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# PANTNAGAR JOURNAL OF RESEARCH

Vol. 24(2)

May-August, 2026

## CONTENTS

- AI and geospatial innovations for Indian agriculture: Applications, policy support and emerging research frontiers (Review)** 233-247  
ABHISHEK DANODIA, SURESH KUMAR and AJEET SINGH NAIN
- Optimizing nitrogen use efficiency and grain quality in basmati rice through Integrated weed management: Insights into nutrient partitioning and Crop-weed competition** 248-255  
ROMAN NISSAR, MANZOOR A GANAI, MEHRAJ UD DIN SOFI, ASHAQ HUSSAIN and F. A. BAHAR
- Performance evaluation of brown top millet (*Brachiaria ramosa* L.) genotypes under rainfed upland ecology of the Bastar plateau of Chhattisgarh** 256-266  
PRAFULL KUMAR
- Effect of pre-harvest sprays of nanofertilizers on fruit quality in litchi (*Litchi chinensis* Sonn.) cv. Rose Scented** 267-274  
NITIN PANWAR, RATNA RAI and RAJEEW KUMAR
- Ethrel induced ripening optimally modulates physicochemical quality, antioxidant properties and shelf life of sapota (*Manilkara zapota* L.) cv. Cricket Ball** 275-285  
ASHOK KUMAR SINGH, KIRTI CHETRI, RISHABH RAJ, S. K. GURU and N. C. SHAHI
- Influence of drip irrigation and mulching practices on fruit set, yield and water use efficiency of litchi cv. rose scented in the Tarai region of Uttarakhand** 286-292  
SHANKAR LAL, N. SINGH, N.K. BIND, PRERNA PRACHI, RAJSHREE, RIYA THAPA and AKHIL RUHELA
- Spatial distribution and survey of soybean anthracnose in the Kumaon region of Uttarakhand, India** 293-300  
MANISHA DEV and K.P. SINGH
- Dimensionality reduction of part milk yield in crossbred cattle using Principal Component Analysis** 301-307  
SIMRAN KAUR, ASHIS KUMAR GHOSH, OLYMPICA SARMA and RAVINDER SINGH BARWAL
- Comparative histomorphological and histochemical studies on the abomasum of adult Bakerwali goats, non-descript goats and non-descript sheep of the Jammu region** 308-318  
JASVINDER SINGH SASAN, SHALINI SURI and KAMAL SARMA
- Gross morphology and biometry on the abomasum of adult Bakerwali goat, non-descript goats and non-descript sheep of Jammu region: A comparative study** 319-325  
JASVINDER SINGH SASAN, SHALINI SURI and KAMAL SARMA



|                                                                                                                                                                                                                                                                                                                                 |         |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| <b>Arterial blood gas analysis in bovine pneumonia: Comparative study of healthy and diseased cattle</b><br>ASMITA NARANG and CHARANJIT SINGH                                                                                                                                                                                   | 326-331 |
| <b>Assessment of commonly used insecticide residues using gas chromatography in buffalo liver samples collected from North India</b><br>S. K. JAIN, VINOD KUMAR, VINAY KANT, PREETI BAGRI, ARCHANA LOHIYA, GAURI CHANDRATRE and GAURAV GUPTA                                                                                    | 332-339 |
| <b><i>In silico</i> molecular docking analysis of squalene with major ageing-related molecular targets: matrix metalloproteinase-1, matrix metalloproteinase-9, cyclooxygenase-2, Tumor Necrosis Factor-<math>\alpha</math>, hyaluronidase-1 and B-cell lymphoma-2</b><br>PAWANESH TAMTA, KULDIP CHANDRA VERMA and APARNA DIXIT | 340-352 |
| <b>Characterization of agro-waste (<i>Parthenium hysterophorus</i>) derived Carbon Dots and to assess their potential as a sustainable adsorbent</b><br>NISHTHA NAUDIYAL and R.K. SRIVASTAVA                                                                                                                                    | 353-360 |
| <b>Assessment of the antibacterial efficacy of endophytic bacteria in combination with neem leaf powder against bacterial pathogens</b><br>SHIVANI SINGH, AJAY VEER SINGH and VELLAICHAMY MAGESHWARAN                                                                                                                           | 361-370 |
| <b>Exploration of endophytic bacterial isolates from Kasni leaves (<i>Cichorium intybus</i>) for Phyto-hormone and Phyto-stimulating metabolite production</b><br>VARSHA MISHRA, SEEMA BISHT, DIVYA PANT, ANUPAMA PANDEY and LAKSHMI TEWARI                                                                                     | 371-382 |
| <b>Mitigation of dexamethasone-induced cardiac oxidative damage by <i>Moringa oleifera</i> and Vitamin E-Selenium: A biochemical study in broilers</b><br>LATA PALIWAL, SONU AMBWANI and TANUJ KUMAR AMBWANI                                                                                                                    | 383-395 |
| <b>Adaptive Neural Networks for dynamic prioritization in autonomous vehicles for road users</b><br>MUMUKSHU BHATT, SUBODH PRASAD, RAJESH SINGH, ASHOK KUMAR and POOJA TAMTA                                                                                                                                                    | 396-403 |
| <b>Assessing the impact of Geopolitical Disturbances on India's agricultural trade performance</b><br>JYOTI, SUMAN GHALAWAT, MEGHA GOYAL, SUNIL KUMAR, AMITA GIRDHAR and SUBODH AGARWAL                                                                                                                                         | 404-409 |
| <b>Black soybean (<i>Glycine max</i> (L.) Merrill): Nutritional and functional analysis of whole seed, dehusked seed and husk fractions</b><br>RITA SINGH RAGHUVANSHI, GUNJAN TEWARI, AABHA RAWAT and APURVA                                                                                                                    | 410-421 |
| <b><i>In silico</i> bioprospecting of bioactive phytochemicals with potential protective effects against exercise-induced muscle stiffness</b><br>NAGARJUN G. S., PAL MURUGAN M., KRISHNANDU MONDAL, PADMASHREE A., WADIKAR D. D. and KUMAR R.                                                                                  | 422-433 |

## Mitigation of dexamethasone-induced cardiac oxidative damage by *Moringa oleifera* and Vitamin E-Selenium: A biochemical study in broilers

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**ABSTRACT:** Intensive broiler production exposes birds to chronic stress that elevates circulating glucocorticoids and drives oxidative injury in the metabolically active myocardium. This study evaluated the cardioprotective potential of dietary *Moringa oleifera* leaf powder (MOLP) and a vitamin E-selenium combination against dexamethasone-induced oxidative stress in the broiler heart. A total of 180 one-day-old Cobb 430 chicks were randomly allotted to six groups: basal control (G1), dexamethasone (G2), 1% MOLP (G3), dexamethasone + MOLP (G4), vitamin E-selenium (G5) and dexamethasone + vitamin E-selenium (G6). Dexamethasone (1 mg/kg body weight) was administered through drinking water on days 7-14. Hearts collected on days 15, 30 and 45 were assayed for lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and total antioxidant capacity (CUPRAC, FRAP) in tissue lysates. Dexamethasone markedly raised malondialdehyde and depleted GSH, SOD, CAT, GST, CUPRAC and FRAP at all sampling points. MOLP produced notable biochemical restoration, exceeding control values for several antioxidant markers, whereas vitamin E-selenium recorded the lowest malondialdehyde and highest glutathione by day 45. Overall, both *Moringa oleifera* and vitamin E-selenium effectively counter dexamethasone-induced cardiac oxidative stress, representing practical nutritional strategies for safeguarding broiler heart health.

