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Anti-oxidant Potentiality of Guduchi (*Tinospora cordifolia*) Supplemented to Lactating Buffaloes under Field Conditions

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ABSTRACT

The study was conducted during the hot dry season (ambient temperature 40–43°C, relative humidity 13–43%, THI 78.7–82.4) under field conditions in the year 2021 at Chapadu, in the Kadapa district of Andhra Pradesh to study the antioxidant and anti-stress potential of *Tinospora cordifolia* (Guduchi/Amruta) against solar radiation-induced heat stress in early lactating Murrah buffaloes. Twenty buffaloes were randomly assigned to control and treatment groups; the treatment group received 120 g day^{-1} of dried *T. cordifolia* plant powder mixed into the concentrate for 30 days. Milk production and composition, somatic cell count (SCC), and erythrocytic oxidative stress parameters-including superoxide dismutase (SOD), reduced glutathione (GSH), and lipid peroxidation (LPO) were measured on days 10, 20, and 30. Supplementation of *T. cordifolia* significantly (p<0.01) increased average daily milk yield by 14.27% and 4% fat-corrected milk (FCM) yield by 18.94% compared to the control. Milk fat, SNF, protein, and total solids were significantly improved, while SCC was markedly reduced (130×10^3 vs. 256×10^3 cells ml^{-1}, p<0.01), indicating enhanced udder health. Antioxidant profiling revealed that SOD activity decreased by 5.44% and 19.78% on days 20 and 30, respectively, and GSH levels declined by 17.0% and 29.5%, reflecting a reduced oxidative burden. LPO was significantly lower in the treatment group throughout the study, with a 31.8% reduction on day 30 (p<0.05). These results demonstrated that *T. cordifolia* supplementation mitigated heat-induced oxidative stress, stabilized cellular redox balance, and improved milk yield and quality in buffaloes. Thus, *T. cordifolia* represented a practical phytogenic strategy to enhance antioxidant defenses and dairy productivity under thermal stress conditions.

KEYWORDS: *T. cordifolia*, heat stress, buffaloes, antioxidant activity, milk yield

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Buffalo (*Bubalus bubalis*) is an important livestock species that produces milk, meat, and is also utilized as a source of draught power, fuel, and leather. Buffaloes are the world's second most important milch animal, making substantial contributions to global milk output; they are distributed across 77 countries and yield over 75 million mt of milk annually, maintaining a growth rate of about 3% (Oghenerume et al., 2024). Their milk is richer in fat, total solids, proteins, caseins, lactose, and ash than milk from other farm animals. India, home to about 108.7 million buffaloes, accounts for roughly 21.23% of the national livestock population (Anonymous, 2019), and has become the world's largest milk producer. Total milk output in India is projected to reach 239.3 million mt by 2025, driven by herd expansion and government initiatives supporting dairy production (Anonymous, 2024). Buffaloes contribute approximately 43.6% of India's overall milk production, underscoring their essential role in the dairy sector (Anonymous, 2025). However, the productivity of buffaloes remains suboptimal due to poor genetic potential, inadequate nutrient supply, unscientific feeding management, and stress (Gupta et al., 2024). Various types of physical and chemical stresses, such as heat/cold, transportation, physiological changes, and diseases reduce productivity primarily through oxidative stress (Gupta et al., 2024). The rising temperature and solar radiation exposure linked with climate change exacerbate heat stress, particularly in tropical countries, negatively impacting buffalo lactation performance (Polsky et al., 2017). Any natural compound with antioxidant properties may help maintain health when continuously ingested as components of dietary foods or drugs (Singh et al., 2000). *Tinospora cordifolia* (Guduchi/Amruta), a member of the family *Menispermaceae,* is a highly regarded medicinal plant in Indian Ayurveda. Guduchi itself means the "one which protects our body," and Amrita means "the nectar that confers immortality". In Hindi, the plant is commonly known as 'giloya', which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and keeps them eternally young. Though every part of the plant has therapeutic value, the stem is used in most of the medicinal preparations. It contains important secondary metabolites such as terpenes, alkaloids, flavonoids, steroids, and glycosides. *T. cordifolia* exhibits robust antioxidant activity and is used traditionally as a general tonic, antispasmodic, antimicrobial, anti-inflammatory, anti-arthritic, antiallergic, antidiabetic, osteo-protective, hepato-protective, and anti-stress agent (Muhammad et al., 2020). In buffaloes, no study has been conducted regarding the supplementation of *Tinospora cordifolia*. *T. cordifolia* has shown many promising biological activities and possesses powerful antioxidant properties. It is used in folk medicine as a general tonic, antispasmodic, antimicrobial, anti-inflammatory, anti-arthritic, antiallergic, antidiabetic, osteo-protective, hepato-protective, and anti-stress agent (Muhammad et al., 2020). Recent studies have demonstrated *T. cordifolia's* ability to mitigate oxidative damage and improve immune response in livestock species subjected to climatic stressors (Sengupta et al., 2009). No studies to date report the effects of T. cordifolia supplementation specifically in buffaloes.

