



Tinospora cordifolia Stem Powder Effects on Antistress and Blood Biochemical Parameters in Heat Stressed Gaddi Goats

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ABSTRACT

Background: Heat stress in animals can occur when ambient temperatures are high and wind speeds are low, resulting in diminished growth, poor health, or even death. The purpose of this study is to evaluate the effect of *Tinospora cordifolia* (TC) on anti-stress and blood biochemical parameters in heat stressed (HS) Gaddi goats.

Methods: Sixteen adult male Gaddi goats were split into four groups, T0H0 (no supplement no HS, control), T1H0 (diet fed with TC stem powder @ 5% of DMI with no HS), T0H1 (no supplement with HS) and T1H1 (diet fed with TC stem powder @ 5% of DMI with HS). The study was planned in a 2x2 factorial design, with supplementation TC stem powder (0 per cent vs. 5 per cent) and heat stress (normal vs. heat exposure) were the key effects to be studied over the 30-d trial period.

Result: There was a significant interaction (supplementxstressxperiod) for oxidative stress indices. At the end of the experiment, the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and cortisol levels were higher in the T0H1 group goats than in the T1H1 group (similar to normal levels). The blood's hematological and biochemical characteristics were equivalent among groups. However, after a 30-d trail, Hb and PCV levels in the blood were low in the stressed group. It can be culminated that, there was no significant change in hematological and blood biochemical parameters across groups, however animals' antioxidant status were improved.

Key words: Antistress, Cortisol, Gaddi, Heat stress, *Tinospora cordifolia*.

INTRODUCTION

Body temperature must be kept within physiological limits of the animal to stay healthy and productive. High ambient temperatures along with low wind speeds can cause heat stress in animals, resulting in decreased growth, poor health, or even death (Chen *et al.*, 2020). As a result, animals must adapt to their surroundings in order to produce more and better. Tropical regions receive more solar energy and have greater temperature than temperate zones but temperate zones are also becoming warmer as a result of global warming and animals adopted in temperate areas now feeling heat stress during summer season. Gaddi (White Himalayan goat) is the most widely known goat breed in the high altitude, Western temperate Himalayas, with its true home tract in Himachal Pradesh's hills but distribution extending to adjoining hilly areas. Studies show that the temperature humidity index (THI), a measure of heat stress faced by animals is projected to rise in many parts of the state and higher THI is leading contributor to thermal stress which leads to oxidative stress (Suong *et al.*, 2022). Although goats are more resistant to thermal stress, they still suffer from heat and cold stress outside of their comfort zone, which for Indian goats is 13-27°C (Gupta *et al.*, 2013). This is the first study of its kind to explore the impact of heat stress on Gaddi goats as temperatures rises continuously due to global warming. Animals have a complex system of antioxidants that includes vitamins C and E, or even the enzymes catalase, superoxide dismutase and glutathione, oxidative stress is

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caused by a lack of antioxidants or the inhibition of antioxidant enzymes, which can damage cells (Devasena and Adilaxamma, 2014). Emphasis has been placed on phytochemical feed additives (PFAs) to mitigate the negative effects of heat stress on both well-being and productivity (Green *et al.*, 2021; Peng *et al.* 2022). *Tinospora cordifolia* (TC) (Family: Menispermaceae), a well-known Ayurvedic medicinal vine also known as Amrita, Guduchi (Sanskrit) and Giloe (Hindi) and it has a broad spectrum of applications in the treatment of a variety of diseases (Devi, 2021). Phytochemical screening of TC indicates the presence of various chemicals, such as alkaloids, diterpenoid, lactones, steroids, glycosides, polysaccharides from different parts. Therefore, the purpose of the study was to determine the antioxidant potential of TC against HS in Gaddi goats.