**Keywords:** Broiler, Dexamethasone, Oxidative stress, *Moringa oleifera*, Vitamin E-selenium, Antioxidant enzymes

The broiler chicken (*Gallus gallus domesticus*) industry constitutes one of the fastest-growing segments of global animal protein production, meeting an ever-increasing demand for affordable, high-quality meat. However, intensive rearing conditions expose birds to a wide spectrum of environmental, nutritional and management-related stressors (Surai *et al.*, 2019). Under such conditions, cellular oxidative balance is frequently disrupted, resulting in excessive generation of reactive oxygen species (ROS) that overwhelm the endogenous antioxidant defence machinery (Yang *et al.*, 2023). Prolonged oxidative imbalance impairs immune competence, compromises organ integrity, reduces growth efficiency and deteriorates meat quality, collectively undermining the economic sustainability of commercial broiler production (He *et al.*, 2025). Broiler chickens are reared under intensive commercial conditions that expose them to a multitude of stressors, including nutritional imbalances, environmental extremes and pathogenic challenges (Oke *et al.*, 2024). These stressors

converge to activate the hypothalamic-pituitary-adrenal (HPA) axis, culminating in elevated circulating glucocorticoid concentrations that, when chronically sustained, exert profoundly deleterious effects on productive performance, immune competence and organ integrity (Huang *et al.*, 2025). To counteract the potentially devastating consequences of ROS accumulation, the myocardium is equipped with a sophisticated, multi-tiered antioxidant defence system comprising both enzymatic and non-enzymatic components (Ighodaro and Akinloye, 2018). The most powerful first-line antioxidant defence system comprises superoxide dismutases (SODs), catalase (CAT) and glutathione peroxidases (GPxs), along with the low-molecular-weight antioxidants glutathione (GSH) and thioredoxin, which serve as cofactors for various antioxidant enzymes (Jomova *et al.*, 2024). Beyond enzymatic defenses, the cardiac antioxidant system also relies on non-enzymatic molecules including vitamin E ( $\alpha$ -tocopherol), vitamin C, glutathione and various polyphenolic compounds that serve as direct



ROS scavengers and membrane-stabilizing agents (Handy and Loscalzo, 2012). Dexamethasone, a potent synthetic glucocorticoid, has been widely employed as an experimental tool to replicate the deleterious physiological consequences of chronic stress in avian species (Fathi *et al.*, 2023). At pharmacological doses, dexamethasone mimics the catabolic cascade triggered by endogenous corticosterone hypersecretion, promoting proteolysis, gluconeogenesis and lipid peroxidation (LPO) while simultaneously attenuating endogenous antioxidant defences (Hayatolghaibi *et al.*, 2025). The heart is particularly susceptible to oxidative injury owing to its high metabolic rate, limited endogenous antioxidant reserves and reliance on membrane-rich, polyunsaturated-fatty-acid-abundant cardiomyocytes. The glucocorticoid-mediated induction of oxidative stress has been particularly well-characterised in cardiac tissue, where dexamethasone-triggered ROS generation through the NOX/NADPH oxidase pathway leads to cardiomyocyte injury, fibrosis and electrophysiological disturbances (Macedo *et al.*, 2020). These adverse effects have stimulated considerable interest in identifying natural dietary interventions capable of mitigating glucocorticoid-induced oxidative damage and preserving cardiac health in poultry. In the search for safe and effective natural interventions against oxidative stress, *Moringa oleifera* Lam. (family Moringaceae) has emerged as a particularly promising candidate. Widely distributed across tropical and subtropical regions and commonly referred to as the “miracle tree” or “drumstick tree” or “sahjan tree”, *Moringa oleifera* is characterised by an exceptionally rich phytochemical profile encompassing flavonoids (notably quercetin and kaempferol), ascorbic acid, carotenoids, glucosinolates, isothiocyanates, tannins and saponins, in addition to an array of essential macro- and micronutrients (da Silva Parente *et al.*, 2025; Abd El-Hack *et al.*, 2022).

Dietary inclusion of *Moringa oleifera* leaf meal or extract in broiler rations has been associated with significantly reduced MDA levels and upregulated antioxidant enzyme activity in tissues, underscoring its translational relevance for mitigating stress-

induced organ pathology in poultry (Husien *et al.*, 2025). Vitamin E is widely regarded as the “headquarters” of the antioxidant defence network in poultry, functioning as the principal lipid-soluble, chain-breaking antioxidant that intercepts free-radical propagation within cell membranes and prevents polyunsaturated fatty acid peroxidation (Surai *et al.*, 2019). Dietary vitamin E supplementation has been consistently shown to increase SOD and CAT activities, elevate total antioxidant capacity and reduce MDA concentrations in broiler tissues, including cardiac muscle (Elgendey *et al.*, 2022). Vitamin E and selenium operate synergistically within the avian antioxidant defence system through complementary and interdependent mechanisms. Vitamin E scavenges lipid peroxyl radicals in the membrane phase, while GPx, requiring selenium as its active-site cofactor, detoxifies the resulting lipid hydroperoxides in the aqueous phase- a biochemical partnership that renders either nutrient more effective in combination than in isolation (Surai *et al.*, 2019; Elgendey *et al.*, 2022).

The present study was therefore designed to evaluate the cardioprotective effects of dietary *Moringa oleifera* leaf supplementation and vitamin E-Selenium against dexamethasone-induced oxidative stress in broiler chickens, with the activities of key antioxidant enzymes in cardiac tissue.

## MATERIALS AND METHODS

### *Materials required*

The feeding trial was conducted in commercial broiler chickens using vitamin E-Selenium and dexamethasone administration. vitamin E-Selenium were provided via the commercial feed additive E-Care Se®. The supplement was administered through drinking water at a dose of 3 g per 30 birds. Dexamethasone was administered as a freshly prepared solution from Dexona® tablets at a dose rate of 1 mg/kg body weight in drinking water for seven consecutive days.