Given the central role of oxidative stress in heat-induced production losses, this study seeks to evaluate the antioxidant potential of *T. cordifolia* in early lactating buffaloes maintained under field conditions (Gupta et al., 2024; Polsky et al., 2017). The findings will help elucidate the role of phytogenic supplementation in improving antioxidant status and production performance of tropical buffaloes, providing an economically viable strategy to mitigate thermal stress (Sharma et al., 2024; Mir et al., 2015; Mallick and Prakash, 2012). Further evidence from related species supports the positive effects of *T. cordifolia* on reducing oxidative biomarkers and enhancing stress resilience (Savant et al., 2021; Soni et al., 2021; Rajneesh et al., 2023; Jain et al., 2023; Soni et al., 2022).

2. MATERIALS AND METHODS

The experiment was conducted for 30 days during the months of May–June (hot dry season with ambient temperature ranging from 40–43°C, relative humidity between 13–43% and temperature humidity index (THI) ranging from 78.7 to 82.4) in Chapadu, in the Kadapa district of Andhra Pradesh. Twenty upgraded Murrah buffaloes of 2nd or 3rd parity, in early lactation (1–8 weeks postpartum) were selected and divided into two groups of ten each.

The plant material of *Tinospora cordifolia* was obtained from Srinivasa Ayurvedic Pharmacy, Srinivasa Mangapuram, Tirupati. The control group was maintained with 6–8 hours of grazing Paddy straw+homemade concentrate mixture (Groundnut cake and rice bran), while the treatment group was maintained with 6-8 hours of grazing Paddy straw+concentrate mixture (Groundnut cake and rice bran), and received *T. cordifloia* plant powder. The animals in the treatment group were fed dried *T. cordifloia* plant powder (120 g day^{-1}) by mixing it in the homemade concentrate mixture for a period of 30 days. Concentrate mixture was offered based on milk production at 1.0 kg per 2.0 kg of milk yield during the morning (6.00 AM) and evening (7.00 PM) at the time of milkings. The buffaloes were hand milked, and the milk yield of each buffalo was recorded at each milking. Milk samples were collected twice early in the morning and evening, aseptically in sterilized milk sampling centrifuge tube of 50 ml from all the animals, and were transported

immediately (collected within one hour) to the laboratory on ice for the milk composition (fat %, SNF %, protein % and total solids %) and milk somatic cell counts (SCCs), which were determined using a Lacto Scan MCC Combo 6030 milk analyzer.

The blood samples were collected in heparinized vials on the 10th, 20th, and 30th day and were used for the estimation of oxidative stress-related blood biochemical parameters in erythrocytes in order to assess the antioxidant and free radical scavenging effects of *T. cordifloia*. Estimation of different oxidative stress indices, viz., superoxide dismutase (SOD), reduced glutathione (GSHx), and lipid peroxidation (LPO), in the erythrocytes was done on the day of blood collection. SOD activity was determined in the erythrocyte haemolysate (Madesh and Balasubramanian, 1998). GSHx was estimated by the 5, 5' dithiobis (2-nitrobenzoic acid) (DTNB) method (Prins and Loos, 1969). LPO was determined in terms of malondialdehyde production (MDA) by the method of Rehman (1984). Data were analyzed by using one-way ANOVA. The significant values were also tested using Duncan's Multiple Range Test (Snedecor and Cochran, 1994).

3. RESULTS AND DISCUSSION

3.1. Milk production and composition

The overall average milk yield (l day^{-1}) and FCM yield (l day^{-1}) of lactating Murrah buffaloes in the treatment group were significantly (p<0.01) higher compared to the control group (Table 1). There was a 14.27% improvement in average milk yield (L/D) and an 18.94% improvement in FCM yield (L/D) in the treatment group compared to the control. The results of the present study were in agreement with the results of Mir et al. (2015), who reported 11.26% and Mallick and Prakash (2012), 10.10%, respectively. The higher percent of milk yield obtained in the present study, as compared to the other reports, could be attributed to the stage of lactation of animals and the length of the study.

