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Integrating infrared thermography with cortisol for diagnosis of inflammatory diseases in dairy cows

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Abstract

Dairy cows' health and welfare are substantially compromised by inflammatory diseases. To visualize real-time, region-specific hyperthermia in cows affected by laminitis, metritis, and mastitis, a study was undertaken to monitor inflammation in dairy cows using infrared thermography (IRT). A total of 48 lactating cows were examined, comprising 12 healthy controls and 12 affected cows of each diseased group. Plasma cortisol levels of each group were quantified by ELISA. In laminitis, significant ($p < 0.05$) surface temperature elevations were localized to the inflamed limb (4.28°C) and coronary band (3.72°C). Metritis cases exhibited significant ($p < 0.05$) increases at the vulval tip (2.26°C) and inner vulval region (1.94°C), while mastitis was characterized by elevated teat (3.58°C) and udder (2.92°C) surface temperatures. Plasma cortisol concentrations were significantly ($p < 0.05$) higher in diseased cows, with the greatest elevations (9.21 ± 0.69 ng/ml) in mastitis and the lowest (1.43 ± 0.23 ng/ml) in healthy animals. The IRT findings enabled differentiation of disease-associated thermal signatures: limb regions for laminitis, reproductive areas for metritis, and udder-associated zones for mastitis. Overall, the integration of IRT with cortisol profiling provided clear evidence of systemic inflammatory responses and disease-specific hyperthermia. IRT can be effectively used alone for diagnosing mastitis and laminitis, while metritis requires support from blood and hormonal analyses for accurate detection.

Keywords: Infrared thermography, dairy cows, laminitis, metritis, mastitis

1. Introduction

Animal diseases are commonly classified into production, endemic, and exotic categories, each carrying major implications for animal health and farm economics. Among the critical challenges in livestock farming, early detection of diseases is essential to mitigate health, welfare, and socio-economic losses. During recent years, thermal imaging has emerged as a promising methodology to strengthen early warning systems by enabling accurate discrimination between healthy and diseased animals, rapid data collection, and timely communication (McManus *et al.* 2022) ^[14]. Infrared thermography (IRT), a non-contact and non-invasive imaging tool, has been successfully used for farm animal health monitoring by various researchers (Luzi *et al.* 2013; Kittur *et al.* 2024; Satheesan *et al.* 2024) ^[12, 10, 19]. Its diagnostic value lies in the detection of localized increases in surface temperature caused by vasodilation, enhanced blood flow, and metabolic activity. Such thermal changes often precede clinical symptoms, making IRT particularly useful for the early diagnosis of inflammatory conditions like laminitis, metritis, and mastitis. With current advances in automation and digital analysis, IRT is increasingly being integrated into routine herd health management. Compared to other imaging modalities, IRT is gaining prominence because it is simple to operate, remote-applicable, and farmer-friendly. Its potential for on-farm use in udder health monitoring has been highlighted as a cost-effective way to detect abnormalities early and reduce treatment expenses (Korelidou *et al.* 2024) ^[11].

Despite encouraging progress, it remains in a developmental stage, with research focused on overcoming inconsistencies observed under field conditions. Importantly, IRT findings have been corroborated by physiological biomarkers of inflammation. Increased plasma cortisol levels indicate hypothalamic-pituitary-adrenal (HPA) axis activation, reflecting systemic stress. In studies carried out in different livestock, cortisol levels have been found to be positively linked with variations in blood flow and surface temperature (Cai *et al.* 2023;

Choudhary *et al.* 2025)^[4, 7]. The aim of this study, carried out in diseased and healthy cows, was to integrate thermal imaging with blood cortisol and evaluate its diagnostic potential as a comprehensive tool for monitoring both local and systemic disease processes.

2. Materials and Methods

2.1 Location of the study

The present research was carried out between January and June 2025 at the Livestock Research Centre (LRC), National Dairy Research Institute, Karnal, Haryana, India. This research location is situated within the Indo-Gangetic alluvial plains at an altitude of approximately 250 meters above mean sea level, located at 29°43'14.4" N latitude and 76°58'55.2" E longitude.