### *Ethical approval*

The present study was conducted with prior approval

from the Institutional Animal Ethical Committee (IAEC), Pantnagar, under approval number IAEC/CBSH/MBGE/529. All experimental procedures involving broiler birds were carried out in strict accordance with the ethical principles governing the humane care, handling and management of laboratory and farm animals.

#### ***Collection and botanical authentication of Moringa oleifera***

Fresh leaves of *Moringa oleifera* were collected during May from areas surrounding the G.B. Pant University campus, Pantnagar. Botanical authentication was performed at the Department of Biological Sciences, College of Basic Sciences and Humanities and a voucher specimen (Accession No. 1570) was submitted to the departmental herbarium. The leaves were shade-dried for 15-20 days and ground into powder form. The *Moringa oleifera* leaf powder (MOLP) was kept in airtight containers at room temperature until incorporation into the experimental diets.

#### ***Birds, housing and experimental design***

A total of 180 one-day-old Cobb 430 broiler chicks were procured from a commercial hatchery and reared at the Instructional Poultry Farm (IPF), Nagla, G.B. Pant University of Agriculture and Technology, Pantnagar. After a 7-day acclimatization period, birds were provided *ad libitum* access to feed and water and maintained under standard management and hygienic conditions. Broiler starter and finisher diets were formulated according to BIS (2007) recommendations. During the first week of brooding, the ambient temperature was kept between 32 and 34°C. After that, it was progressively lowered to 24°C with a relative humidity of about 60%. Every experimental technique was carried out in compliance with CPCSEA regulations.

Following a 7-day acclimatization period, the chicks were randomly assigned to six treatment groups, each comprising three replicates of ten birds. Group 1 received the basal diet alone. Group 2 was administered dexamethasone. Group 3 received the basal diet supplemented with 1% MOLP. Group 4 was provided dexamethasone along with MOLP

supplementation. Group 5 received vitamin E-Selenium supplementation, while Group 6 was administered dexamethasone together with vitamin E-Selenium. The experimental trial lasted for six weeks; however, dexamethasone treatment in Groups 2, 4 and 6 was administered only for seven consecutive days (days 7-14).

#### ***Slaughter schedule and cardiac tissue collection***

To evaluate temporal changes in the cardiac antioxidant defence system over the production cycle, birds were sampled at three predetermined time periods: the 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day. At each scheduled interval, two birds per replicate from each of the six experimental groups were randomly selected and slaughtered following standard operating procedures. Immediately after slaughter, the thoracic cavity was opened and the heart was rapidly excised. Each cardiac tissue sample was freed of adherent pericardial fat and connective tissue, rinsed briefly in ice-cold normal saline to remove residual blood.

#### ***Preparation of cardiac tissue homogenate***

To estimate antioxidant indices in cardiac tissue homogenates, 200 mg of the harvested heart tissue was collected and suspended in 2 ml of ice-cold phosphate-buffered saline (PBS) with a pH of 7.4. Tissue homogenization was performed under cold conditions using a tissue homogenizer to prevent enzymatic degradation and maintain the integrity of antioxidant constituents. The resulting homogenates were subsequently centrifuged at 3000 rpm for 10 minutes and the clear supernatant thus obtained was carefully collected for further analysis of antioxidant parameters. For the purpose of measuring GSH, tissue samples were separately collected in a solution containing 0.02 M EDTA to protect the free sulfhydryl groups from oxidation during processing.

#### ***Estimation of antioxidant parameters***

The cardiac tissue lysates were subjected to a panel of seven antioxidant assays encompassing markers of oxidative damage, enzymatic antioxidant defence and non-enzymatic reducing capacity. All the estimations were performed using two samples each collected from the replicates and are presented as

mean $\pm$ standard error (SE).

### Membrane lipid peroxidation (LPO)

LPO was quantified following the method of Li *et al.* (2014). Briefly, 0.1 ml of tissue lysate was incubated with 1.9 ml phosphate buffer (0.1 M, pH 7.4) at 37°C for 1 h. After incubation, 350  $\mu$ l of 5% TCA was added and the mixture was centrifuged at  $2300 \times g$  for 15 min. An aliquot of the resulting lysate (0.5 ml) was reacted with an equal volume of 1% TBA and heated in a boiling water bath for 15 min. Following cooling and dilution with distilled water, absorbance was measured at 535 nm. The results were expressed as nmol MDA/ml tissue lysate.

### Reduced glutathione (GSH)

GSH levels were determined according to Nanto-Hara *et al.* (2021). Tissue lysate (0.1 ml) was mixed with 0.9 ml of 5% TCA and centrifuged at  $2300 g$  for 15 min at 4°C. Subsequently, 0.5 ml of the clear lysate was combined with 1.5 ml of 0.01% DTNB solution and absorbance was recorded at 412 nm. GSH concentration was expressed as mM GSH/ml tissue lysate.

### Superoxide dismutase (SOD)

SOD activity was assessed using the procedure outlined by Paseban *et al.* (2019) with slight modifications. The assay mixture contained 0.65 ml PBS, 30  $\mu$ l MTT, 75  $\mu$ l pyrogallol and 10  $\mu$ l tissue lysate. Following incubation at room temperature for 5 min, the reaction was halted by adding 0.75 ml DMSO. Absorbance was measured at 570 nm and enzyme activity was expressed as SOD units/mg protein.

### Catalase (CAT)

The spectrophotometric approach of Aebi (1984) was used to determine catalase activity. A fresh 50 mM phosphate buffer was used to prepare a 30 mM  $H_2O_2$  solution. Briefly, a quartz cuvette was filled with 20  $\mu$ l of cell lysate and 2 ml of phosphate buffer. 1 ml of 30 mM  $H_2O_2$  was added to start the reaction. A UV-visible spectrophotometer was used to track the absorbance decline at 240 nm at 30-second intervals for two minutes. Catalase activity was calculated using the molar extinction coefficient of

$H_2O_2$  and expressed as mM  $H_2O_2$  utilized  $min^{-1} mg^{-1}$  protein.

### Glutathione-S-Transferase (GST)

The Katerji *et al.* (2019) method was used to test GST activity. In 0.1 M sodium phosphate buffer (pH 6.0), the final substrate concentrations were 1 mM CDNB (1-chloro- 2, 4 dinitrobenzene) and 5 mM GSH. 60  $\mu$ l of cell lysate and 1.2 ml of substrate solution were combined to create a reaction mixture. 60  $\mu$ l of distilled water was used in place of the cell lysate for the blank preparation. An ELISA reader was used to measure the increase in absorbance at 340 nm for five minutes after aliquots (210  $\mu$ l) were placed in a 96-well plate. GST activity was calculated using the molar extinction coefficient of  $9.6 mM^{-1} cm^{-1}$  and expressed as  $\mu$ mol GSH-CDNB conjugate formed  $min^{-1} ml^{-1}$  cell lysate.

