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Effect of amla powder supplementation on haematological parameters, ceruloplasmin and transferrin levels in summer stressed murrah buffaloes

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Abstract

The effect of amla powder supplementation was investigated on oxidative stress, metaloproteins viz; plasma ceruloplasmin and transferrin levels and haematological parameters i.e. packed cell volume (PCV) and haemoglobin (Hb) levels of summer stressed buffaloes. 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 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), a significant rise was observed in the erythrocytic lipid peroxidation level and ceruloplasmin whereas decrease in plasma transferring, PCV and Hb was observed. Supplementation of amla powder to summer stressed buffaloes was able to lower lipid peroxidation levels and superoxide dismutase activity coupled with the increase in transferrin, PCV and Hb levels coupled with decrease in ceruloplasmin concentration. It was concluded that amla powder supplementation can ameliorate the adverse effects of summer stress in Murrah buffaloes.

Keywords: Amla, Antioxidant status, Buffaloes, haematological parameters, metaloproteins, Oxidative stress.

Stress is a condition which arises when an animal is exposed to sudden changes in its environment. Physiological equilibrium is maintained mainly by blood in the body (Ahmed *et al.,* 2003). Changes in the haematological indices are the indicator of physiological and pathological state of the animal (Genesar *et al.,* 1986). Dietary ascorbate has been shown to interfere with copper and

iron absorption in a number of animal species and in humans (Jacob *et al.*, 1987). Since ascorbate appears to influence the free metal and 90-95% of copper found in serum is bound to ceruloplasmin (Gubler *et al.*, 1953), one might expect ascorbate to have an influence on this protein. Also ascorbate modifies transport of iron by modulating the activity of transferrin. There is growing evidence that oxidative stress significantly alters physiological, metabolic, biochemical and haematological parameters of the body.

Heat stress modulates metabolic reactions through free radicals and produces oxidative stress (Kataria *et al.*, 2010), leading to adverse effects on haematological status of the animals. 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. Vitamin C supplementation has been found to ameliorate the adverse effects caused by 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 has 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 stress in buffaloes

Materials and Methods

The study was conducted on 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 6 each viz. Pre-Summer group (group I): No supplementation; Summer Control Group (Group II): No supplementation; 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 ^{O}C and RH = Average relative humidity)

Blood samples (8-10 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. The blood samples were analyzed shortly after collection for hematological parameters viz. hemoglobin (Hb) and packed cell volume (PCV) and rest of samples were processed for the separation of plasma and preparation of hemolysate. Blood level was marked in the vial; plasma was separated and stored in small aliquots at -20°C for ceruloplasmin 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) and superoxide dismutase activity according to (Placer *et al.,* 1966). Haemoglobin (Hb) and PCV were analysed using met-haemoglobin method and microcapillary method respectively.

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. Significant increase in LPO level, SOD activity and ceruloplasmin and decrease in PCV, Hb and plasma transferrin levels (Table 1) were observed in summer stressed buffaloes (Group-II) as compared to the pre-summer group (Group-I). Marked increase in LPOlevels during summer season has been previously reported by Sunilkumar *et al.* (2010) in heat stressed Murrah buffaloes. Increased *lipid peroxidation is associated 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.

Altan *et al.* (2003) also demonstrated that increase in lipid peroxidation due to heat stress might be associated with production of large number of free radicals which are capable of initiating peroxidation of polyunsaturated fatty acids. 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 a positive effect of supplementation of amla powder in relieving the negative effect of heat stress on buffaloes.

Table 1: Oxidative stress and haematological indicators in summer stressed buffaloes supplemented with amla powder

Sampling	Lipid Peroxidation (nmol MDA produced/g/Hb)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment *)**
1	212.17±12.29	319.67±33.16	268.67±7.31
2	204.33±14.16	357.67±39.37	238.67±9.13
3	225.33±12.44	391.50±15.25	221.67±9.07
Mean	**213.94±07.35**[a]	**356.28±18.25**[b]	**243.00±6.61**[c]
	Hemoglobin (g %)		
Sampling	**GROUP I (Pre summer)**	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	13.75±0.75	10.50±0.58	12.08±0.72
2	14.64±0.29	11.42±0.67	12.68±0.68
3	13.83±0.42	11.10±0.56	12.61±0.83
Mean	**14.07±0.30**[a]	**11.01±0.34**[c]	**12.43±0.41**[b]
	Packed Cell Volume (%)		
Sampling	**GROUP I (Pre summer)**	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	42.83±1.40	33.83±1.76	38.67±1.23
2	44.33±1.48	34.50±1.52	36.67±1.41
3	40.17±1.70	36.50±1.02	40.67±1.02
Mean	**42.44±0.93**[a]	**34.94±0.84**[b]	**38.67±0.78**[c]