2.2 Experimental design

For experimentation, a total of 48 cows were selected from the institutional farm's lactating herd and experimental animals were grouped as 12 number of healthy cows and compared with 12 number of laminitis affected cows, 12 number of metritis affected cows, 12 number of mastitis affected cows. Randomization was performed while ensuring uniformity in parity (3-5) and lactation stage (60-90 days in milk). Blood samples for blood cortisol analyses, as well as thermal images from various thermal windows, were collected on the same day from all the cows. The cows were fed as per the standard feeding practices (ICAR, 2013) followed at the Livestock Research Centre (LRC) of ICAR-National Dairy Research Institute, Karnal. They were offered *ad libitum* green fodder: Maize (*Zea mays*), Jowar (*Sorghum bicolor*) and Hybrid Napier Grass (*Pennisetum purpureum* × *Pennisetum glaucum*). In addition to green fodder, the cows were supplemented with a concentrate mixture formulated to contain approximately 70% Total Digestible Nutrients and 20% Crude Protein. This high-energy, high-protein mixture was adjusted based on individual milk yield, following the formula of 1 kg of concentrate per 3 kg of milk produced, with a standard milk fat content of 4%. The concentrate mixture consisted of 33% maize, 21% ground nut cake, 12% mustered cake, 20% wheat bran, 11% de-oiled rice bran, 2% mineral mixture and 1% common salt. *Ad libitum* drinking water is accessed to all cows for 24 h.

2.3 Blood sampling and plasma separation

Approximately 10 ml of blood was collected aseptically from the jugular vein using sterile, heparinized vacutainer tubes (Vacuette®-Na-heparin, Greiner Bio-One GmbH, Austria), ensuring minimizing animal disturbance. Blood samples were centrifuged at 3000 rpm for 15 minutes at room temperature to separate plasma. Roughly 3-4 ml of plasma was obtained per sample and stored at -80 °C for further hormonal analysis.

2.4 Quantification of blood plasma cortisol

Plasma cortisol concentrations were determined using bovine-specific competitive ELISA kits (Bioassay Technology Laboratory, Shanghai, China). Optical density (OD) readings were obtained with a Tecan® Nano Quant ELISA reader (Infinite M200 PRO, Bio Screen Instruments Pvt. Ltd). The cortisol assay (Catalog No. EA0021Bo) had a sensitivity below 0.2 ng/ml and a detection range of 0.5-200 ng/ml. Both intra-and inter-assay coefficients of variation

(CV) were under 12%. All reagents were prepared and the assay was performed according to the manufacturer's instructions. Final OD values were recorded at 450 nm using an ELISA plate reader (MultiSkan GO, Thermo Scientific).

2.5 Infrared imaging

The body surface temperatures of various areas were measured using a Fluke Tix 580 infrared thermal camera (Fluke Corp., Everett, WA, USA), capable of capturing 1280 × 960 resolution images and operating within a calibration range of -25.0 °C to 105.0 °C. Prior to data collection, the camera was calibrated, and its emissivity was set to 0.95. This device is equipped with a 240° rotating screen, a 640 × 480 display, a thermal sensitivity of 0.1 °C, and an imaging range of approximately 1-20 m. It utilizes an uncooled microbolometer with a 120 × 120 focal plane array for image scanning. Thermal images were obtained following the methods described by Choudhary *et al.* (2025)^[7] and were subsequently analyzed using the Fluke® Systems Inc. Smart View Classic 4.4 software (<https://assets.fluke.com/SmartView/TUupdates/Setup.exe>).

A high-contrast color scale was applied, and the "square marker" tool was manually used to measure the average temperatures (°C) of the cows' thermal windows. To maintain uniformity, the camera was positioned and captured exactly 1 meter from the animals. All thermal images were captured by the same operator to reduce variability associated with operator differences. Imaging was conducted in shaded areas to prevent direct sunlight interference and to limit environmental variation, with all photos taken on the same day as blood sampling. Wind effects were minimized by selecting sheltered spots, and the cows were given time to acclimate to the imaging environment before recording. Although no anti-reflective coatings were used, these standardized measures ensured uniform imaging conditions throughout the experiment.