### Ferric reducing antioxidant power (FRAP)

According to Aremu *et al.* (2019), FRAP activity was assessed. 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 50 mM HCl and 20 mM  $FeCl_3 \cdot 6H_2O$  solution were combined in a 10:1:1 (v/v) ratio to create the fresh FRAP reagent. For the purpose of creating standard curves, various concentrations of  $FeSO_4 \cdot 7H_2O$  (20–100  $\mu$ g/ml) were prepared. 100  $\mu$ l of cell lysate and 3 ml of freshly made FRAP reagent were combined for the test, which were then allowed to incubate at room temperature for 30 minutes in the dark. An ELISA reader was used to detect absorbance at 593 nm. Results were expressed as  $FeSO_4$  equivalents ( $\mu$ g/ml cell lysate).

### Cupric ion reducing antioxidant capacity (CUPRAC)

Apak *et al.* (2005) used the CUPRAC method to measure total antioxidant capacity. Briefly, 7.5 mM neocuproine solution, 1 M ammonium acetate buffer (pH 7.0) and 10 mM  $CuCl_2 \cdot 2H_2O$  were made. One millilitre of  $CuCl_2$  solution, one millilitre of ammonium acetate buffer and one millilitre of neocuproine solution made up the reaction mixture. After that, 25  $\mu$ l of cell lysate was added and distilled water was used to bring the total amount down to 4.1 ml. After 30 minutes of incubation in dark at

room temperature, the mixture's absorbance at 450 nm was measured. Results were expressed as quercetin equivalents ( $\mu\text{g/ml}$  cell lysate).

### **Statistical analysis**

To verify the findings, SPSS statistical software (version 26.0) was used, all data were subjected to two-way analysis of variance (ANOVA) and Tukey's post-hoc test for multiple comparisons among groups at each time period. Mean $\pm$ SEM was used to express the results and differences were viewed as statistically significant at  $p < 0.05$ . For heat map visualization, the data were standardized using Z-score transformation. In order to ensure that higher Z-scores consistently indicate a more favourable antioxidant profile across all metrics, lipid peroxidation data were inverted before normalization. The standardized values were used to generate the heat map, where a blue-white-red colour gradient represented low, mean, and high standardized values, respectively.

## **RESULTS AND DISCUSSION**

### **Oxidative stress and antioxidant status**

The cardiac redox status of all six groups was assessed by measuring LPO, along with both enzymatic and non-enzymatic antioxidant markers in heart tissue lysates. These included GSH, SOD, CAT and GST. In addition, total antioxidant capacity was evaluated using the CUPRAC and FRAP assays (Fig. 1). The findings are summarized in Table 1.

The Z-score standardized antioxidant parameter heatmap showed clear treatment-specific trends in all of the experimental groups (Fig. 2). Lower normalized values for antioxidant measures and higher values for LPO were more common in the dexamethasone-treated group (G2), suggesting increased oxidative stress. On the other hand, the vitamin E-Selenium (G5) and *Moringa oleifera* (G3) groups demonstrated better antioxidant status as seen by lower lipid peroxidation and higher standardized antioxidant values. The intermediate profiles of the co-treatment groups (G4 and G6) suggested a partial reduction in the oxidative stress caused by dexamethasone. Overall, the oxidative stress profiles

of the birds treated with dexamethasone and those supplemented with antioxidants were significantly differentiated by the heat map.

### **LPO**

Over the course of the trial, LPO levels varied considerably between the treatment groups. While *Moringa oleifera* (G3) and vitamin E-Selenium (G5) supplementation maintained significantly reduced LPO levels, the dexamethasone-treated group (G2) continuously showed the highest lipid peroxidation, indicating increasing oxidative damage. Lipid peroxidation mediated by dexamethasone was partially attenuated in both antioxidant co-treatment groups (G4 and G6). All supplemented groups showed a slow decrease in LPO between Day 15 and Day 45, indicating a steady decrease in oxidative stress over time.

### **GSH**

Treatment had a substantial impact on GSH content. Throughout the trial, the treatment of dexamethasone considerably reduced GSH levels, while supplementing with vitamin E-Selenium and *Moringa oleifera* greatly increased GSH levels. When compared to the stressed group, birds who received antioxidants in addition to dexamethasone also showed significant recovery and vitamin E-Selenium supplementation produced the highest GSH concentration. All treatment groups saw a gradual increase in GSH levels from Day 15 to Day 45, with the supplemented birds showing the most significant improvement.

### **Superoxide dismutase (SOD)**

Throughout the investigation, there were notable differences in SOD activity between the treatment groups. While *Moringa oleifera* supplementation (G3) maintained the highest activity at all sampling intervals, the dexamethasone-treated group (G2) continuously showed the lowest SOD activity. While both co-treatment groups (G4 and G6) shown some recovery from dexamethasone-induced suppression, vitamin E-Selenium supplementation (G5) also markedly increased SOD activity in comparison to the stressed group. From Day 15 to Day 45, SOD activity increased gradually in both groups, but it



was more noticeable in the birds who received antioxidant supplements. This suggests that endogenous antioxidant defense improves with age and ongoing supplementation.

### Catalase (CAT)

Following the treatment of dexamethasone, catalase activity was markedly decreased; however, greater enzyme activity was maintained by supplementing with vitamin E-Selenium and *Moringa oleifera*. The group supplemented with *Moringa oleifera* consistently had the highest CAT activity, followed by the group treated with vitamin E and selenium. In birds given dexamethasone, co-treatment with antioxidants partially restored CAT activity. Antioxidant-supplemented birds maintained considerably higher activity than the stressed group throughout the research, despite a modest drop in CAT activity by Day 45 compared with earlier sampling in most groups.

### Glutathione-S-Transferase (GST)

Dietary interventions have a considerable impact on GST activity. While supplementing with *Moringa oleifera* consistently produced the highest GST activity on all sampling days, dexamethasone significantly decreased GST activity. When compared to the stressed group, vitamin E-Selenium likewise increased GST activity; G4 and G6 showed intermediate values. From Day 15 to Day 45, GST activity in the supplemented groups increased slightly, suggesting that sustained supplementation would improve detoxifying capability.