MATERIALS AND METHODS

The current study was conducted at the ICAR-IVRI regional station in Palampur, Himachal Pradesh. Sixteen adult male Gaddi goats (Age, 3-4 years and body weight, 30-35 kg) were divided into four groups (T0H0, T1H0, T0H1 and T1H1) in a 2×2 factorial design. Animals were housed in two separate well-ventilated sheds in a standard management setting with individual feeding and watering facilities, two groups T0H0, T1H0 were kept at normal temperature and T0H1, T1H1 were heat-treated group in which animals were subjected to a constant temperature of 33±2°C for 30 days for 6 h (10 AM to 4 PM). Group T0H0 was used as control (no treatment no HS), while T1H0 (animals fed on diet supplemented with TC stem powder @ 5% of DMI with no HS), T0H1 (no treatment with HS) and T1H1 (animals fed on diet supplemented with TC stem powder with HS) group. TC stem was purchased from local market, Palampur (H.P.) sun dried powdered and well mixed with concentrate mixture before giving to Gaddi goats. The minimum and maximum temperature and relative humidity (RH) of the shed were measured with the aid of a dry bulb-wet bulb thermometer and expressed in °C. THI is thought to be a sensitive indicator of climatic stress (Adjassin *et al.*, 2022), the THI equation for goats was as follows:

$$\text{THI} = \text{db}^\circ\text{C} - [(0.31-0.31\text{RH}) * (\text{db}^\circ\text{C} - 14.4)]$$

Where

db°C = Dry-bulb temperature in °C.

RH= Relative humidity percentage/100.

A THI value of less than 27.8 was deemed to indicate the absence of heat stress (Marai *et al.*, 2001).

On days 0 and 30, blood samples were collected from the jugular vein before the morning feeding (09:00 h) with the purpose of determining hematological and blood biochemical parameters. Packed cell volume (PCV) and hemoglobin (Hb) concentration were determined by microhematocrit and cyanmethemoglobin methods, respectively. The concentrations of cortisol and activity of GPx and SOD were determined by using kits by (Cayman Chemical Company, Ann Arbor, MI, USA.). And other serum biochemicals were analyzed using an ELISA plate following the protocol from the biochemical assay kit (Tulip Diagnostics (P) Ltd., India).

All of the analysis were repeated three times. The results were expressed as means. A difference of P<0.05 was deemed statistically significant. The data generated from the experiment were analyzed statistically through SPSS (20) software programme version 20.0. (SPSS Inc., Chicago, IL, USA) by following the procedure described by Snedecor and Cochran (1994). Parameters like blood biochemical parameters were analyzed through two-way ANOVA and statistical differences between treatment group means were determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

Micro climatic conditions inside experimental house

Means of estimating the extent of heat stress was proposed using both ambient temperature and relative humidity,

termed as the THI (Marai *et al.*, 2001). The values obtained indicate the following: <27.8 = absence of heat stress and 28.9 and more = severe heat stress. In the present study during experimental period average temperature were 14.3°C versus 33°C and average relative humidity were 72% versus 38% in normal and heat exposure group respectively. Hence, THI values were 14.29 and 29.40 in normal and heat stress group of animals, which designate all the experimental animals were in severe heat stress.

Oxidative stress indices

The antioxidative enzyme system is the first line of antioxidant defense and even minor changes in antioxidative enzyme activity can shift the balance between the production of reactive oxygen species (ROS) and the antioxidant system (Ibtisham *et al.*, 2019). SOD, catalase and GPx are antioxidant enzymes that aid in the reduction of oxidative stress in various parts of the cell. These antioxidants may exert their effects by interacting directly with ROS, quenching them and/or chelating the catalytic metal ions (Yasooob *et al.*, 2021). The result indicated that SOD activity was significantly lower in TC supplemented group. The activity of SOD increased in the control group (T0H0) and T0H1 and decreased notably in TC supplemented groups (T1H1 and T1H0) on 30th days. No interaction effects have been obtained between supplement×stress, stress×period and supplement×period, only individual stress effects have been seen in a small increased level of SOD in the heat stressed groups (Table 1) as a result of providing defense against oxidative damage caused by heat stress. Higher activity of SOD in summertime than in wintertime have been reported earlier in cattle and buffaloes (Yatoo *et al.*, 2014). Tiwari and Sahni (2012) reported a decline in the level of SOD in goats under heat stress, which resumed to normal status following treatment with *Withania somnifera* root powder. Improved performance of the T1H0 and T1H1 groups is related to TC, which could have provided exogenous antioxidants for ROS scavenging. The activity of GPx improved slightly from 0 to 30 days in the control group (T0H0) and increased considerably (T0H1) while it increased moderately (P<0.05) in the TC fed groups (T1H0 and T1H1). Glutathione is considered a master antioxidant and is present in almost all the body's living cells. Supplementation of TC may have enhanced the development of hepatic antioxidant enzymes, *i.e.*, SOD and GSH (Goel *et al.*, 2002) and reduced oxidized vitamin C and vitamin E to detoxify toxins to preserve cellular redox potential and the erythrocyte membrane integrity altered in the event of heat stress (Ramnath *et al.*, 2008). The antioxidant activity of TC in the reduction of GSH rates in livestock subjected to heat stress and in rats in the event of induced myocardial damage (Raj *et al.*, 2010) supports our assertion. Tiwari and Sahni (2012) reported a reduction in GSH levels in goats under heat stress, which returned to normal status following treatment with *Withania somnifera* root powder. However, (Habeeb, 2018) reported that hyperthermia causes a temporary decrease followed by an increase in GSH levels

