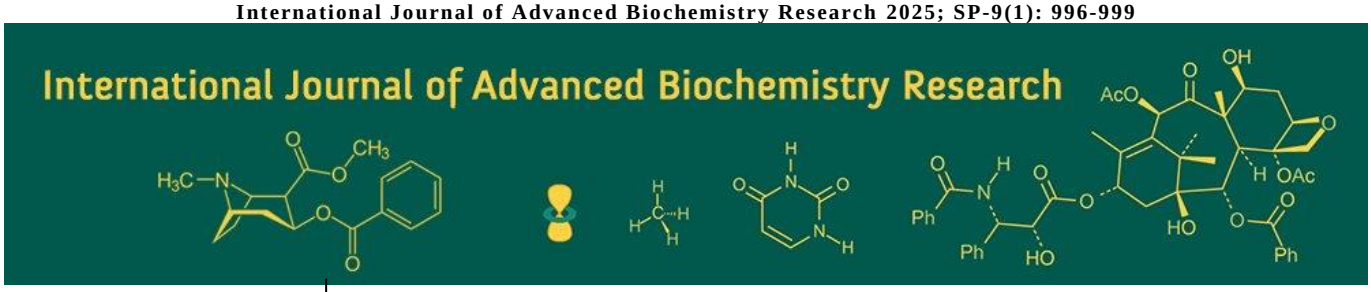

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; SP-9(1): 996-999
www.biochemjournal.com
Received: 08-11-2024
Accepted: 10-12-2024

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Histopathological effects of heat stress and the protective role of dried tamarind pulp powder and vitamin C in broilers

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DOI: https://doi.org/10.33545/26174693.2025.v9.i1Sm.3664

Abstract
The study evaluated histopathological alterations caused by heat stress in broilers and the potential mitigating effects of dried tamarind pulp powder (DTPP). A total of 150 broilers were divided into six groups (T_1–T_6) and reared for 35 days. T_1 group served as the healthy control, and T_2 as the heat-stressed (HS) control group. Groups T_3 and T_4 received DTPP (2.5 g/kg feed) and herbal Vitamin C (250 mg/kg feed) without heat stress, respectively. Groups T_5 and T_6, exposed to heat stress, were supplemented with DTPP and herbal Vitamin C, respectively.
Gross pathological changes in heat-stressed broilers included liver discoloration, congestion, vacuolar degeneration, and necrosis, impairing metabolism. Kidneys showed congestion, intertubular hemorrhages, and glomerular atrophy, reducing excretory efficiency. The heart exhibited petechial hemorrhages, myofibrillar degeneration, and pale coloration, compromising cardiovascular function. Enlarged spleens with lymphoid depletion, atrophied thymus and bursa of Fabricius weakened immunity. Intestinal congestion, villi desquamation, and epithelial exfoliation impaired nutrient absorption. Lungs displayed congestion and edema, while muscle degeneration affected growth and meat quality of broiler birds.
Histopathological evaluation revealed pronounced degenerative changes in heat-stressed birds (T_2), including cardiac myofibrillar degeneration, vascular congestion, liver centrilobular necrosis, widened sinusoidal spaces, and kidney tubular degeneration with necrotic foci. Spleen and thymus tissues exhibited lymphoid depletion and architectural distortion, while the bursa of Fabricius showed severe lymphoid population reduction and degeneration. However, broilers in T_5 (DTPP with HS) and T_6 (Vitamin C with HS) exhibited reduced severity of histopathological changes, with improved lymphoid populations in the spleen and bursa of Fabricius, and less intestinal degeneration of broiler birds.
Overall, DTPP supplementation significantly mitigated heat stress-induced pathological changes across multiple organ systems, highlighting its potential as an effective dietary intervention to enhance resilience in broilers under heat stress conditions.