### CUPRAC

The CUPRAC assay revealed substantial differences in the treatment group's total antioxidant capacity. *Moringa oleifera* supplementation maintained the greatest values throughout the experiment, while the dexamethasone-treated group continuously

**Table 1: Effect of dietary supplementation of *Moringa oleifera* leaf powder and Vitamin E-Selenium on cardiac oxidative stress indicators and antioxidant status of dexamethasone-induced broiler chickens.**

| Group  | LPO<br>(nM<br>MDA/ml)           | GSH<br>(mM/ml)                 | SOD(U/mg<br>of protein)         | CAT( $\mu$ M H <sub>2</sub> O <sub>2</sub><br>utilized/min/<br>mg of protein) | GST (mM of<br>GSH-CDNB<br>conjugate/min/ ml) | CUPRAC<br>( $\mu$ g/ml)         | FRAP<br>( $\mu$ g/ml)           |
|--------|---------------------------------|--------------------------------|---------------------------------|-------------------------------------------------------------------------------|----------------------------------------------|---------------------------------|---------------------------------|
| Day 15 |                                 |                                |                                 |                                                                               |                                              |                                 |                                 |
| G1     | 1.970 $\pm$ 0.019 <sup>b</sup>  | 0.707 $\pm$ 0.007 <sup>b</sup> | 45.47 $\pm$ 1.250 <sup>b</sup>  | 65.03 $\pm$ 1.16 <sup>b</sup>                                                 | 0.499 $\pm$ 0.003 <sup>b</sup>               | 5.615 $\pm$ 0.496 <sup>ab</sup> | 2.372 $\pm$ 0.092 <sup>b</sup>  |
| G2     | 3.521 $\pm$ 0.019 <sup>a</sup>  | 0.366 $\pm$ 0.005 <sup>d</sup> | 27.42 $\pm$ 1.180 <sup>d</sup>  | 38.18 $\pm$ 1.42 <sup>d</sup>                                                 | 0.243 $\pm$ 0.004 <sup>d</sup>               | 2.505 $\pm$ 0.606 <sup>c</sup>  | 1.178 $\pm$ 0.067 <sup>c</sup>  |
| G3     | 1.656 $\pm$ 0.044 <sup>c</sup>  | 0.820 $\pm$ 0.007 <sup>b</sup> | 64.09 $\pm$ 2.820 <sup>a</sup>  | 81.66 $\pm$ 4.08 <sup>a</sup>                                                 | 0.817 $\pm$ 0.017 <sup>a</sup>               | 7.112 $\pm$ 0.398 <sup>a</sup>  | 4.260 $\pm$ 0.174 <sup>a</sup>  |
| G4     | 3.231 $\pm$ 0.165 <sup>a</sup>  | 0.547 $\pm$ 0.005 <sup>c</sup> | 36.70 $\pm$ 1.810 <sup>c</sup>  | 52.57 $\pm$ 1.77 <sup>c</sup>                                                 | 0.367 $\pm$ 0.007 <sup>b</sup>               | 3.578 $\pm$ 0.606 <sup>bc</sup> | 1.773 $\pm$ 0.056 <sup>bc</sup> |
| G5     | 1.488 $\pm$ 0.049 <sup>c</sup>  | 0.902 $\pm$ 0.007 <sup>a</sup> | 49.18 $\pm$ 1.890 <sup>b</sup>  | 69.82 $\pm$ 3.27 <sup>b</sup>                                                 | 0.560 $\pm$ 0.043 <sup>b</sup>               | 6.071 $\pm$ 0.460 <sup>a</sup>  | 2.996 $\pm$ 0.319 <sup>b</sup>  |
| G6     | 3.065 $\pm$ 0.178 <sup>a</sup>  | 0.594 $\pm$ 0.006 <sup>c</sup> | 30.40 $\pm$ 1.070 <sup>cd</sup> | 46.43 $\pm$ 3.20 <sup>c</sup>                                                 | 0.315 $\pm$ 0.004 <sup>c</sup>               | 3.181 $\pm$ 0.608 <sup>c</sup>  | 1.340 $\pm$ 0.106 <sup>c</sup>  |
| Day 30 |                                 |                                |                                 |                                                                               |                                              |                                 |                                 |
| G1     | 2.056 $\pm$ 0.052 <sup>b</sup>  | 0.828 $\pm$ 0.007 <sup>b</sup> | 56.53 $\pm$ 1.320 <sup>b</sup>  | 50.22 $\pm$ 1.16 <sup>b</sup>                                                 | 0.545 $\pm$ 0.005 <sup>b</sup>               | 6.304 $\pm$ 0.320 <sup>ab</sup> | 3.202 $\pm$ 0.233 <sup>ab</sup> |
| G2     | 3.748 $\pm$ 0.015 <sup>a</sup>  | 0.502 $\pm$ 0.005 <sup>d</sup> | 35.74 $\pm$ 0.900 <sup>d</sup>  | 31.19 $\pm$ 0.70 <sup>d</sup>                                                 | 0.339 $\pm$ 0.009 <sup>d</sup>               | 4.287 $\pm$ 0.595 <sup>b</sup>  | 1.997 $\pm$ 0.237 <sup>b</sup>  |
| G3     | 1.446 $\pm$ 0.119 <sup>c</sup>  | 0.921 $\pm$ 0.007 <sup>b</sup> | 80.25 $\pm$ 3.640 <sup>a</sup>  | 78.57 $\pm$ 1.56 <sup>a</sup>                                                 | 0.893 $\pm$ 0.067 <sup>a</sup>               | 8.276 $\pm$ 0.543 <sup>a</sup>  | 4.862 $\pm$ 0.419 <sup>a</sup>  |
| G4     | 2.970 $\pm$ 0.020 <sup>b</sup>  | 0.659 $\pm$ 0.006 <sup>c</sup> | 51.27 $\pm$ 1.510 <sup>b</sup>  | 45.64 $\pm$ 1.62 <sup>c</sup>                                                 | 0.447 $\pm$ 0.007 <sup>c</sup>               | 5.612 $\pm$ 0.564 <sup>ab</sup> | 2.802 $\pm$ 0.310 <sup>ab</sup> |
| G5     | 1.294 $\pm$ 0.079 <sup>c</sup>  | 1.034 $\pm$ 0.008 <sup>a</sup> | 61.81 $\pm$ 4.080 <sup>b</sup>  | 63.46 $\pm$ 2.01 <sup>b</sup>                                                 | 0.646 $\pm$ 0.051 <sup>b</sup>               | 7.132 $\pm$ 0.429 <sup>a</sup>  | 3.872 $\pm$ 0.504 <sup>a</sup>  |
| G6     | 2.665 $\pm$ 0.165 <sup>b</sup>  | 0.718 $\pm$ 0.006 <sup>c</sup> | 43.29 $\pm$ 1.210 <sup>c</sup>  | 39.75 $\pm$ 2.09 <sup>c</sup>                                                 | 0.365 $\pm$ 0.006 <sup>c</sup>               | 5.044 $\pm$ 0.562 <sup>ab</sup> | 2.200 $\pm$ 0.156 <sup>b</sup>  |
| Day 45 |                                 |                                |                                 |                                                                               |                                              |                                 |                                 |
| G1     | 1.675 $\pm$ 0.029 <sup>b</sup>  | 0.859 $\pm$ 0.021 <sup>b</sup> | 58.67 $\pm$ 1.750 <sup>b</sup>  | 41.19 $\pm$ 2.11 <sup>bc</sup>                                                | 0.536 $\pm$ 0.006 <sup>b</sup>               | 5.831 $\pm$ 0.546 <sup>ab</sup> | 3.437 $\pm$ 0.067 <sup>b</sup>  |
| G2     | 2.872 $\pm$ 0.189 <sup>a</sup>  | 0.594 $\pm$ 0.005 <sup>d</sup> | 42.81 $\pm$ 0.820 <sup>d</sup>  | 25.23 $\pm$ 0.91 <sup>d</sup>                                                 | 0.312 $\pm$ 0.032 <sup>d</sup>               | 4.467 $\pm$ 0.619 <sup>b</sup>  | 2.579 $\pm$ 0.115 <sup>c</sup>  |
| G3     | 1.309 $\pm$ 0.051 <sup>c</sup>  | 0.965 $\pm$ 0.058 <sup>b</sup> | 86.20 $\pm$ 3.260 <sup>a</sup>  | 72.68 $\pm$ 1.31 <sup>a</sup>                                                 | 0.943 $\pm$ 0.057 <sup>a</sup>               | 7.462 $\pm$ 0.616 <sup>a</sup>  | 5.191 $\pm$ 0.492 <sup>a</sup>  |
| G4     | 1.817 $\pm$ 0.164 <sup>b</sup>  | 0.812 $\pm$ 0.005 <sup>c</sup> | 61.16 $\pm$ 0.920 <sup>b</sup>  | 44.02 $\pm$ 1.73 <sup>b</sup>                                                 | 0.553 $\pm$ 0.033 <sup>c</sup>               | 5.594 $\pm$ 0.353 <sup>ab</sup> | 3.608 $\pm$ 0.238 <sup>b</sup>  |
| G5     | 1.097 $\pm$ 0.027 <sup>c</sup>  | 1.097 $\pm$ 0.016 <sup>a</sup> | 65.46 $\pm$ 3.170 <sup>b</sup>  | 58.15 $\pm$ 2.90 <sup>b</sup>                                                 | 0.728 $\pm$ 0.021 <sup>b</sup>               | 6.357 $\pm$ 0.546 <sup>ab</sup> | 4.485 $\pm$ 0.386 <sup>ab</sup> |
| G6     | 1.530 $\pm$ 0.112 <sup>bc</sup> | 0.854 $\pm$ 0.006 <sup>b</sup> | 56.34 $\pm$ 1.820 <sup>c</sup>  | 38.33 $\pm$ 1.24 <sup>c</sup>                                                 | 0.459 $\pm$ 0.029 <sup>c</sup>               | 4.952 $\pm$ 0.320 <sup>b</sup>  | 3.366 $\pm$ 0.223 <sup>b</sup>  |