2.6 Statistical Analysis

The statistical analysis of data was performed using IBM SPSS Statistics software, version 22.0 (SPSS Inc., Chicago, IL, USA). Within-group variations over time were evaluated using one-way ANOVA, and the Tukey HSD multiple comparison test was applied to identify significant differences. Results are presented as mean ± standard error (SE), and statistical significance was set at $p < 0.05$. Associations between variables were assessed through Pearson's bivariate correlation, with two-tailed significance levels considered at $p < 0.05$ and $p < 0.01$. The magnitude and direction of correlations were interpreted according to the classification of Landis and Koch (1977), where correlation coefficients (r) were defined as: very weak ($0 \leq r < 0.2$), weak ($0.2 \leq r < 0.4$), moderate ($0.4 \leq r < 0.6$), strong ($0.6 \leq r < 0.8$), and very strong ($0.8 \leq r < 1$).

3. Results and Discussion

The present study carried out in diseased and healthy dairy cow's highlights the diagnostic potential of infrared thermography (IRT) in detecting region-specific surface temperature changes associated with laminitis, metritis, and mastitis, supported by haematological and endocrine profiles. The results obtained from this study demonstrated distinct thermal patterns depending on the underlying inflammatory condition, confirming that IRT can detect localized hyperthermia in tissues associated with

inflammation and circulatory changes (Casas-Alvarado *et al.* 2020; Machado *et al.* 2021) [6, 13].

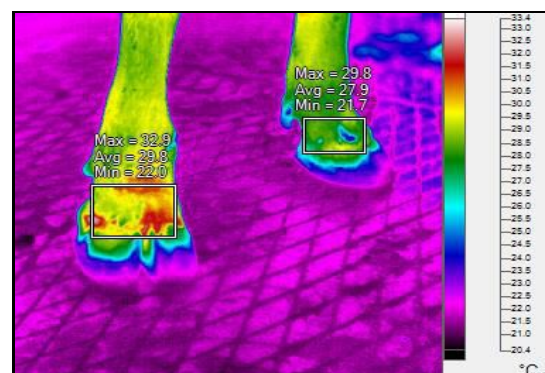
Figure 2 and Figure 1.a illustrates the variations recorded in surface temperature across different thermal windows in cows affected by laminitis compared with healthy cows. The inflamed leg region showed the greatest increase in temperature, showing a significant ($p < 0.05$) rise of 4.28 °C compared to the healthy cows. This was followed by the coronary band region, which exhibited a significant ($p < 0.05$) increase in temperature of 3.72 °C. The forehead and left eye also demonstrated significant ($p < 0.05$) increases of 2.18 °C and 2.03 °C, respectively. Other regions, including the muzzle (1.87 °C), right eye (1.67 °C), cheek (1.65 °C), ear (1.22 °C), and abdomen (1.16 °C), displayed relatively lower temperature elevations. Overall, limb-associated areas (leg region and coronary band region) showed the most pronounced thermal changes with laminitis-affected cows. In the laminitis-affected cows, the most pronounced temperature increases were observed in the inflammatory leg region and coronary band, followed by modest elevations in the forehead and ocular regions (Bobić *et al.* 2025) [3]. This is consistent with the inflammatory pathogenesis of laminitis, where vasodilation, increased peripheral blood flow, and inflammatory mediators release cause notable limb hyperthermia (Thompson *et al.* 2023) [25]. The heightened thermal signals in the coronary band region are of particular diagnostic interest as they could precede visible signs of hoof pathology, a finding corroborated by research indicating that early laminitis is detectable by identifying a temperature rise of >2 °C in hoof-associated regions (Alsaad *et al.* 2014; Bobic *et al.* 2024) [1, 2].

Figure 3 and Figure 1.b show the variations observed in surface temperature across different thermal windows in cows affected by metritis compared with healthy cows. A significant ($p < 0.05$) increase in temperature was observed at the vulval tip with a rise of (2.26 °C), followed by the inner vulval region (1.94 °C) and the left eye (1.94 °C), respectively. The right eye exhibited a significant ($p < 0.05$) rise of 1.63 °C, while the outer vulval area also showed a significant ($p < 0.05$) increase of 1.47 °C. Moderate temperature elevations were recorded in the muzzle (0.89 °C), ear (0.80 °C), forehead (0.77 °C), and cheek (0.76 °C). The abdomen showed the smallest increase of 0.75 °C. Overall, the vulval regions and the eyes exhibited the most pronounced thermal deviations in metritis-affected cows compared with the healthy cows. In cows having metritis, the vulval tip and inner vulval region were the main hotspots, with ocular temperatures also significantly elevated. These findings align with reports that reproductive tract infections induce localized vascular and metabolic activity that is detectable by IRT in the perineal region (Sheldon *et al.* 2006; Talukder *et al.* 2015) [22, 24]. The significant ocular temperature rise may reflect systemic inflammatory fever responses, as eye-region IRT has been validated as a reliable non-invasive indicator of core body temperature (Shu *et al.* 2022) [23].