Keywords: Tamarind powder, vitamin C, stress, broiler and histopathology

Introduction
The poultry industry has emerged as a rapidly growing segment of India's agricultural sector, with egg production increasing at a remarkable annual rate of 8–10%, far outpacing the overall agricultural growth rate of 1.5–2%. India now ranks as the third-largest egg producer globally, driven by factors such as rising per capita income, urbanization, and declining poultry prices. Poultry meat production has also experienced significant growth, becoming the fastest-growing category in the global meat market. This expansion is particularly evident in India, where an expanding middle class and vertically integrated production systems have contributed to improved efficiency and affordability (APEDA, 2014) [3]. Poultry farming is a crucial source of animal protein globally, yet its growth in tropical and subtropical regions is hindered by challenges such as heat stress. The combination of high environmental temperatures and humidity disrupts the thermoregulatory mechanisms of birds, which lack sweat glands and naturally have higher body temperatures. Heat stress leads to physiological and behavioral changes, including panting, reduced feed intake, lethargy, and increased susceptibility to diseases.

These effects compromise productivity and increase mortality rates, especially in broiler birds, which are more prone to heat stress than layers (Sahin *et al*., 2009; Safdar and Maghami, 2014) [13, 12].

Heat stress is defined as the inability of birds to dissipate excess body heat effectively, often resulting in oxidative stress. This stress negatively impacts their immunity, feed efficiency, weight gain, and egg production, leading to economic losses. Behavioral signs such as prolonged rest, reduced movement, and increased drinking further indicate the detrimental impact of heat stress on broilers (Lara and Rostagno, 2013) [8]. In response to these challenges, alternative approaches, including the use of natural antioxidants and herbal remedies, have gained attention. Tamarind (*Tamarindus indica* Linn.), a tree native to tropical Africa and widely cultivated in India, contains polyphenols such as tannins and anthocyanidins, known for their anti-inflammatory and antioxidant properties. Tamarind pulp and extracts have shown potential to improve feed intake and weight gain under thermal stress conditions (Pumthong, 1999; Shinde *et al*., 2018) [11, 14]. Similarly, ascorbic acid (vitamin C) is a potent antioxidant that alleviates metabolic stress, improves immunity, and enhances performance. Supplementing poultry diets with vitamin C has proven to mitigate the adverse effects of heat stress by reducing oxidative damage and improving thermoregulation (Abidin and Khatoon, 2013) [1]. This study aims to explore the efficacy of tamarind pulp and vitamin C in mitigating heat stress, offering sustainable solutions to enhance productivity in the poultry sector under high-temperature conditions.

Materials and Methods

Experimental Design

The study involved a total of 150 broilers divided into six treatment groups, each consisting of 25 birds. The T_1 served as the healthy control group, receiving no induced stress or additional treatments. The T_2 comprised birds subjected to induced heat stress, where the temperature was maintained continuously above 35 °C from the 10th to the 35th day of age, acting as the positive control group. The T_3 birds were fed dried tamarind pulp powder at a concentration of 2.5 g/kg of feed from the 0th to the 35th day. In T_4, birds received a standard drug containing herbal vitamin C at a dosage of 250 mg/kg of feed throughout the same period. The T_5 consisted of induced heat-stressed broilers that were supplemented with dried tamarind pulp powder at 2.5 g/kg of feed, while T_6 included induced heat-stressed broilers supplemented with the standard drug containing herbal vitamin C at 250 mg/kg of feed.

IAEC Approval

The birds were reared according to standard management practices and the experimental protocol was approved from Institutional Animal Ethics Committee (IAEC) before start of experiment. All the birds in different groups were kept under close observation during whole experimental period.

Gross lesions

The gross pathological lesions in the visceral organs were recorded from dead and sacrificed birds at 7th day (15th day of age), 14th day (22nd day of age) and 21st day (29th day of age) of experiment.

Microscopic lesions

Pieces of suitable thickness of heart, lungs, trachea, liver, spleen, kidneys, intestine and air sacs were collected from the sacrificed birds of all the groups and those died due to infection and the samples were preserved in 10% neutral formal saline. The tissues were embedded in paraffin and processed as per the standard procedure. The sections were cut at 4 to 5 µ thickness and were stained with Mayer's haematoxylin and eosin for microscopic examination (Culling, 1974) [4]. The detail microscopic findings in the tissue sections were recorded for each group at 7th day (15th day of age), 14th day (22nd day of age) and 21st day (29th day of age) of experiment.