Values are expressed as mean $\pm$ SEM (n = 6). G1 = Control; G2 = Dexamethasone; G3 = *Moringa oleifera*; G4 = *Moringa oleifera* + Dexamethasone; G5 = Vitamin E-Selenium; G6 = Vitamin E-Selenium + Dexamethasone. Different superscript letters within the same day indicate significant differences (P < 0.05)

displayed the lowest antioxidant capacity. Supplementing with vitamin E–Selenium also greatly increased antioxidant capacity and both co-treatment groups showed some recovery from oxidative damage caused by dexamethasone. With only slight variations between sampling days, CUPRAC levels stayed comparatively constant throughout the trial.

### **FRAP**

Treatments had a considerable impact on FRAP readings. The highest FRAP values were consistently achieved by supplementing with *Moringa oleifera*, while exposure to dexamethasone produced the lowest ferric reducing antioxidant power. In comparison to the stressed group, vitamin E–Selenium supplementation also increased ferric reducing capacity and antioxidant co-treatment partially restored FRAP activity. From Day 15 to Day 45, FRAP increased gradually in most groups, especially in the birds who received supplements, suggesting a long-term improvement in overall antioxidant status.

The present study demonstrates that dexamethasone-induced oxidative stress profoundly disrupts the cardiac antioxidant defense system of broiler chickens, while dietary supplementation with *Moringa oleifera* leaf powder and the vitamin E–Selenium combination confer significant and, in several parameters, near-complete cardioprotection. models.

The marked elevation of MDA and the parallel depletion of GSH, SOD, CAT, GST, CUPRAC and FRAP in the dexamethasone-challenged group confirm that sustained glucocorticoid exposure imposes severe oxidative burden on broiler cardiac tissue. The heart's high mitochondrial density makes it especially susceptible to oxidative damage, continuous contractile activity and abundant polyunsaturated fatty acid content in cardiomyocyte membranes, which serve as ready substrates for LPO (Tsutsui, 2001). In a seminal study directly relevant to the present work, Rajashree and Puvanakrishnan (1998) demonstrated that dexamethasone administration in rats significantly elevated LPO and

simultaneously depleted the activities of SOD, CAT, GSH, GPx and GST in heart and kidney tissues. The near-doubling of cardiac MDA in the dexamethasone-challenged group relative to control is further consistent with Gao *et al.* (2010), who reported that dexamethasone treatment significantly augmented plasma TBARS and compromised overall antioxidant status in broiler chickens. Also, a study by He *et al.* (2025) reported that broilers treated with dexamethasone exhibited a significant reduction in antioxidant status in cardiac tissue, characterized by lower total antioxidant capacity and SOD activity, together with increased MDA and LDH concentrations. Lin *et al.* (2004) documented that even sub-chronic corticosterone administration in broilers caused a sustained, cumulative reduction of antioxidant enzyme activities reflecting continuous pro-oxidant pressure exceeding the capacity for compensatory de novo enzyme synthesis. The severe depression of GST activity in dexamethasone-challenged group deserves particular attention in the cardiac context. The severely reduced CUPRAC and FRAP values in dexamethasone-challenged group reflect the comprehensive exhaustion of both enzymatic and non-enzymatic reducing equivalents, consistent with the marked depletion of antioxidant potential previously documented in dexamethasone-treated laying hens (Eid *et al.*, 2008) and broilers (Fathi *et al.*, 2023).