Group I: Pre-Summer group; Group II: Summer Control Group; Group III: Summer Treatment Group (supplemented with amla powder @ 200 mg/Kg body wt. /day)

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

Packed Cell Volume and Heamoglobin

Treatment wise average packed cell volume (PCV) in pre summer, heat stressed and amla supplemented buffaloes was 42.44±0.93, 34.94±0.84 and 38.67±0.78% and hemoglobin concentration (g %) was 14.07±0.30, 11.01±0.34 and 12.43±0.41 in Groups I, Group II and Group III respectively. Studies revealed that mean PCV and Hb were found to be lower in summer control group (Group II) as compared to pre-summer group (Group I). Singh *et al.,* (2008) studied the effects of housing system on blood constituents and reported that PCV level decreased significantly ($p<0.05$) in Marwari in subtropical climate when animals were exposed to sun during summer season as compared to animals kept in shed. Bhan *et al.* (2012) reported that the

packed cell volume (PCV) and hemoglobin (Hb) levels were higher during winter than other seasons in Sahiwal cattle. Similar fall in PCV as a result of heat stress has been reported in sheep (Srikandakumar *et al.,* 2003) and goats (Abdel-Samee *et al.,* 1992). Reduction in PCV level could either be due to increased attack of free radicals on the RBC membrane resulting in their lysis or inadequate nutrient availability for erythropoiesis as the animal consume less feed under heat stress (Srikandakumar *et al.,* 2003). At high ambient temperature, peripheral vasodilation and redistribution of cardiac output are associated with expansion of blood volume and hemo-dilution in goats and sheep (Silanikove 2000; Ganong 2003). Effect of amla powder supplementation on Packed Cell Volume (Mean ± S.E.) in summer stressed Murrah buffaloes increased respiratory rate/panting, causing increased oxygen intake and excess elimination of CO_2 (West *et al.,* 1991). The increased partial pressure of oxygen in blood results in decreased erythropoiesis thus, reducing the number of circulating erythrocytes and haemoglobin values.

The studies have revealed a significant increase in PCV and Hb in Group III (summer treatment group) as compared to Group II (summer control group). These findings are in accordance to Ghanem *et al.,* (2007) who stated that vitamin C supplementation alleviated the effect of dehydration on PCV. Similar findings were reported by Ul-Haq *et al.* (2013) in crossbred dairy cows that hemoglobin and packed cell volume (PCV) were significantly ($P<0.05$) lower in control group (8.41 g%, 29.02%) than in ascorbic acid (9.77 g%, 32.96%) and amla powder supplemented group (10.10 g %, 33.43%). The results indicated that amla powder supplemented to heat stress buffaloes can be useful in order to maintain hematological parameters.

Plasma Ceruloplasmin

Mean Ceruloplasmin concentrations were found to be 5.14±0.34, 5.54±0.27 and 4.69±0.24 mg/dl (Fig. 1) in Groups I (pre summer group), II (summer control group) and III (amla supplemented), respectively.

The mean ceruloplasmin concentration was more during summer season as compared to pre summer. This increase in ceruloplasmin concentration might be due to reduced feed intake during summer heat stress. Similarly, Gursel *et al.* (2010) reported higher ceruloplasmin levels in ewes fed low energy diet as compared to normal energy feed. While on amla powder supplementation, the ceruloplasmin level declined in Group III as compared to Group II and were comparable to pre-summer values of ceruloplasmin (Group-I). Mateeseu *et al. (*1995) stated that ascorbate enhances copper transport and decreases