Results and Discussion

Gross Pathology

Evaluation of gross pathological alterations revealed varying degrees of organ modifications across the treatment groups. The T_1 (healthy control), T_3 (tamarind pulp), and T_4 (herbal vitamin C) groups exhibited minimal pathological changes, consistent with their relatively unstressed conditions. However, the T_2 group, comprising broilers subjected to induced heat stress, exhibited remarkable gross pathological changes. The lungs were congested and edematous, while the liver displayed congestion and a pale, yellowish discoloration. The heart presented with moderately hypertrophic ventricles, blood-filled chambers, petechial hemorrhages, and pale coloration. The kidneys were slightly swollen and showed minimal congestion. A cut-section examination of the intestines revealed moderate congestion, and the spleen was enlarged and dark, with increased blood volume. Additionally, the thymus in the T_2 group exhibited marked atrophic changes, in contrast to the mild atrophy observed in groups T_5 and T_6. Necropsy findings in broilers that succumbed during the experiment revealed a partially cooked muscle appearance with mild hemorrhages and discoloration, alongside hepatomegaly and a fragile liver. These findings aligned with previous reports by Aengwanich and Simaraks (2004) [2], who documented similar pathological changes in heat-stressed broilers, including ventricular hypertrophy, liver congestion, and necrosis. Lola *et al*. (2018) [9] also observed atrophy of the bursa of Fabricius under heat stress.

Histopathological Alterations

Heat stress was a critical issue in poultry farming, causing significant physiological and pathological alterations in broiler organ systems. This study evaluated the gross and histopathological changes induced by heat stress in broilers and investigated the protective effects of *Tamarindus indica* and vitamin C supplementation. The findings revealed that heat stress impacted various organs, including the heart, liver, kidneys, spleen, intestines, thymus, and bursa of Fabricius, while supplementation with *Tamarindus indica* and vitamin C alleviated these pathological changes to varying degrees. Histopathological examination of cardiac tissues in the heat-stressed T_2 group demonstrated pronounced myofibrillar degeneration, moderate infiltration of mononuclear cells (MNCs), focal hemorrhages, and blood vessel congestion. These changes aligned with previous findings by Aengwanich and Simaraks (2004) [2] and Hamza *et al*. (2008) [5], who reported similar cardiac damage under heat stress conditions. In contrast, treatment groups T_5 and T_6 showed reduced severity, with mild

myocardial degeneration, myofiber separation, and less pronounced congestion and hemorrhages. The T_4 group exhibited nearly normal cardiac histoarchitecture, with minimal degenerative changes, suggesting the cardioprotective potential of *Tamarindus indica* and vitamin C supplementation during heat stress. The liver tissue of the T_2 group displayed extensive damage, including widened sinusoidal spaces, sinusoidal hemorrhages, granular and vacuolar degeneration, centrilobular necrosis, pyknotic nuclei, and focal coagulative necrosis. Such changes were consistent with studies by Mohamed *et al*. (2015) [10] and Xie *et al*. (2019) [15], which highlighted sinusoidal dilation and necrosis as hallmarks of heat stress. Supplementation with *Tamarindus indica* (T_3) and herbal vitamin C (T_4) preserved hepatic parenchyma and mitigated degenerative changes, with T_4 showing the most significant hepatoprotective effect. Groups T_5 and T_6 exhibited moderate protection, characterized by reduced congestion and localized lymphoid aggregation. Kidney sections from the T_2 group revealed severe pathological changes, including congestion, intertubular hemorrhages, granular and vacuolar degeneration in the tubular epithelium, glomerular atrophy, and focal tubular necrosis. These findings aligned with studies by Huang *et al*. (2018) [6] and Aengwanich and Simaraks (2004) [2], who observed similar renal alterations under heat stress. Groups T_5 and T_6 showed reduced severity of these changes, indicating a protective effect of *Tamarindus indica* and vitamin C. The T_3 and T_4 groups demonstrated minimal alterations, with relatively normal renal parenchyma, suggesting that these supplements effectively counteracted heat stress-induced renal damage.

