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Effect of Feeding of *Tinospora cordifolia* on Immune Response in Cattle

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ABSTRACT

Thirty two apparently healthy local non descript cows were experimentally divided into two groups *viz.* treatment and control group having 16 animals each. The animals under treatment group were fed dried stem powder of *Tinospora cordifolia* (100 mg/kg BW) by mixing it in concentrate mixture for a period of 5 days. The control group animals were fed equal amount of the basal concentrate mixture without *T. cordifolia* supplementation. Blood and serum samples collected on day 0, 15, 30 and 45 day after feeding of *T. cordifolia* were subjected for concentration and purification of IgG, phagocytic activity by neutrophils, T cell count and haemolytic complement activity to assess the level of immune response in animals. Significant increase ($P<0.05$) of total serum immunoglobulin and mean phagocytic index was recorded in treatment group as compared to control group; however complement activity and T cell count did not vary significantly ($P>0.05$) between treatment and control group. In conclusion, it can be stated that *T. cordifolia* feeding had significant immunomodulatory effect in cows.

Keywords: *Tinospora cordifolia*, Cow, IgG, Phagocytic activity, T cell count, Complement activity

Chhattisgarh state has 1.27 crore livestock animals out of which cattle population is highest with 64% of the total population (Ann, 2007). Only 20% of these animals have been classified as recognized breeds in India and the rest are generally considered non-descript (ND) and hence undocumented (Tomar, 2010) but these non-descript animals play an important role in the economy of the region in which they are present. Hence, information about health status parameters, which unfortunately scanty in present perspectives need more emphasis, once situations to compromise with production traits arises. Though peak production potential of local cattle is less, the relative cost of milk production largely depends on the total population of animals coupled with minimum cost over medication and vaccination of animals. The best option is to use an herb, an efficient feed supplement with potential health advantages. Herbal immunomodulators efficiently cause functional alteration of the immune effectors cells and employed for modifying the immunological status of stressed livestock (Blecha, 1988). Unfortunately, we have little peer-reviewed research to support use of tropical herbs in specific nutritional recommendations to optimize immunity in ND cattle. Among the medicinal plants *T. cordifolia* (commonly called '*Guduchi*') has been evaluated for its immunomodulatory properties (Krishna *et al.*, 2009). Due to shifting of attention towards herbal alternatives a fairly large number of studies have been done more recently on therapeutic potential of *T. cordifolia* (Spelman, 2001; Singh *et al.*, 2011). However most of the researches have been done either in rats (Stanely *et al.*, 2000), mice (Sharma *et al.*, 2011) or human beings (Nemmani *et al.*, 2002). Keeping in view the need to work for upliftment of local ND cattle of Chhattisgarh using medicinal potential of *T. cordifolia* as diet supplementation, the present investigation was undertaken to study the effect of feeding of *T. cordifolia* on immune response of cattle.

MATERIALS AND METHODS

Experimental animals

Experimental animals included 32 apparently healthy local ND cows reared under standard managemental

practices at Shree Krishana Gaushala, Chhatagarh, Durg (Chhattisgarh). The animals were apparently free from ectoparasite infestation. The faecal sample examination for parasitic eggs did not reveal presence of any gastrointestinal parasites. All animals under study were also administered with preventive dose of oral antiparasitic medicines as a regular deworming programme.

Source of herbal immunomodulator

The fresh cylindrical stem pieces of *T. cordifolia* were collected from the Government Science College, Durg (Chhattishgarh). Authentication of the stem was performed at Department of Botany, Government Girls College, Durg (Chhattisgarh) and was deposited at Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Anjora, Durg (Chhattisgarh).

Preparation of plant material

The plant materials were thoroughly cleaned and made into small pieces and then dried in shade for two months. The dried stems were powdered in high speed electronic grinding machine, sieved through a BSS Mesh No. 85 sieve, weighed and packed in an airtight polythene bag for further use.

