ANTI-OXIDANT POTENTIALITY OF TINOSPORA CORDIFOLIA AS EVIDENCED FROM THE BLOOD BIOCHEMICAL PROFILE OF NELLORE ZODPI MALE SHEEP EXPOSED TO SPONTANEOUS SOLAR RADIATION ON RANGE

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ABSTRACT

Sheep in India are vulnerable to solar radiation induced heat stress, while grazing on range during summer, due to overproduction of reactive oxygen species (ROS) with resultant detrimental effect on their health, production, and reproduction. This paper explores the antioxidant property of Tinospora Cordifolia (Tc) in sheep exposed to heat stress on range, since there was no such study earlier. The experiment was conducted on twelve Nellore Zodpi adult male sheep divided into three groups with four animals in each group under three different feeding systems. The first group of animals (T-1) was kept under extensive system, which allowed free range grazing for 6-8 hours in a day in summer, when the ambient temperature was 40-43 °C, and relative humidity was 45-52 percent. The second group (T-2) was given commercially available Tc whole plant powder orally @ 600 mg/ kg body weight/ day besides grazing on range. The third group (T-3) was managed under semi-extensive system, and was given the same Tc treatment as to T-2, in addition to ad-lib post-grazing supplementation of black gram (Vigna mungo) straw and green tree leaves. The duration of the experiment was 30 days. The oxidative stress indices in terms of concentration of SOD (units/ g Hb), GSH (µM/ g Hb), and LPO (nM MDA/ ml of packed erythrocytes) of the experimental animals were estimated on 10th, 20th and 30th day of administration of Tc. The study indicated increase (P ≥ 0.05) in the level of anti-oxidants (SOD and GSH) as well as pro-oxidant (LPO) with increase in duration of exposure of the T-1 animals (control) to solar radiation, and indicated heat stress. Administration of Tc caused significant (P ≤ 0.05) reduction in SOD and GSH levels with restrained increase (P ≥ 0.05) in the level of MDA in T-2 and T-3 animals, demonstrating the antioxidant potential of Tc. It is concluded that Tc conferred significant protection against heat stress to grazing sheep on range during summer. The benefit was more pronounced in the animals, provided with post grazing forage supplementation along with Tc.

KEYWORDS

Antioxidant, Heat stress, Nellore sheep, Tinospora cordifolia

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INTRODUCTION

Sheep in India get their sustenance from grazing on range. In the summer, when the ambient temperature soars high, the animals suffer from hyperthermia and heat stress (oxidative stress) due to incessant exposure to solar radiation on range, that stimulates production of reactive oxygen species (ROS) such as free radicals and peroxides in large amounts (Salo et al., 1991) overriding the innate antioxidant defense mechanism of the body, and damage the body tissues (Sahin et al., 2001), due to peroxidation of biomolecules, such as, lipids, proteins, and nucleic acids (Maybauer et al., 2006). Generation of ROS beyond the threshold puts the animal in an unhealthy disposition triggering several diseases and viral infections (Irshad and Chaudhuri, 2002), besides attenuation of production and reproduction performance (Ganaie et al., 2013).

The body activates the innate antioxidative defense mechanism by triggering production of enzymatic antioxidants, like, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) etc., for scavenging the circulating free radicals in the system (Kumar et al., 2011). Exogenous anti-oxidants like vitamins C & E with selenium (Kumar et al., 2010) and minerals like zinc and chromium in the form of drugs and/ or dietetics can confer protection against oxidative stress (Kumar et al., 2011).

Tinospora cordifolia, known as guduchi in Sanskrit and gurcha in Hindi, is a native to the tropical forests of Indian subcontinent (Dastur, 1962). It is used in folk medicine for treatment of several ailments (Upadhyay et al., 2010). However, the anti-oxidant potential of the plant has been sparsely studied (Bhwaya and Anilakumar, 2010), and virtually none in respect of heat stress.

This maiden study was undertaken to evaluate the blood biochemical parameters of Nellore Zodpi male sheep exposed to spontaneous solar radiation during range grazing in summer in the semi-arid tropical region of India with or without supplementation of T. cordifolia in the diet.