The spleen tissue of the T_2 group exhibited mild to moderate degenerative changes, including lymphoid depletion, immature germinal centers, indistinct red and white pulp boundaries, and thickened arteries. These findings were consistent with studies by Iraqi *et al*. (2013) [7] and Mohamed *et al*. (2015) [10], who documented similar splenic damage under heat stress. Supplementation with *Tamarindus indica* and vitamin C improved splenic integrity, with T_5 and T_6 showing reduced lymphoid depletion and better-preserved lymphoid follicles. The T_4 group displayed the most significant protection, characterized by intact germinal centers and minimal alterations, emphasizing the potential of these supplements in maintaining immune function during heat stress. Microscopic analysis of the duodenum from the T_2 group revealed moderate degenerative changes in the lamina propria, desquamation, blunt villi, epithelial exfoliation, and inflammatory infiltration. These observations corroborated findings by Xie *et al*. (2019) [15] and Lola *et al*. (2018) [9], who reported intestinal congestion and villi architectural loss under heat stress. Groups T_5 and T_6 exhibited improved intestinal integrity, with preserved villi morphology and glandular structures. The T_3 and T_4 groups demonstrated near-normal intestinal histoarchitecture, suggesting the role of *Tamarindus indica* and vitamin C in enhancing intestinal health and nutrient absorption during heat stress. The thymus of the T_2 group showed atrophic changes, focal necrosis, hemorrhages, and vacuolar alterations in the medulla, indicative of severe damage due to heat stress. These findings were consistent with Lola *et al.* (2018) [9], who documented thymic atrophy under similar conditions. Supplementation with *Tamarindus indica* and vitamin C mitigated these effects, with T_5 and T_6 showing mild degenerative changes and better-preserved thymic parenchyma. The T_4 group demonstrated relatively normal thymic histoarchitecture, highlighting its potential in maintaining immune competence during heat stress. Histological analysis of the bursa of Fabricius in the T_2 group revealed significant atrophy, lymphoid depletion, necrosis, and loss of interfollicular connective tissue. These results aligned with findings by Iraqi *et al*. (2013) [7] and Lola *et al*. (2018) [9], who reported similar bursal damage under heat stress. Groups T_5 and T_6 exhibited milder alterations, with less severe lymphoid depletion and better-preserved follicle architecture. The T_4 group showed minimal degenerative changes, characterized by uniform bursal follicles and a well-maintained lymphoid population, suggesting its efficacy in preserving immune organ function. The gross and histopathological findings from this study underscored the detrimental effects of heat stress on broiler organ systems. The observed pathological changes in the T_2 group highlighted the vulnerability of broilers to heat-induced damage, consistent with previously reported findings. Supplementation with *Tamarindus indica* and vitamin C (T_5 and T_6) mitigated these effects to varying degrees, demonstrating their potential as protective agents. The T_4 group (herbal vitamin C) showed the most significant preservation of histoarchitecture across multiple organs, followed by T_3 (tamarind pulp). These findings suggested that *Tamarindus indica*, alongside vitamin C, held promise for reducing heat stress-induced damage in broilers. Further research was warranted to elucidate the underlying mechanisms of these protective effects and validate these findings across diverse poultry populations and environmental conditions. Investigating optimal dosage, timing, and combinations of *Tamarindus indica* and vitamin C supplementation could provide valuable insights for improving poultry health and productivity under heat stress. Additionally, exploring the bioactive compounds in *Tamarindus indica* responsible for these effects may contribute to the development of targeted interventions for managing heat stress in poultry farming.

Conclusion

The study demonstrated that heat stress significantly impacted broiler health, leading to pathological changes across multiple organ systems. However, supplementation with *Tamarindus indica* pulp powder and herbal vitamin C alleviated these effects, particularly in the heart, liver, kidneys, spleen, and immune organs. The findings suggest that these natural supplements hold promise in enhancing broiler resilience to heat stress, warranting further research into their optimal use.

Acknowledgement

Authors wish to thankful to the Associate Dean, College of Veterinary and Animal Sciences, Parbhani (MAFSU, Nagpur) for conducting the experiment and providing facilities required for the same.

Conflict of interest

None

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