*Moringa oleifera* leaf powder supplementation produced the most pronounced reversal of dexamethasone-induced cardiac oxidative injury among all treatment groups. *Moringa oleifera* not only restored but substantially exceeded normal control values in SOD, CAT, GST, CUPRAC and FRAP - indicating that the phytochemical constituents of *Moringa oleifera* provide cardio protection beyond mere normalization of the redox equilibrium. This cardiac-specific protective profile is directly supported by Aju *et al.* (2019), who demonstrated that the methanolic extract of *Moringa oleifera* leaves significantly increased cardiac SOD, CAT, GPx, glutathione reductase and GSH content while simultaneously reducing TBARS, hydroperoxides and conjugated dienes in the hearts

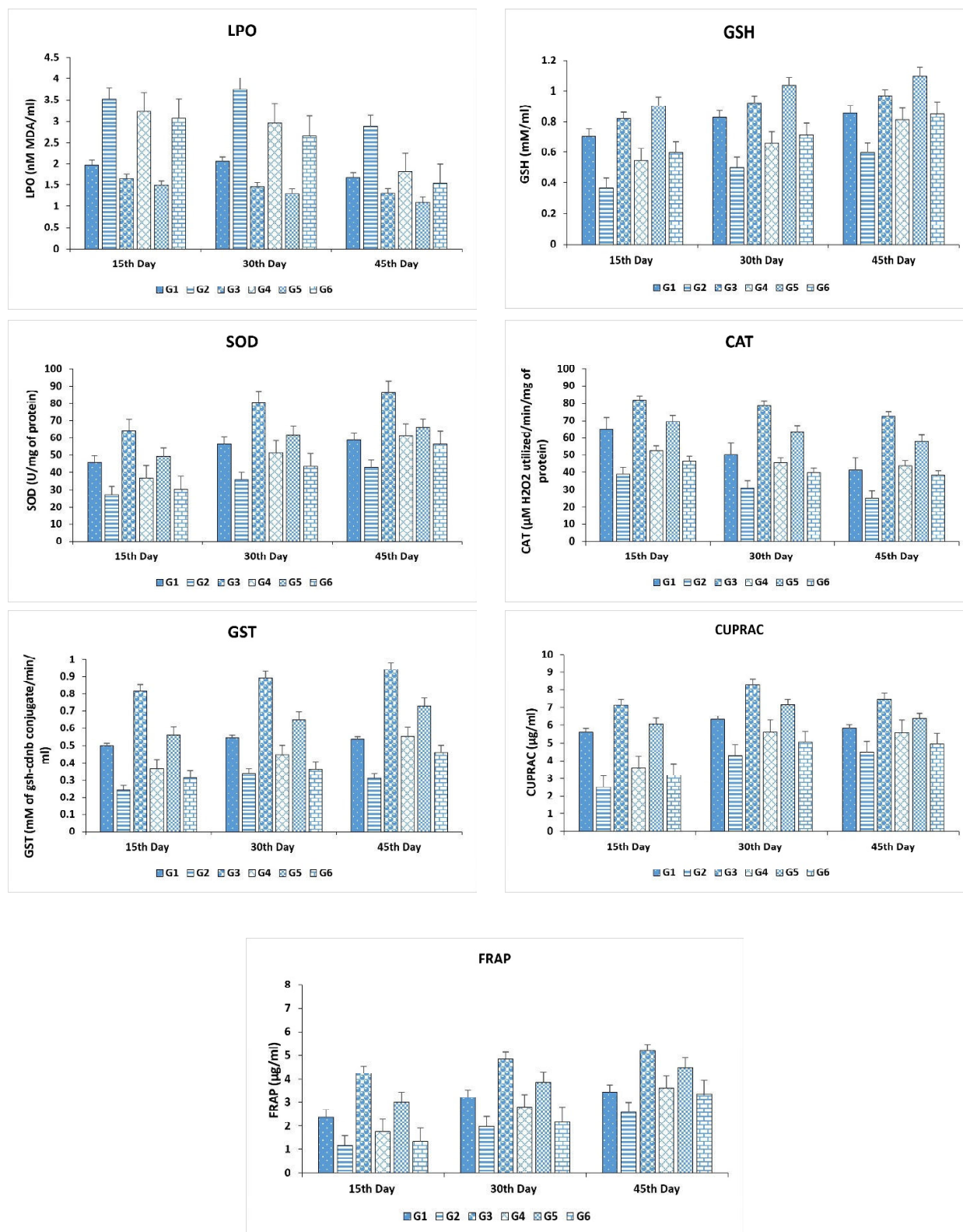
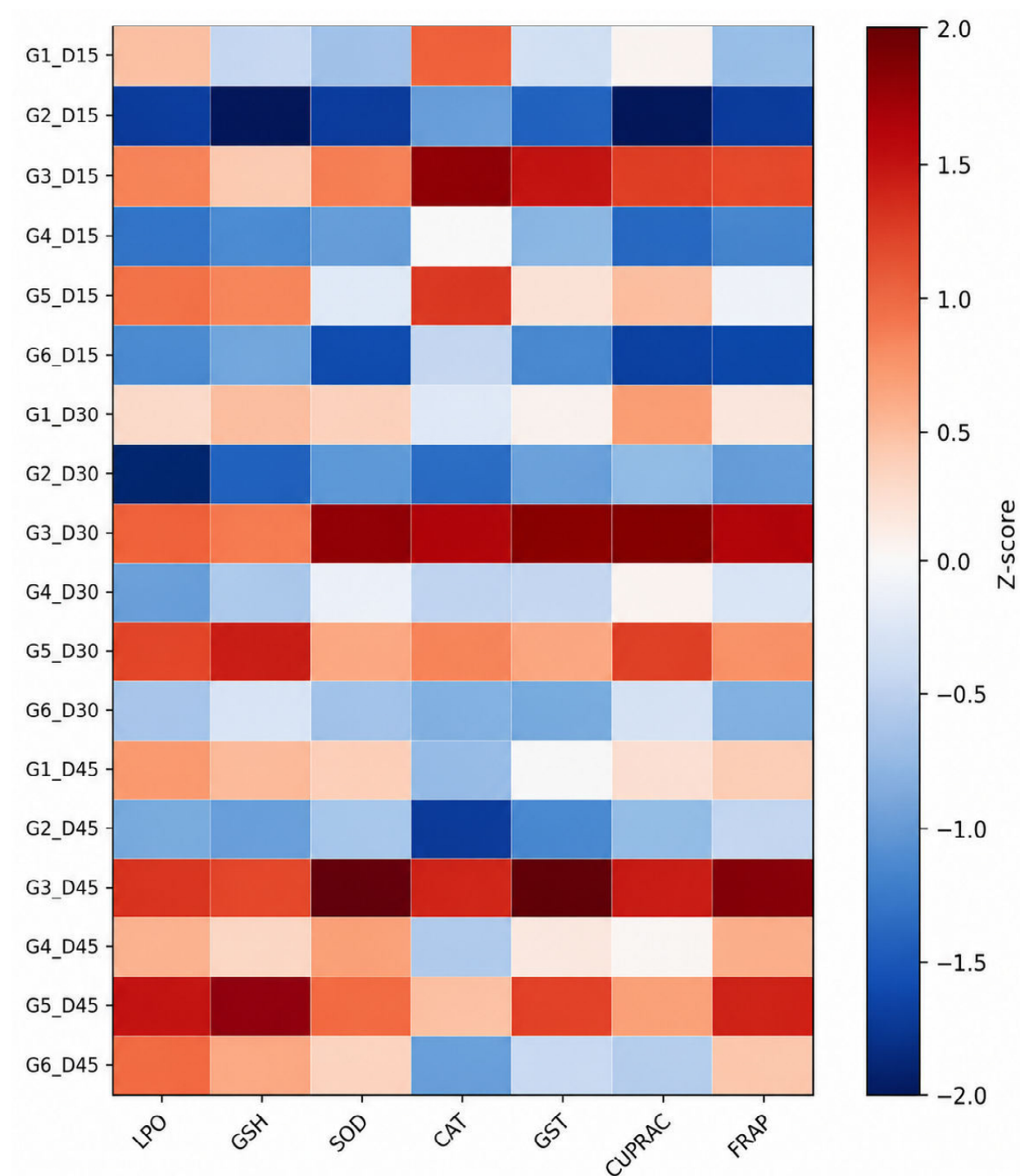