Experimental design

Cows were randomly divided into two groups based on their age *viz.* Group 1 (six month – two years) and Group 2 (above two years). Each group was further divided into treatment and control group (8 animals in each group). The animals under treatment group (Subgroup T-1 and T-2) were fed dried stem powder of *T. cordifolia* (100 mg/kg BW) by mixing it in concentrate mixture for a period of 5 days. The control group animals (Subgroup C-1 and C-2) were fed equal amount of the basal concentrate mixture without *T.cordifolia* supplementation.

Sampling

Serum samples were collected on day 0 and after feeding of *Guduchi* stem powder at days 15, 30, and 45 and stored at -20^{0}C till further use. Simultaneously the peripheral blood samples were also collected separately in heparinized vacutainer tubes (BD vacutainer®).

Isolation of bovine IgG

The salt (ammonium sulphate) precipitation and purification technique was used to isolate IgG from bovine serum. The IgG was isolated according to the method described by Hay and Westwood (2003) with little modifications. Protein concentration of crude IgG was estimated by UV light absorption at 280 nm (Systronics

Table 1: Effect of feeding *T. cordifolia* on total serum IgG concentration

Major group	Sub-group	IgG concentration mg/ml (Mean±SEM)				Level of significance
		Day 0	Day 15	Day 30	Day 45	
Group1	C-1	16.07±0.48	16±0.47	15.71±0.23	15.8±0.38	NS
(6month - 2 year)	T-1	15.9±0.24^c	20.05±0.22^a	18.07±0.46^b	17.71±0.22^b	**
Group 2	C-2	17.85±0.39	17.76±0.44	17.36±0.69	17.41±1.37	NS
(above 2 year)	T-2	17.5±0.83^b	21.07±0.98^a	19.03±0.15^{ab}	18.72±0.84^{ab}	*
Between C-1 and T-1		NS	**	**	**	
Between C-2 and T-2		NS	NS	NS	NS	
Between C-1 and C-2		*	*	*	*	
Between T-1 and T-2		NS	NS	NS	NS	

Note: The values are mean±SEM of 8 animals in each group. The mean values in a row bearing different superscript differ significantly *P<0.05, **P<0.01

NS= Non-significant

UV-vis spectrometer). Specificity of the bovine IgG was confirmed by known anti-bovine IgG (Mumbai GeNei) using Agar gel immuno diffusion test (Bhambani and Krishnamurty, 1963).

Estimation of IgG concentration

The total serum IgG concentration was estimated using single radial immuno diffusion (SRID) method as described by Mancini *et al.* (1965). The appearance of concentric ring around the well was observed and the diameter (mm) was measured. Graph for reference bovine IgG *i.e.* diameter of ring (on Y-axis) versus concentration of reference IgG (on X-axis) was plotted on a semi-log graph sheet. The concentration of IgG in the sample serum was estimated by allocating the corresponding point on the graph according to the diameter of ring for each serum sample.

Measurement of phagocytic activity

Mean phagocytic index was taken as a simple assay to measure the ability of phagocytes to bind and internalize or phagocytose bacteria (Ordonez *et al.* 2008). Opsonised bacteria and neutrophils were mixed in suspension and rotated to give optimal condition for interaction. Extracellular bacteria were then removed by washing or centrifugation. The amount of phagocytosis was determined by examining the stained cells under oil immersion microscope.

The 'antigen' (fresh whole bacterial cell suspension) was prepared from heavily inoculated trypticase soy agar slants by *Staphylococcus aureus* (maintained at microbiology laboratory). Count of bacteria ingested by each of the first 100 polymorphonuclear leukocytes was recorded. The phagocytic index was estimated as the average number of bacteria ingested per neutrophil. Each sample was set up in duplicate and results were expressed as mean of two determinations.

T cell count

T lymphocytes were enumerated by the E- rosette technique employed by Wadley (1977) with little modifications. The surface of T lymphocytes contains the receptor (CD_2) of sheep erythrocytes, which is called E receptor and so, T lymphocytes form E-rosette with sheep erythrocytes. The number of 'E rosettes' imply T lymphocytes number. This test was used to differentiate T lymphocytes from B-lymphocytes and enumerate T lymphocytes in peripheral blood.