Milk composition parameters, including fat, SNF, protein, and total solids, differed significantly (p<0.01) between treatment and control groups. The fat percentage increased from 5.59% in the control group to 5.92% in the treatment group. Similarly, SNF content improved from 8.73% to 8.86%, protein from 4.13% to 4.76%, and total solids from 15.00% to 15.90%. These significant improvements indicated that T. cordifolia supplementation not only enhanced quantitative milk production but also positively influenced milk quality. This was aligned partly with the findings of Yadav et al. (2013), who suggested that while protein and lactose contents remain relatively stable across feeding regimens, fat, SNF, and total solids are more variable. In this study, however, protein also showed a marked increase, suggesting the positive metabolic influence of the supplementation under heat stress conditions.

Table 1: Effect of *T. cardifolia* on milk production and composition in lactating buffaloes exposed to solar radiation-induced heat stress

Parameter	Control	Treatment
Milk yield (L/D)**	7.64^{a}±0.05	8.73^{b}±0.04
FCM (L/D)**	9.50^{a}±0.03	11.30^{b}±0.02
Fat %**	5.59±0.04	5.92±0.01
SNF %**	8.73±0.01	8.86±0.01
Protein %**	4.13±0.01	4.76±0.01
Total solids %**	15.00±0.01	15.90±0.01
SCC ($X10^3$ cells ml^{-1})**	256^{b}±0.13	130^{a}±0.01

The means with different superscripts in rows differ significantly (p<0.01)

Milk somatic cell count (SCC) was significantly (p<0.01) lower in the treatment group (130×10^3 cells ml^{-1}) compared to the control group (256×10^3 cells ml^{-1}). The reduction in SCC indicates an enhancement in udder health and a potential immunity-boosting and anti-inflammatory effect of *T. cordifolia*. Similar results were reported by Mir et al. (2015) in buffaloes and Mallick and Prakash (2012), stating that somatic cell count was significantly higher in untreated control cows throughout the period of the experiment as compared to the guduchi-supplemented group of cows. Likewise, Mukherjee et al. (2006) observed an initial rise in SCC after intramammary infusion of *T. cordifolia* extract in bovine subclinical mastitis cases, followed by a significant reduction during the treatment period. The marked decrease in SCC highlights the immune-modulatory and antioxidant properties of *T. cordifolia* (Rajneesh et al., 2023), which protect the mammary gland, mitigate oxidative stress, and aid in the maintenance of udder health. This is particularly beneficial under solar radiation-induced heat stress conditions, where animals are predisposed to stress-related mammary disorders.

3.2. Antioxidant profile

The results indicated that Superoxide dismutase (SOD) activity (units/g HB) was significantly (p<0.05) lower in the *T. cordifolia* treated group (Table 2). On day 10, no major difference was observed, but at day 20 and 30, the treatment group exhibited 5.44% and 19.78% reductions, respectively, compared to the control group. The decreased SOD activity is likely related to reduced oxidative challenge in erythrocytes of the supplemented buffaloes. Heat stress was known to elevate SOD activity initially as a

Table 2: Effect of *T. cordifolia* on antioxidant enzymes in lactating buffaloes exposed to solar radiation-induced heat stress.

Group	DAY 10	DAY 20	DAY 30
Superoxide dismutase (units g^{-1} Hb)			
Control	1035± 0.31	1048± 0.26	1056± 0.261
Treatment (*T. cordifolia*)	1049^{a}± 34.8	991± 35.9^{b}	847^{c}± 24.9^{c2}
Reduced glutathione (μM g=1 Hb)			
Control	3.46± 0.01^{a}	3.24± 0.01^{b1}	3.9± 0.02^{c1}
Treatment (*T. cordifolia*)	3.46^{a}± 0.01^{a}	2.69^{b}± 0.01^{b2}	2.75c± 0.5^{c2}
Lipid peroxidation (nM MDA ml^{-1} erythrocytes)			
Control	21.40^{c}± 0.18^{a1}	26.2^{b}± 0.27^{b1}	28.3^{a}± 0.07^{c1}
Treatment (*T. cordifolia*)	18.40± 0.26^{a2}	19.8± 0.27^{bc2}	19.3± 0.13^{c2}

The means with different superscripts in rows differ significantly (p<0.05); The means with different superscripts in a column differ significantly (p<0.05)

compensatory mechanism (Megahed et al., 2008), since SOD protected tissues against damage caused by superoxide radicals generated by auto-oxidation of hemoglobin and enzymatic processes, including cytochrome P450 reductase and xanthine oxidase (Vásquez et al., 2008). *T. cordifolia* supplementation appeared to alleviate the burden of reactive oxygen species (ROS), thereby downregulating the need for higher SOD activity. This suggested that the plant might have lowered intracellular ROS levels, reducing the requirement for endogenous enzymatic scavenging.

