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Effect of Heat Stress on Humoral Immunity and its Amelioration by Amla Powder (*Emblica officinalis*) Supplementation in Buffaloes

Preeti Lakhani*, Rajesh Jindal and Shashi Nayyar

Department of Veterinary Physiology and Biochemistry, GADVASU, Ludhiana, INDIA

*Corresponding author: P Lakhani; Email: preetilakhani@yahoo.in

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ABSTRACT

The effect of amla powder supplementation on heat stress and immunoglobulin (Ig) levels of summer stressed buffaloes were investigated. The study was carried out with 24 apparently healthy Murrah buffaloes during pre-summer and summer seasons. The animals were divided into 3 groups of 8 animals each viz. Pre-Summer group (Group I); Summer Control group (Group II); Summer Treatment group (Group III). Group III animals were supplemented with amla powder @ 200 mg/Kg body wt. / day for 30 days. In summer stressed buffaloes (Group II), there was a significant rise in erythrocytic lipid peroxidation level and superoxide dismutase activity along with a decrease in plasma IgM and increase in IgG levels. Supplementation of amla powder to summer stressed buffaloes was able to lower lipid peroxidation levels and superoxide dismutase activity coupled with the increase in IgM and decrease in IgG levels. It was concluded that amla powder supplementation can ameliorate the adverse effects of summer stress on humoral immunity in Murrah buffaloes.

Keywords: Amla, Antioxidant status, Buffaloes, Humoral immunity, Oxidative stress

An animal undergoes heat stress when there is an imbalance between heat production within the body and its dissipation. Summer stress evokes a series of drastic changes in biological functions of buffaloes that include depression in intake and utilization of feed, disturbed metabolic processes, variation in hormonal secretions and enhanced oxidative stress (Marai and Habeeb, 2010). There is growing evidence that oxidative stress significantly impairs growth, production, immunological status and reproductive performance of the animal and a tight relationship between oxidative stress and impaired fertility has been reported in buffalo-cows (Ahmad *et al.* 1995). Heat stress modulates metabolic reactions through generation of free radicals and produces oxidative stress leading to adverse effects on the immune status of the animals. (Kataria *et al.* 2010), In order to ameliorate the adverse effects of the summer stress, the common strategies like providing shade, use of sprinklers, fans, etc. are not only capital intensive but also partially effective under semi-intensive system (Sivakumar *et al.* 2010). Thus, antioxidant supplementation as an alternative approach for reducing the oxidative stress to the animal has been tried in several species. Supplementation of vitamin has been found to ameliorate the adverse effects of heat stress in goats (Kumar *et al.* 2010), cows (Ul-Haq *et al.* 2013) and buffaloes (Sunilkumar *et al.* 2010). Amla powder (Indian gooseberry), the most potent source of Vitamin C and rich in tannins and flavinoids have been reported to reduce the adverse effects of summer stress in goats (Randhawa, 2013) and cattle (Ul-Haq *et al.* 2013). Therefore, the investigations have been made to assess the effect of amla powder as an antioxidant supplement for ameliorating heat and humoral immunity in buffaloes

MATERIAL AND METHODS

The study was conducted with 24 apparently healthy adult female Murrah buffaloes maintained under standard management conditions during pre-summer (March-April; Mean THI=68.5) and summer (June –August; Mean THI=83.5) seasons. The animals were divided into three groups of 8 each viz. Pre-Summer group (group I; No

supplementation), Summer Control Group (Group II; No supplementation) and Summer Treatment Group (Group III; Supplemented with amla powder @ 200 mg/Kg body wt. / day for 30 days). Shed temperature and humidity was recorded with thermo hygrometer. Temperature humidity index (THI) of the animal shed was calculated using the formula:

$$THI = (0.81 \times Ta) + \{(RH \div 100) \times (Ta - 14.4)\} + 46.6$$

(Where, Ta = Average ambient temperature in ˚C and RH = Average relative humidity)

Blood samples (6-8 ml each) were collected thrice from all the animals at weekly intervals in heparinized glass vials by jugular vein puncture. In summer treatment group (Group III) animals, the sample collection was commenced after one week of the start of the supplementation. Blood level was marked in the vial; plasma was separated and stored in small aliquots at -20°C for immunoglobulin analysis. The erythrocyte pellet was washed thrice with normal saline, distilled water was added up to the marked level and the resulting hemolysate was stored at -20°C till analyzed for erythrocytic lipid peroxidation (LPO) (Placer *et al.* 1966) and superoxide dismutase (SOD) activity **(**Nishikimi *et al.* 1972). IgG and IgM were analysed spectrophotometrically using the kits from SPINREACT (Spain).

The data were subjected to analysis of variance (ANOVA) for comparison of means among different groups, and group-differences were detected by the Fisher's least-significant-difference test. All analyses were performed with the statistics package SYSTAT VERSION 6.0.1 Copyright (c) 1996, SPSS INC.