in the blood, owing to an increase in hepatic GSH excretion which supports our finding of increased level of GPx in heat stressed (T0H1) goats.

Improved output is due to the T1H0 and T1H1 groups, which could have provided exogenous antioxidants for ROS scavenging. Cortisol hormone is essential to several

biological processes, including energy production, thermal regulation, lactogenesis and milk production regulation (Slimen *et al.*, 2019). Cortisol levels rose slightly ($P>0.05$) with an increase in the number of days of heat stress exposure in the T0H1 group compared to other groups such as T0H0, T1H1 and T1H0. Individual effect of supplement

Table 1: Effect of *T. cordifolia* on serum antioxidant activity in goats exposed to heat stress.

Attributes	SOD (U/ml)		GPx (nmol/min/ml)		Cortisol (ng/ml)	
	0	30	0	30	0	30
T0H0	16.91	17.23 ^b	68.52	69.01 ^b	10.23	09.51 ^b
T1H0	15.80	13.41 ^c	62.83	67.52 ^{bc}	11.20	08.72 ^c
T0H1	15.52	23.76 ^a	67.62	97.3 ^a	09.55	15.83 ^a
T1H1	15.74	12.51 ^c	73.00	66.30 ^c	10.30	08.93 ^c
SEM	0.60	0.51	0.67	3.50	0.26	0.34
P-value	0.234	0.001	0.453	0.001	0.675	0.001

T0H0 = No supplement no heat stress, control, T1H0 = Animal fed on diet supplemented with TC @5% of DMI with no heat stress, T0H1 = No supplement with heat stress and T1H1= Diet supplemented with TC with heat stress.

^{abc} Means bearing different superscripts in a column differ significantly for a parameter.

Table 2: Effect of *T. cordifolia* on blood biochemicals in goats exposed to heat stress.

Attribute	Days	T0H0	T1H0	T0H1	T1H1	SEM	P-value
HB (g/dl)	0	10.40	10.80	10.41	10.65	0.18	0.841
	30	11.03	11.33	8.60	9.03	0.38	0.903
PCV %	0	32.23	32.38	32.6	32.43	0.20	0.724
	30	32.5	33.05	30.03	30.43	0.44	0.909
Total protein (g/dl)	0	5.84	6.01	6.01	6.17	0.06	0.949
	30	6.28	6.28	6.47	6.39	0.08	0.820
Albumin (g/dl)	0	2.92	2.91	2.62	2.73	0.05	0.572
	30	3.17	3.14	2.83	3.06	0.08	0.728
Globulin (g/dl)	0	2.92	3.10	3.39	3.44	0.08	0.665
	30	3.11	3.14	3.65	3.34	0.11	0.700
A:G ratio	0	0.92	0.95	0.78	0.80	0.03	0.928
	30	1.16	0.114	0.81	0.95	0.05	0.391
Glucose (mg/dl)	0	49.46	50.96	48.50	50.14	0.52	0.948
	30	53.95	53.69	49.10	50.53	0.90	0.611
BUN (mg/dl)	0	15.51	16.68	17.41	15.80	0.54	0.242
	30	15.74	16.72	17.55	16.86	0.36	0.263
Creatinine (mg/dl)	0	1.16	1.19	1.19	1.11	0.03	0.734
	30	1.18	1.19	1.54	1.54	0.05	0.601
Total cholesterol (mg/dl)	0	82.22	90.3	86.06	83.88	2.63	0.379
	30	81.25	77.31	79.7	85.53	3.86	0.573
HDL (mg/dl)	0	28.52	28.03	29.13	27.39	0.99	0.790
	30	26.85	31.44	28.37	30.32	0.87	0.800
LDL (mg/dl)	0	32.87	33.83	28.34	32.30	1.81	0.704
	30	32.6	32.09	32.04	41.67	1.87	0.497
ALT (IU/L)	0	32.61	32.73	34.85	33.8	0.56	0.567
	30	33	36.47	40.78	44.3	0.46	0.867
AST (IU/L)	0	140.7	145.1	142.4	140.4	2.58	0.436
	30	141.3	145.3	170.6	180.4	3.45	0.216
ALP (IU/L)	0	136.7	140.8	143.6	141.9	2.53	0.234
	30	145.0	149.7	133.8	131.7	2.43	0.925