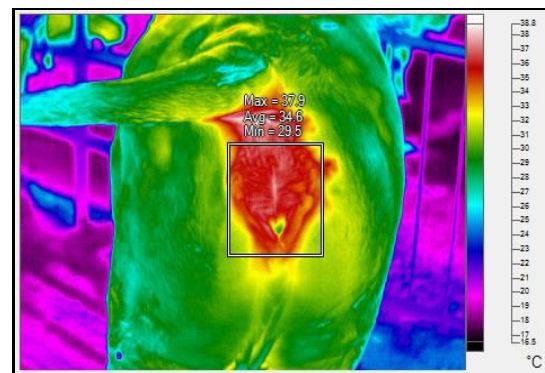
Figure 4 and Figure 1.c depicts the variations observed in surface temperature across different thermal windows in cows affected by mastitis compared with healthy cows. The Teat Skin Surface Temperature (TSST) region showed significant ($p < 0.05$) increase in temperature, rising by 3.58 °C compared with healthy cows, followed by the right Udder Skin Surface Temperature (USST) of 2.92 °C and

Teat Apex Temperature (TAT) of 2.85 °C. Other regions exhibited a significant ($p < 0.05$) increase in temperature, included the Teat Barrel Temperature (TBT) 2.27 °C, left USST (2.15 °C), and left eye temperature (2.08 °C). Moderate significant ($p < 0.05$) increase in temperature were observed in the muzzle (1.94 °C), right eye (1.93 °C), ear temperature (1.55 °C), and forehead (1.41 °C). The cheek showed a smaller significant ($p < 0.05$) rise of 1.19 °C, while the abdomen recorded the lowest temperature variation, at just of 0.52 °C. Overall, the udder associated regions particularly TSST, USST, TAT, TBT displayed the most pronounced temperature elevations in mastitis-affected cows, reflecting marked thermal responses linked to the mastitis condition.

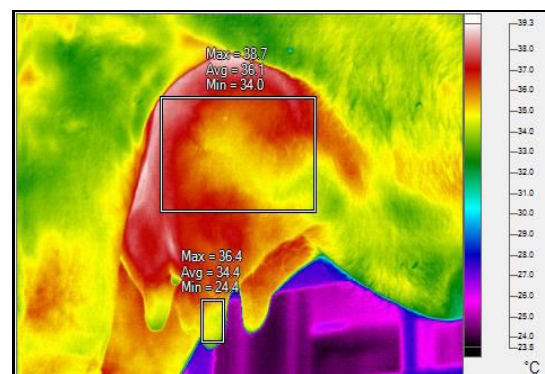
Blood cortisol levels increased significantly ($p < 0.05$) in mastitis cows, reaching 9.21 ± 0.69 ng/ml, compared to 4.66 ± 0.52 ng/ml in laminitis cows, 3.71 ± 0.43 ng/ml in metritis cases, and only 1.43 ± 0.23 ng/ml in healthy animals (Figure 5).