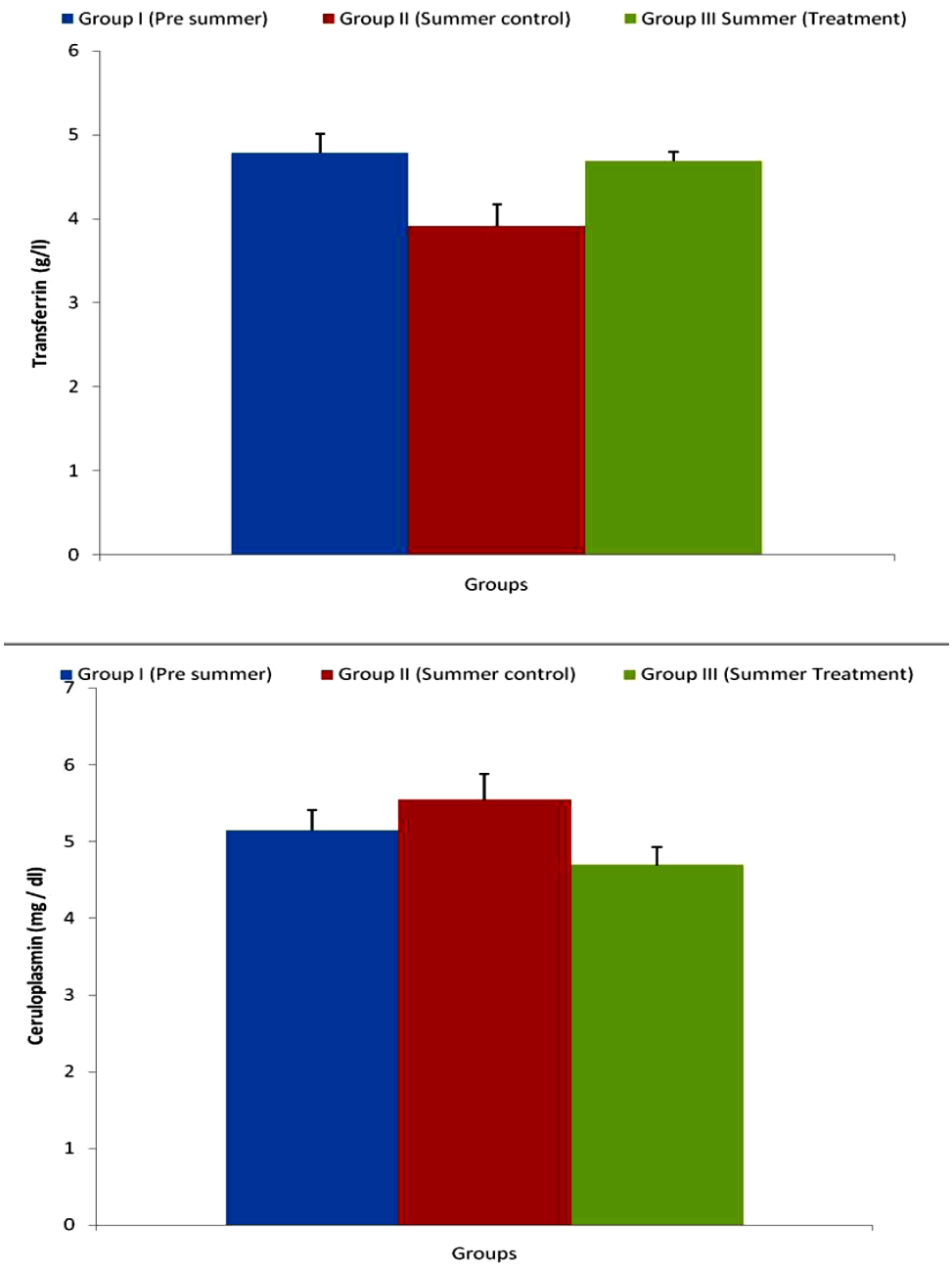

Fig. 1: Effect of amla powder supplementation on plasma transferrin and ceruloplasmin levels in summer stressed Murrah buffaloes

the ceruloplasmin activity as copper could protect against the deleterious effects of oxygen free radicals. Finley and Cerklewski (1983) reported that supplementation of 150 mg ascorbic acid/d for 2 months resulted in a significant drop in serum ceruloplasmin activity in humans. The antioxidant property of ceruloplasmin is through its oxidase activity, which is directed towards aromatic amines, phenols as well as ferrous ions (ferroxidase activity). The antioxidant activity of amla powder may be involved in the lowering ceruloplasmin level in Group III buffaloes.

Plasma Transferrin

Mean Transferrin concentrations were found to be 4.78±0.23, 3.91±0.26 and 4.79±0.11g/l in Groups I (pre summer group), II (summer control group) and III (amla supplemented), (Fig. 1) respectively.

In the present study, there was a significant ($p<0.05$) decrease in plasma transferrin during summer as compared to pre summer season. On supplementation of amla powder, there was significant increase in transferrin level in Group III. Free iron is known to be toxic to the cell; it can cause cellular damage, particularly lipid peroxidation. Iron is transported between different compartments by plasma transferrin. The transfer of iron from transferrin to the cells requires internalization of the iron-laden transferrin molecule (Kaneko 2004). Higher animals transport iron in a protein complex, transferrin, to render it nontoxic. As in Group II the level of transferrin has declined possibly leading to liberation of more free iron causing cellular toxicity which might be related to increase in lipid peroxidation activity. Vitamin C increases the size of cellular iron compartment (Bridges and Hoffman, 1986) and on supplementation of amla powder, level of transferrin increased thus combating oxidative stress. However, Bridges (1987) reported a decline in transferrin on ascorbate supplementation.

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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