MATERIALS AND METHODS

The present study was conducted on twelve Nellore Zodpi adult male sheep aged about 2-2.5 years of age, and weighing on an average 29.00 ± 0.56 kg, and maintained on range grazing during the months of March and April 2013, when the ambient temperature ranged between 40-43°C, and relative humidity ranged between 45-52 percent. The animals were selected from the farmers’ flocks in Kadapa district of Andhra Pradesh. The experimental animals were dewormed by oral administration of albendazole @5mg/ kg body weight. The duration of the experiment was 30 days.

Tinospora cordifolia plant in pulverized form was obtained from Srinivasa Ayurvedic Pharmacy, Srinivasamangapuram, Tirupati,
Andhra Pradesh. The experimental animals were divided into three groups with four animals in each group. The control animals (T-1) were maintained on extensive system, i.e., 6-8 hours of grazing on range. The second group of animals (T-2) were maintained under extensive system, but supplemented with Tinospora cordifolia plant powder @600 mg/ kg body weight per os per day. The third group of animals (T-3) was maintained under semi-extensive system with post-grazing supplementation of black gram (Vigna mungo) straw and green tree leaves (Sesbania/ Subabul/ Moringa), besides oral administration of Tinospora cordifolia plant powder @600 mg/ kg body weight per day.

The oxidative stress indices, in terms of superoxide dismutase (SOD), reduced glutathione (GSH), and lipid peroxidation (LPO), in the erythrocytes of the experimental animals were estimated on 10th, 20th and 30th day of administration of Tinospora cordifolia.

SOD activity was determined in the erythrocyte haemolysate (Madesh and Balasubramanian, 1998). GSH was estimated by the 5, 5’ dithiobis (2-nitrobenzoic acid) method (Prins and Loos, 1969). LPO was determined in terms of malondialdehyde (MDA) production by the method adopted by Rehman (1984). Data were analyzed by the method of complete randomized design using one way ANOVA (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The erythrocytic concentration of SOD (units/ g Hb), GSH (µM/ g Hb) and LPO ((nM MDA/ ml of packed erythrocytes) are depicted in Table-1.

Superoxide dismutase: The concentration of SOD marginally increased (P>0.05) from 10-30 days in the control group (T-1), while it decreased significantly (P≤0.05) in Tinospora cordifolia supplemented groups (T-2 and T-3). The magnitude of reduction was the highest (26.75%) in T-3 group at 30 days.

Superoxide is formed in the RBC by auto oxidation of haemoglobin to methaemoglobin. It confers protection against damage to the tissues caused by superoxide anionic radical. Higher concentration of SOD in summer than in winter has been reported earlier in cattle (Chandra, 2009) and in buffaloes (Lallawmkimi, 2009).

Tiwari and Sahni (2012) had reported reduction in the level of SOD in goats under heat stress, which regained the normal status after treatment with Withania somnifera root powder. Better performance of T-3 group is attributed to mineral rich post-grazing supplemented diets (Shinde et al., 2009), which could have provided exogenous antioxidants for scavenging ROS (Kumar et al., 2011).

Reduced Glutathione: The concentration of GSH marginally increased (P>0.05) from 10-
Table-1: Effect of *T. cordifolia* on SOD, GSH, and LPO in sheep exposed to solar radiation induced heat stress.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of exposure</th>
<th>Change (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 days</td>
<td>20 days</td>
<td>30 days</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td></td>
<td>1041.2±502</td>
<td>1059.5±143</td>
<td>1066.1±251</td>
</tr>
<tr>
<td>T-1 (Extensive)</td>
<td></td>
<td>1050.6±216</td>
<td>992.9±160</td>
<td>851.7±143</td>
</tr>
<tr>
<td>T-2 (Extensive + Tc)</td>
<td></td>
<td>1241.46±118</td>
<td>1021.28±116</td>
<td>909.42±47</td>
</tr>
<tr>
<td>Reduced glutathione</td>
<td></td>
<td>2.64±0.4</td>
<td>2.67±0.5</td>
<td>2.69±0.3</td>
</tr>
<tr>
<td>T-1 (Extensive)</td>
<td></td>
<td>2.4±0.2</td>
<td>1.76±0.4</td>
<td>1.51±0.6</td>
</tr>
<tr>
<td>T-2 (Extensive + Tc)</td>
<td></td>
<td>2.46±0.2</td>
<td>1.52±0.3</td>
<td>1.48±0.5</td>
</tr>
<tr>
<td>Lipid peroxidation</td>
<td></td>
<td>23.46±2.2</td>
<td>28.62±6.2</td>
<td>27.53±4.5</td>
</tr>
<tr>
<td>T-1 (Extensive)</td>
<td></td>
<td>21.48±6.1</td>
<td>25.65±4.3</td>
<td>26.78±3</td>
</tr>
<tr>
<td>T-2 (Extensive + Tc)</td>
<td></td>
<td>20.08±5.6</td>
<td>24.42±7.6</td>
<td>24.23±9.8</td>
</tr>
</tbody>
</table>