Haemolytic complement activity

The activity of the classical pathway of complement activation (hemolytic complement) was assayed by the

Table 2: Effect of feeding *T. cordifolia* on Phagocytic index

Major group	Sub-group	PI (Mean±SEM)				Level of significance
		Day 0	Day 15	Day 30	Day 45	
Group 1 (6 month - 2 year)	C-1	3.75±0.25	3.5±0.42	3.87±0.29	3.62±0.32	NS
	T-1	3.87±0.71	4.62±0.49	4.5±0.32	4.12±0.39	NS
Group 2 (above 2 year)	C-2	4.12±0.54	4±0.46	3.87±0.39	3.75±0.45	NS
	T-2	3.87±0.69^{b}	6.25±0.61^{a}	5.87±0.54^{a}	4.37±0.73^{ab}	*
Between C-1 and T-1		NS	NS	NS	NS	
Between C-2 and T-2		NS	*	**	NS	
Between C-1 and C-2		NS	NS	NS	NS	
Between T-1 and T-2		NS	*	*	NS	

Note: The values are mean±SEM of 8 animals in each group. The mean values in a row bearing different superscript differ significantly *P<0.05, **P<0.01

NS = Non-significant

Table 3: Effect of feeding *T. cordifolia* on T cell count

Major group	Sub-group	T cell % (Mean±SEM)				Level of significance
		Day 0	Day 15	Day 30	Day 45	
Group 1	C-1	70.50±1.37	71.75±2.46	68.00±2.43	69.25±1.97	NS
(6 month - 2 year)	T-1	68.62±3.38	72.12±2.20	69.50±1.52	68.37±1.96	NS
Group 2	C-2	68.75±2.50	69.37±1.91	70.25±2.20	71.50±1.82	NS
(above 2 year)	T-2	69.87±2.09	73.12±1.12	69.75±2.87^{NS}	69.12±3.06^{NS}	NS

Note: The values are mean±SEM of 8 animals in each group. Mean values within a row and between C-1 and T-1; C-2 and T-2; C-1 and C-2; T-1 and T-2 within a column did not differ significantly (P>0.05)

NS= Non-significant

methods of Coligan *et al*. (1994) with slight modification. Further, complement activity was quantified by determining the serum dilution required to lyses 50% of the cells in the assay mixture. The reciprocal of above dilution was expressed as CH_{50} U per ml of serum.

Statistical analysis

Within the same age group the comparison between the control and treatment group was made by applying independent mean 't' test as per Snedecor and Cochran (1967). Similarly independent mean't' test was also be applied to study the effect of age between the corresponding groups (control with control group and treatment with treatment group). To see the effect of time within the treatment group's data was subjected to analysis of variance (ANOVA) one way classification.

RESULTS AND DISCUSSION

Total Serum IgG concentration

Significant increase was recorded in IgG concentration in T-1 groups calves after feeding of *T. cordifolia* as compared to C-1 groups (Table 1). Almost identical trend was observed when the IgG concentration of adult animals from groups C-2 and T-2 were compared. The effect of age was however not appreciable as rise in IgG concentration between C-1 and C-2 and between T-1 and T-2 yielded non-significant (P>0.05) results. In agreement with present findings, Kapil and Sharma, (1997); Acharya *et al.* (2002) and Nagalakshmi and Dhanalakshmi (2008) also reported enhanced immunoglobulin G (IgG) concentration in the serum of mice, cows and lamb; respectively after *T. cordifolia* supplementation.