T0H0 = No supplement no heat stress, control, T1H0 = Animal fed on diet supplemented with TC @5% of DMI with no heat stress, T0H1 = No supplement with heat stress and T1H1= Diet supplemented with TC with heat stress.

significantly lower cortisol concentration in supplemented group. Higher temperature causes heat stress during the summer and cortisol levels may rise as a result. Herbal antioxidants such as TC have been shown to decrease the increased levels of oxidants in rats under disease-induced stress (Rasool and Varalakshmi, 2008) and in solar radiation induced thermal stress in goats (Tiwari and Sahni, 2012), while TC could provide protection against isoproterenol induced cardiac stress in rats (Raj *et al.*, 2010) and corroborated with the present finding in Goat. The improved performance of the T1H0 and T1H1 groups is attributable to the presence of exogenous antioxidants for scavenging ROS.

Blood biochemical

Blood biochemicals are vital indicators for envisaging the health status of animals and dietary nutrient absorption and metabolism can define the features of blood biochemical parameters (Ban *et al.*, 2022). All the blood parameters were within the normal range both at 0 and 30 days (Table 2). Individual effect of heat stress on Hb and PCV levels were significantly lower (9.99 vs 10.56 and 31.5 vs 32.4) as compared to normal condition. Heat stress has been shown to alter hematological parameters (Rojas-Downing *et al.* 2017). There is a wide range of variation in these parameters among the goat breeds. Heat stress causes animals to consume more oxygen by increasing their respiration rate. Higher oxygen intake raises the partial pressure of oxygen in the blood, which reduces erythropoiesis and, as a result, the number of circulating erythrocytes, PCV and Hb values (Gupta and Mondal, 2021). The main effect on blood albumin, globulin, creatinine, BUN, glucose and total protein level was non-significant, however higher concentration of these parameters were obtained (3.04 g/dl, 3.45 g/dl, 1.33 mg/dl, 16.68 mg/dl 51.82) due individual effect of heat stress as compared to normal groups. In the present study, an increase in total protein concentration indicated a shift in protein metabolism from anabolic to catabolic, which was evident with a decrease in body weight of the animals. Perhaps the need for energy to maintain homeothermy was met by increased tissue protein catabolism, which resulted in increased serum protein and creatinine. A prior study found that exposing goats to heat stress for an extended period of time increases plasma total protein, albumin and globulin levels (Okoruwa, 2014), this has been attributed to increase in plasma volume as a result of heat stress. On the contrary, the level of total plasma protein, albumin and globulin decreases in heat stressed goats (Shaji *et al.*, 2017). This might be due to vasoconstriction and dehydration that occurs due to increased respiration rate during heat stress. Triglyceride, total cholesterol, HDL and LDL levels were comparable among the groups irrespective of heat stress or supplementation. There was no main effect of TC stem on serum enzyme levels; only an individual heat stress effect was noticed, whereas ALP activity was reduced and ALT and AST activity were significantly

increased. Heat stress causes an increase in ALT and AST (Srikandakumar *et al.*, 2003) and a decrease in AKP activity (Marai *et al.*, 2009), which validates our findings. Increase in ALT, AST and decrease in ALP due to heat stress has been reported by Rathwa *et al.* (2017). Overall, no change in serum biochemical profile might be due to the mild heat stress which animals could have tolerated and also suggested supplementation of TC at 5% DMI should be considered safe for Gaddi goats. Further, TC did not show any ability to improve the serum biochemical profile.

CONCLUSION

The study can be culminated that the heat stress in goats has been shown to be triggered by high ambient temperatures. There was no significant difference in hematological and blood biochemical parameters among the groups due to overall interaction (effect of supplement, stress and period). However, supplementing Gaddi goats with TC at 5% DMI augmented their antioxidant status.

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Conflict of interest: None.

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