Note: (1) The figures are presented as Mean±SEM. (2) The means of a particular feeding system for a particular parameter at different days of exposure bearing different superscripts differed significantly at *P*≤0.05.

30 days in the control group (T-1), while it decreased significantly (*P*≤0.05) in *Tinospora cordifolia* supplemented groups (T-2 and T-3). The magnitude of reduction was the highest (38.62%) in T-3 group at 30 days.

Glutathione is considered as a master antioxidant, and is located in almost all living cells of the body. Supplementation of *Tinospora cordifolia* could have boosted the production of hepatic antioxidant enzymes, *viz.* SOD and GSH (Goel et al., 2002), and reducing oxidized vitamin C and vitamin E to detoxify toxins to maintain cellular redox potential and erythrocyte membrane integrity altered in the event of heat stress (Ramnath et al., 2008).

Antioxidant action of *Tinospora cordifolia* in decreasing the level of GSH in cattle exposed to heat stress (Lakritz et al., 2002) and in rats in case of induced myocardial damage (Alpha Raj et al., 2010) support our contention. Tiwari and Sahni (2012) had reported reduction in the level of GSH in goats under heat stress, which regained the normal status after treatment with *Withania somnifera* root powder.

Better performance of T-3 group in our study is attributed to mineral rich post-grazing supplemented diets (Shinde et al., 2009), which could have provided exogenous antioxidants for scavenging ROS (Kumar et al., 2011).

Lipid Peroxidation: The level of LPO marginally increased (*P*≥0.05) with increase in number of days of exposure to heat stress in T-1 (control) as well as in T-2 and T-3.
groups. The increment in T-2 group was less in 20-30 days (0.87%) over 10-20 days (23.78%). The trend was similar in T-3 group, which showed less increment between 20-30 days (0.77%) than between 10-20 days (21.61%). The increment was relatively less in T-3 group than in T-2 group.

Lipid peroxidation in animals under heat stress induces elevation of malondialdehyde (MDA), the most important product of fatty acid decomposition with concurrent reduction in SOD and GSH signifying attenuation of the oxidative defense mechanism of the body (Song et al., 2008), which is harmful for the viability of cells, and is implicated in the pathogenesis of several diseases and different types of viral infections (Irshad and Chaudhuri, 2002).

It is reported that herbal antioxidants like Withania somnifera could control the augmented level of LPO in rats under disease induced stress (Bhattacharya et al., 2001; Rasool and Varalakshmi, 2008) and in solar radiation induced thermal stress in goats (Tiwari and Sahni, 2012), while Tinospora cordifolia could provide protection against isoproterenol induced cardiac stress in rats (Alpha Raj et al., 2010), and corroborated with the present finding in sheep. Better performance of T-3 group is attributed to mineral rich post-grazing supplemented diets (Shinde et al., 2009), which could have provided exogenous antioxidants for scavenging ROS (Kumar et al., 2011).

**CONCLUSION**

It is concluded that solar radiation in the form of high ambient temperature caused heat stress in sheep on range. The administration of indigenous medicinal plant T. cordifolia conferred significant protection against oxidative stress induced by high ambient temperature. The benefit was more pronounced in the animals, which were given post-grazing supplementation of fodder (gram straw) and tree leaves.

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