Phagocytic activity

Phagocytic index were significantly (P<0.05) enhanced in the T-1 and T-2 groups as compared to C-1 and C-2, respectively (Table 2), which indicates positive effect of *T. cordifolia* feeding in calves and adult cows both. The magnitude of phagocytic activity was significantly (P<0.05) higher in adult cows (T-2) and gradually increased from day 0 (3.87±0.69) to day 15 (6.25±0.61) and day 30 (5.87±0.54). The enhanced phagocytic activity of polymorphonuclear cells in the peripheral blood treated with herb powder in ND cows indicated immunomodulatory and chemoattractent properties of the herb which could be due to the presence of bioactive substances like polysaccharide and glycoproteins in the stem powder. Present findings is in accordance with observation of Mukherjee *et al.* (2010) who reported enhanced phagocytic activity in diseased cows fed with the *T. cordifolia* extract on day 7 as compared with 0 day value. Likewise, Singh *et al.* (2003) reported significant leukocytosis, predominant neutrophilia and enhanced phagocytosis in murine model due to effect of *T. cordifolia.*

T cell count

Non significant (P>0.05) variations were recorded in T

Table 4: Effect of feeding *T. cordifolia* on complement activity

Major group	Sub-group	CH_{50} U/ml (Mean±SEM)				Level of significance
		Day 0	Day 15	Day 30	Day 45	
Group 1	C-1	158.02±8.34	158.02±9.73	158.02±9.59	159.02±5.19	NS
(6 month - 2 year)	T-1	156.02±6.83	148.02±8.01	152.02±4.05	154.02±2.29	NS
Group 2	C-2	178.02±7.48	181.02±6.81	172.02±6.90	167.02±8.41	NS
(above 2 year)	T-2	181.02±6.90	167.02±5.69	170.02±8.58	166.02±10.24	NS

Note: The values are mean±SEM of 8 animals in each group. Mean values within a row and between C-1 and T-1; C-2 and T-2; C-1 and C-2 ; T-1 and T-2 within a column did not differ significantly (P>0.05)

NS= Non-significant

cell count between control and treatment group. In group T-1, the T cell count enhanced on day 15 and day 30 to an extent of 1.9 and 2.2 %, respectively as compared to group C-1, followed by reduction on day 45 (1.3%). Similarly, non-significant increase (P>0.05) was observed in sub group T-2 on day 15. Increase in T cell count might be attributed to polysaccharides ingradients of *T. cordifolia* which activated different subsets of the lymphocytes such as natural killer cells (331%), T cells (102%), and B cells (39%) at 100 µg/ml concentration (Nair *et al.* 2004). The mechanism of action of the immunostimulatory compounds present in *T. cordifolia* stem powder might be due to similarity of these compounds with the pathogen associated molecular patterns for the activation of immune system. Toll like receptor mediated nuclear factor-kB (NF-kB) pathway activation has been shown so far to be the principal mechanism used by *T. cordifolia* (Nair *et al.* 2006). In contrast with the present study, Catherine *et al.* (2010) and Shakya *et al.* (2013) observed significantly enhanced non-specific humoral and cell mediated immune response effect of *T. cordifolia.*

Complement Activity

The haemolytic complement activity (CH_{50}) for the *T. cordifolia* fed and control animals before and after feeding is presented in Table 4. In group T-1, there was no significant effect (P>0.05) of feeding *T. cordifolia* on complement activity (148.02±8.01 to 154.02±2.29), and moreover the complement activity reduced marginally. In adult animals (T-2) magnitude of the complement activity was high (between 166.02±10.24 to 181.02±6.90). The complement system belongs to the non-specific humoral defense system. In the present study slightly decreased complement activity was recorded in both T-1 and T-2 groups which is almost similar with the findings of Kapil and Sharma (1997). The reduced immunohaemolysis might be due to inhibition of C3 convertase of the classical complement pathway. In contrast to present finding, Nair *et al.* (2004) and Catherine *et al.* (2010) reported increased complement activity through feeding of same plant.

It can be concluded from present study that the stem powder of *T. cordifolia* have potential to modulate immune response in non-descript animals, thus indicating its immunomodulatory activity. It is therefore opined that repeated feeding of *T. cordifolia* @ 100 mg/kg BW per day for five days at every 3 weeks interval may help in sustenance of immunoglobulin level and phagocytic activity. Further studies regarding mechanism of immunomodulation and probable use in experimental animals are still to be investigated and their use as an adjuvant during vaccination programs in order to reduce number of non-responder to vaccines can be explored.

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