a. Thermogram of the limb region of laminitis cow



b. Thermogram of the vulval region of metritis cow



c. Thermogram of the udder of mastitis cow

Fig 1: Thermograms of dairy cows a) Laminitis b) Metritis c) Mastitis

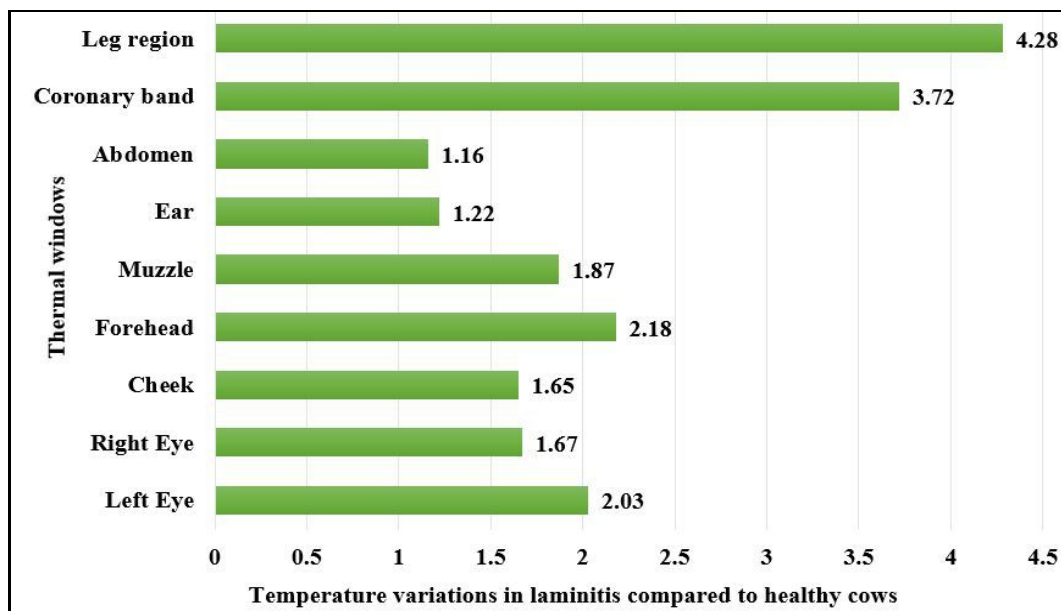


Fig 2: Surface temperature differences (°C) observed in the thermal windows of diseased cows (laminitis) compared to the healthy cows.

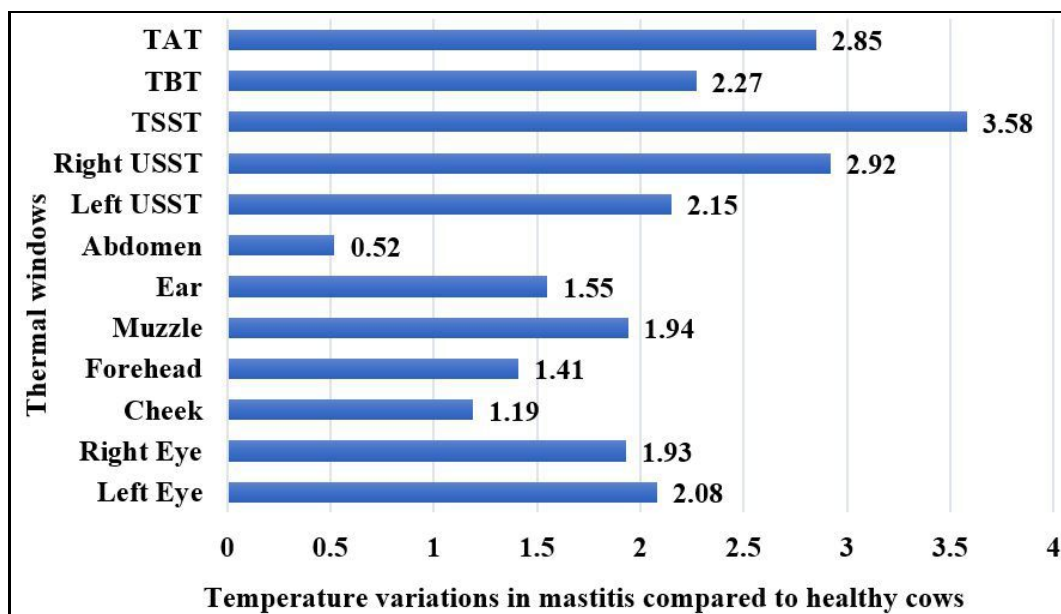


Fig 3: Surface temperature differences (°C) observed in the thermal windows of diseased cows (metritis) compared to the healthy cows.

