 0.04 nmol/ 10^8 spermatozoa, respectively. These values did not differ significantly between groups Patel *et al.*, 2016 (172.70 ± 0.41).

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