Fig. 1: Effect of different dietary treatments on cardiac antioxidant status in broiler chickens on days 15, 30 and 45 of the experiment



**Fig. 2: Z-score heatmap illustrating the antioxidant status of cardiac tissue across experimental groups at days 15, 30 and 45. Data were standardized independently for each biomarker using Z-score normalization. Lower to higher standardized values are indicated by a colour gradient from blue to red, with increasing intensity corresponding to higher standardized values**

of diabetic rats. Similarly, Mabrouki *et al.* (2020) reported that *Moringa oleifera* leaf extract significantly restored cardiac CAT, GPx and SOD activities and reduced cardiac MDA to near-normal levels in high-fat diet-induced obese rats, with restored normal myocardial architecture and alleviated myocardial fibre disorganization, oedema, inflammation and fibrosis induced by a high-fat diet. The superior cardiac antioxidant status observed in *Moringa oleifera* supplemented group is mechanistically attributable to the exceptionally rich and diverse phytochemical profile of *Moringa oleifera* leaves, which contain high concentrations of flavonoids (quercetin, kaempferol, rutin), phenolic acids (chlorogenic acid, gallic acid, caffeic acid), ascorbic acid,  $\alpha$ -tocopherol and glucosinolates-compounds that collectively provide direct free radical scavenging, metal chelation and Nrf2-dependent upregulation of endogenous antioxidant enzymes (Louisa *et al.*, 2022). The improvement in antioxidant status following *Moringa oleifera* leaf powder supplementation may be attributed, at least in part, to the antioxidant phytoconstituents present in its leaves. Ruj *et al.* (2026) similarly demonstrated reduced lipid peroxidation together with enhanced GSH levels and SOD and catalase activities in *Moringa oleifera* leaf extract -treated chicken lymphocytes, providing cellular-level evidence consistent with its capacity to reinforce endogenous antioxidant defence. *Moringa oleifera* leaves contain glucosinolates and are rich in flavonoids/polyphenols that activate the Nrf2-Keap1 pathway, augmenting antioxidant defenses and GST expression (Ndlovu *et al.*, 2023). Abu Hafsa *et al.* (2020) demonstrated that *Moringa oleifera* leaf powder supplementation significantly increased serum GST activity in broilers, attributing this effect directly to the plant's thiol compound content. The consistently elevated CUPRAC and FRAP values in *Moringa oleifera*, which showed a progressive upward trajectory even at Day 45 when enzymatic activities exhibited a modest decline reflect the accumulation of non-enzymatic reducing equivalents derived from dietary polyphenols, tocopherols and ascorbate that serve both as direct antioxidants and as regenerators of oxidized glutathione and tocopherol radicals. The findings of the present study agree with those of Manjunath *et al.* (2023), who demonstrated

that a standardized *Moringa oleifera* leaf preparation activated the NRF2 signaling pathway and enhanced the expression of NRF2-responsive antioxidant genes, thereby reinforcing endogenous antioxidant defense mechanisms.

The vitamin E-Selenium combination consistently delivered robust cardio protection, with values closely trailing *Moringa oleifera* and importantly, recording the lowest MDA and highest GSH concentrations at Day 45. This pattern is mechanistically coherent and reflects the complementary antioxidant actions of these two micronutrients. Vitamin E is the principal chain-breaking, lipid-soluble antioxidant that intercalates directly into cardiac mitochondrial and sarcolemmal membranes, where it donates its phenolic hydrogen to lipid peroxy radicals, terminating the autocatalytic propagation of LPO at the membrane level (Wallert *et al.*, 2019). Selenium, as an essential cofactor incorporated as selenocysteine into the active site of GPx and thioredoxin reductase, catalyzes the glutathione-dependent reduction of hydrogen peroxide and lipid hydroperoxides in the aqueous cytosolic compartment (Zoidis *et al.*, 2018).

Elgendey *et al.* (2022) demonstrated that a vitamin E-enriched diet combined with selenium significantly increased liver CAT, SOD activity and reduced MDA in broilers compared to the basal diet, while Kumbhar *et al.* (2018) confirmed that dietary selenium and vitamin E significantly enhanced SOD and CAT activities and their corresponding mRNA levels in the breast muscles of heat-stressed broilers. Gao *et al.* (2010) demonstrated that high-dose vitamin E effectively suppressed LPO in dexamethasone-treated broilers, further corroborating the low MDA trajectory observed vitamin E-Selenium supplemented group. Vitamin E supplementation improved antioxidant defenses in broilers by increasing SOD and GSH-Px activities and decreasing MDA levels, as shown by Ebrahimzadeh *et al.* (2018).

## CONCLUSION

The present findings demonstrate that dexamethasone challenge induces a severe,



multisystem collapse of the cardiac antioxidant defence network in broiler chickens- characterized by elevated LPO and depletion of enzymatic (SOD, CAT, GST) and non-enzymatic (GSH, CUPRAC, FRAP) antioxidant reserves. Dietary supplementation with MOLP conferred the most comprehensive cardio protection, restoring and exceeding normal antioxidant status through upregulation of endogenous enzymes and direct free-radical scavenging by its rich phytochemical constituents. The vitamin E-Selenium combination provided robust, mechanistically complementary protection, particularly evident in the suppression of LPO and elevation of glutathione levels. The cardioprotective effects are likely mediated by enhanced antioxidant defences and suppression of oxidative stress-induced inflammatory responses. Overall, the findings suggest that *Moringa oleifera* and vitamin E-Selenium can serve as effective nutritional strategies for protecting the broiler heart against dexamethasone-induced oxidative damage.

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