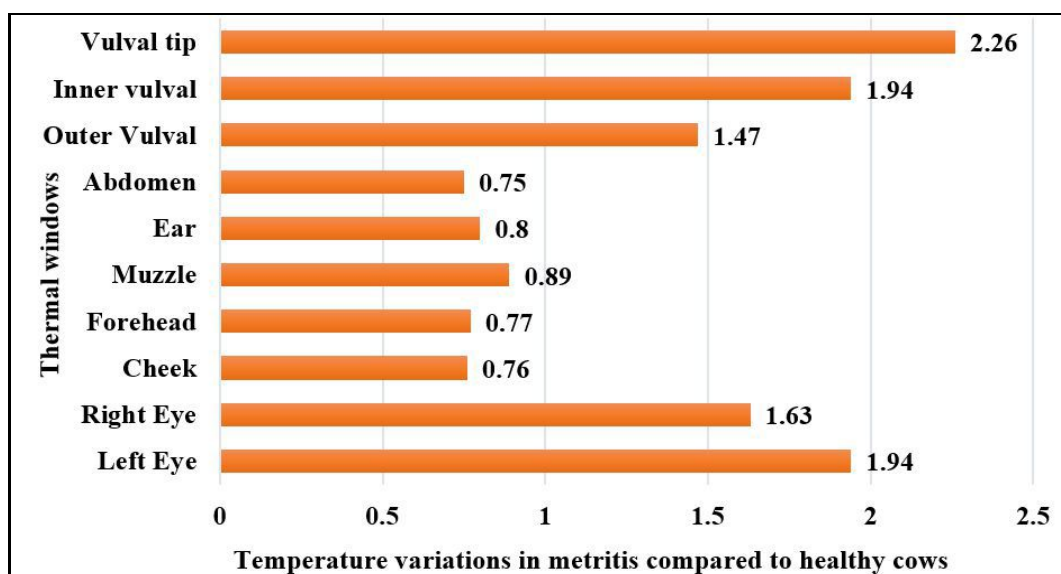


Fig 4: Surface temperature differences (°C) observed in the thermal windows of diseased cows (mastitis) compared to the healthy cows.

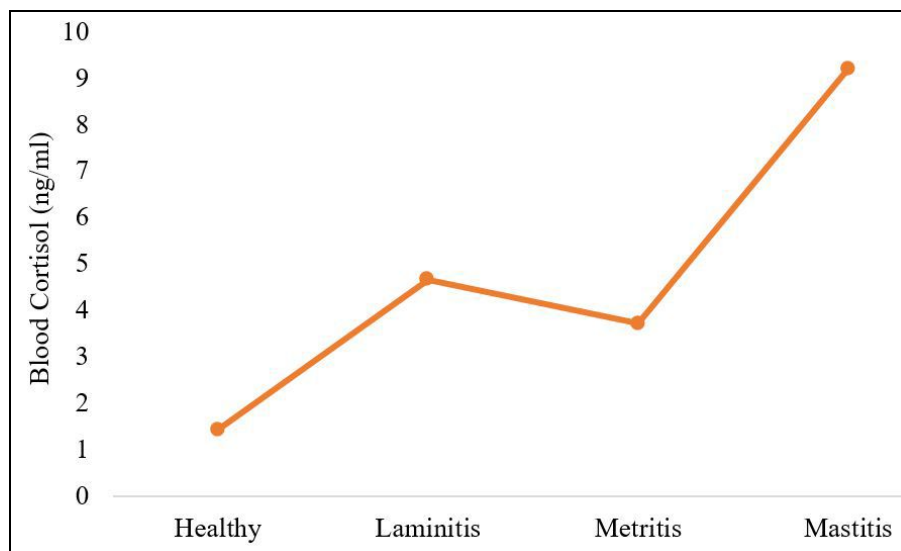


Fig 5: Blood cortisol level (ng/ml) observed in the healthy and diseased cows.

4. Conclusion

The present study confirms that IRT effectively detects disease-specific thermal signatures in laminitis and mastitis-affected dairy cows, with pronounced temperature elevations in the leg, coronary band, and udder regions. However, IRT alone demonstrated limited diagnostic sensitivity for metritis, as internal uterine inflammation restricts surface thermal expression, and supportive diagnostic parameters are required for its confirmatory diagnosis. Blood cortisol levels were significantly elevated in diseased cows, particularly in mastitis cases, validating the thermal findings and reflecting systemic HPA axis-mediated stress responses. Overall, integration of IRT with complementary biochemical and clinical parameters offers a reliable, non-invasive, and comprehensive approach for early disease detection and routine herd health monitoring in dairy cattle.

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6. Ethics approval

Experimental approval for conducting the present investigations was obtained from the Institutional Animal Ethics Committee according to the Committee for Control and Supervision of Experiments on Animals rules, laid down by the Government of India No. 50-IAEC-23-09 dated: 28.11.2023.

7. Conflict of Interest: The authors declare no competing interests.

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