The leaf extract of *T. cordifolia* was reported to have potent antioxidant and ROS-scavenging activities, which might have been attributed to its content of alpha-glucosidase inhibitor (saponarin) (Sengupta et al., 2009). As a result results in downregulation of SOD and GPx activity (Sivakumar and Rajan, 2010). Similar findings were observed in goats with Withania somnifera supplementation (Shireen and Sahni, 2012).

Reduced glutathione (GSH, μM g^{-1} Hb) levels also declined significantly (p<0.05) in the treatment group compared to the control, with reductions of 17.0% on day 20 and 29.5% on day 30. GSH was recognized as a master intracellular antioxidant that maintained redox potential, detoxified peroxides and xenobiotics, and helped in the recycling of oxidized vitamins C and E (Ramnath et al., 2008). Although a reduction was generally viewed as unfavorable, here it might have indicated a lower oxidative burden, leading to less mobilization of reduced glutathione from reserves. Decreased GSH under heat stress was reported in both cattle (Lakritz et al., 2002) and sheep (Devasena and Adilaxmamma, 2014). *T. cordifolia* exerted free radical scavenging properties against hydroxyl, peroxynitrite, and nitric oxide radicals (Shivananjappa and Muralidhara, 2012). While overall levels of GSH decreased in our study, the literature suggested that supplementation with *T. cordifolia* could normalize thiol metabolism and improve redox balance over longer supplementation periods. This functional adaptation could have protected cells via non-enzymatic pathways, contributing to improved resilience under heat stress.

Lipid peroxidation (LPO, nM MDA ml^{-1} erythrocytes), an indicator of oxidative damage, was significantly (p<0.05) lower in the treatment group than in the control throughout the study. By day 30, the treatment group exhibited 31.8% lower MDA levels compared to the control, indicating decreased membrane lipid oxidation. Elevated LPO reflected compensatory changes in response to oxidative stress, as excess free radicals oxidized polyunsaturated fatty acids in erythrocyte membranes, generating malondialdehyde (MDA) as a stable biomarker Vásquez et al., 2008. By maintaining lower LPO, *T. cordifolia* supplementation prevented oxidative degradation of lipids, stabilized cell membranes, and protected cellular functions in buffaloes exposed to solar heat stress. Similar protective effects of antioxidant-rich supplements lowering LPO were reported with *Withania Somnifera* (Rasool and Varalakshmi, 2008; Shireen and Sahni, 2012) and *T. cordifolia* in rat cardioprotection studies (Ekbbal et al., 2022).

Overall, the data confirmed that solar radiation-induced heat stress disrupted antioxidant status in buffaloes, as reported earlier by Marai and Haeeb (2010), who linked heat exposure with impaired enzymatic reactions, hormonal balance, and nutrient metabolism. In this context, *T. cordifolia* supplementation substantially reduced oxidative stress indices, as evidenced by reduced requirements for enzymatic antioxidants (SOD, GSH) and significantly lowered lipid peroxidation levels. This dual, i.e., lower oxidative load and protection of membrane integrity, indicated that *T. cordifolia* works as both an antioxidant and an adaptogen under stressful conditions.

Such effects might have explained explain the improved production performance observed in parallel trials, suggesting that phytogenic supplementation could have be a promising strategy for managing heat stress in lactating buffaloes.

4. CONCLUSION

The study concluded that solar radiation in the form of high ambient temperature had caused heat stress

in buffaloes. Supplementation of *Tinospora cordifolia* had increased milk yield and improved milk quality by reducing somatic cell count. Moreover, administration of the indigenous medicinal plant had offered significant protection against oxidative stress induced by high temperature, as indicated by normalization of glutathione (GSH), superoxide dismutase (SOD), and lipid peroxidation (LPO) levels under field conditions.

5. ACKNOWLEDGEMENT

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