RESULTS AND DISCUSSION

Heat stress is the most common stressor in tropical countries. Adaptation to heat stress involves integration of physiological, endocrine and immune systems (Altan et al. 2003). Significant increase in LPO, SOD and IgG and decrease in IgM levels (Table 1) were observed in summer stressed buffaloes (Group-II) as compared to the pre-summer group (Group-I). Amla, being a rich source of vitamin C and having antioxidant properties, may have an ameliorative effect on the heat stress of animals.

Marked increase in LPO levels and SOD activity during summer season has been previously reported by Altan *et al.* (2003) in heat stressed dairy animals. Increased *lipid peroxidation is associated with increased production of free radicals during heat stress which initiates peroxidation of polyunsaturated fatty acids.* Lipid peroxidation is the indicator of oxidative stress in cells and tissues. Similar finding were reported by Yarovan (2008) in cows and Kumar *et al.* (2010) in buffaloes. LPO level and SOD activity were significantly (P<0.05) lowered by supplementation with amla powder (Group III); and the LPO and SOD levels dropped to values comparable with those found in pre-summer group of animals, suggesting

Table 1: Humaral immunity and oxidative stress indicators in summer stressed buffaloes supplemented with amla powder

Groups	Erythrocytic lipid peroxidation (nmol MDA produced/g Hb)	Erythrocytic Superoxide dismutase activity (U/mgHb)	IgM (mg/dl)	IgG (mg/dl)	Cortisol (ng/ml)
I Pre-Summer group	213.94 ±7.35^{a}	19.56 ±1.06^{a}	145.00 ±0.19^{a}	600.04 ±0.32^{a}	9.48±0.70 ^{a}
II Summer Control Group	356.28 ±18.25^{b}	23.45 ±0.93^{b}	138.70 ±0.10^{b}	1070.30 ±0.74^{b}	15.46±1.11 ^{b}
III Summer Treatment Group: (supplemented with amla powder @ 200 mg/Kg body wt. / day)	242.00 ±6.60^{a}	18.93 ±1.68^{a}	141.70 ±0.33^{a}	720.30 ±0.33^{a}	9.39±0.37 ^{a}

Means bearing different superscripts (a, b, c) differ significantly (P<0.05) within the columns

a positive effect of supplementation of amla powder in relieving the negative effect of heat stress on buffaloes.

Erythrocytic SOD activity was significantly ($p<0.05$) higher in Group-II (23.45±0.93 U/mg Hb) as compared to Group I (19.56±1.06 U/mg Hb). This physiological up-regulation of this enzyme may be an attempt to diminish the superoxide radical challenge. Findings are in accordance with Lallawmkimi (2009) who reported that levels of erythrocyte SOD and catalase increased significantly in Murrah buffaloes in response to heat stress. Superoxide dismutase (SOD) is considered the first defence against pro-oxidants (Halliwal and Chirico, 1993) which scavenges both extracellular and intracellular superoxide radicals by acting in conjugation with glutathione peroxidase and catalase (Agarwal and Prabhakaran, 2005).

In this study, a significant increase in IgG was observed in Group II as compared to Group I buffaloes indicating effect of heat stress on this parameter. Amla powder supplementation to summer stressed buffaloes lowered the levels of IgG may be the effect mediated by mediated by the decreased cortisol concentration and increased the level of IgM (Table 1). The effect of heat stress can be neutralized by complex antioxidant system that organisms can develop (Mac Arthur, 2000). Vitamin C and trace minerals like zinc have proved to play a vital role as modulators of antibody response (Vegad and Katiyar, 1995).

Al-Busaidi *et al. (*2008) also observed increase in IgG levels in Dhonfari goats during summer. Johnson *et al.* (1997) showed seasonal fluctuations with an increase in immunoglobulin levels during the hot dry season in Brazilian goats. However, increase in IgG level may possibly is associated with increase in level of cortisol. An elevation of plasma cortisol levels has also been reported to result in increased susceptibility to infectious diseases by with an increased production of antibodies especially IgG as it possesses phagocytic activity (Masaki *et al.* 1994).

The supplementation of amla powder caused a significant ($p<0.05$) alteration in levels of LPO and SOD activity in summer stressed buffaloes. *The decreased LPO levels and SOD activity in treatment group indicated that supplementation of amla powder ameliorated the influence of heat stress. Similar decrease in LPO levels and SOD activity by vitamin E and C supplementation was reported by Randhawa (2013) and Kumar et al. (2012) in goats under heat stressed conditions.* The effects of antioxidant vitamins directly alter thermal set point by decreasing prostaglandin output, especially of PGE series whose turnover increases during stress (Hadden, 1987) and which has a direct effect on the hypothalamic thermoregulatory zone (Ganong, 2001).

It is concluded that amla powder, an easily available and cost effective supplement can be used as an alternative/ additional approach to ameliorate the adverse effects of heat stress in Murrah buffaloes. However, further research is necessary regarding the minimum effective dose under field condition where heat stress is a challenge to the optimal production of livestock.

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