Journal of Animal Research: v.7 n.2, p. 293-298. April 2017

DOI: 10.5958/2277-940X.2017.00042.0

Effect of Supplementing *Cassia tora* Leaf Extract on Immunological and Haematological Parameters of Broiler Birds

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Received: 22 September, 2016 **Revised:** 13 Feb., 2017 **Accepted:** 20 Feb., 2017

ABSTRACT

The effect of supplementing methanolic extract of *Cassia tora* on immunological and haematological parameters of broiler bird was investigated. Day-old broiler birds of Vencobb strain (n = 36) were randomly assigned into 3 treatments with 3 replicates each, 4 birds (2 each for cell mediated and humoral immune response) in each replicate. The birds of group T1 (Control) received basal diet, whereas birds of group T2 (Standard) received an antibiotic (Lincomycin @ 0.05% in feed) in addition to basal diet. The birds of group T3 (Test) received methanolic extract of leaves of *Cassia tora* (CSE) @ 0.4 g/l in drinking water in addition to basal diet. The treatment was given to birds of all the groups for 6 weeks. Cell mediated immune response was measured by 2, 4-Dinitro-fluorobenzene (DNFB) skin sensitization test whereas humoral immune response was assessed by micro haemagglutination test against sheep red blood cells. The cell mediated, humoral immune response and haematological parameters were significantly ($p \leq 0.05$) altered in CSE treated broiler birds when compared to birds of control and standard groups.

Keywords: *Cassia tora*, immune response, haematology, broiler bird

Herbal drugs are known to possess immuno-modulatory properties and generally act by stimulating or suppressing both specific and non-specific immunity. Some of these stimulate both humoral and cell mediated immunity while others stimulate only the cellular components of the immune system, i.e. phagocytic function without affecting the humoral mediated immunity. In mice, contact hypersensitivity has been studied using haptens such as dinitrofluorobenzene (DNFB), FITC, and oxazolone and the response is thought to be driven mainly by T cells. The production of antigen-specific antibodies represent a major defense mechanism of humoral immune responses. Micro haemagglutination test has been a tool to measure humoral immune response in animal model.

Many plants with potential immuno-modulatory activity such as *Curcuma longa, Panax ginseng, Tinospora cordifolia, Withania somnifera, Zingiber officinale, Ocimum sanctum, Moringa oleifera* and *Cajanus indicus* are reported, some of these have already been undertaken for evaluation of their activities in animals, and also to some extent in human (Jain *et al*., 2010; Desmukh *et al*., 2015; Sahu *et al*., 2016). Since ancient times *Cassia tora* (Common name: Charota/Chakunda) has been a subject of considerable interest as herbal medicine worldwide. *Cassia tora,* plant of Caesalpiniaceae family, is an important legume weed in Indian sub-continent, Eastern Africa, and Central America.

The leaves and seeds of *Cassia tora* are reported to have curative effect in leprosy, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders, skin diseases and liver disorders (Shakywar *et al.*, 2011). *Cassia tora* leaves posses phytoconstituents viz, rhein, aloe-emodin, chrysophanol, resins, cathatrine, calcium, iron, phosphorus, 1,3,5-trihydroxy-6-7-dimethoxy-2-

methylanthroquinone, beta-sitosterol, naptho-alpha-pyrone-toralactune, chrysophanol, physcion, emodin, rubrofusarin, cchrysophonic acid-9-anthrone, tricontan-1-0l, stigmasterol, b-sitosteral-b-D-glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids uridine, quercitrin, isoquercitrin (Rao and Chatterjee, 2016). Thus, study was aimed to evaluate effect of supplemention of methanolic extract of *Cassia tora* leaves on immuno-modulatory potential and haematological parameters of broiler birds.

MATERIALS AND METHODS

Plant extract and experimental design

The *Cassia tora* leaves used in the study were collected locally, identified, authenticated, shade dried and powdered using an electric grinder. The methanol extract from the powder was prepared by using Soxhlet's apparatus by hot extraction technique. All the animals and procedure employed were approved by the Institutional Animals Ethics Committee. A total of 36, day-old broiler birds were randomly divided into 3 treatment groups and each group divided into 3 replicates of 4 birds each (2 each for cell mediated and humoral immune response). Each bird of different group was individually identified by using leg bands. All the groups were maintained as per the following treatment schedule for 6 weeks: Control Group (T1): Basal diet, Standard Group (T2): Basal diet supplemented with Lincomycin @ 0.05% w/w and Test Group (T3): Basal diet and CSE @ 0.04% w/v in drinking water. Basal diet of standard composition and clean wholesome water were provided *ad libitum* to birds of all group throughout the study period.

Limit test

Acute oral toxicity study (Limit test) was performed as per OECD guidelines (OECD, 2001) for testing of CSE in Wistar albino rats with upper limit dose of 2000 mg/kg bwt.

Cell mediated immune response

Cell mediated immune response was measured by 2, 4-Dinitro-fluorobenzene (DNFB) skin sensitization test. Featherless area was marked on both sides of abdomen, cleaned thoroughly with acetone and air dried. Right lateral side of abdomen was used for DNFB application whereas left side served as control. 2000 µg of DNFB in 0.1 ml of acetone and olive oil (4:1) was applied on the marked area on the right abdomen using a plastic ring to avoid spillage. The sensitized birds were challenged with 50µg of DNFB in 0.1 ml of acetone and olive oil (4:1) on the same area on day 14th after sensitization. The response to DNFB was assessed by measuring the skin thickness in millimeter using engineer's micrometer on 0, 24 and 48 hours post challenge with three readings each and the overall mean skin thickness was calculated (Tamang *et al*., 1988).

Humoral immune response

Humoral immune response was assessed by micro haemagglutination test against sheep red blood cells. Sheep blood was collected in equal volume of Alsevier's solution and allowed to stabilize for one week. Sheep RBCs obtained after centrifugation was washed thrice in normal saline solution and finally 7% suspension of SRBC was prepared. For immunization, 1ml of SRBC suspension was injected intravenously in six birds aged 30 days from each group and the birds were bled on day 10th following injection. The blood was allowed to clot at room temperature for 2-4 hours and serum was harvested. Serum was heated in a boiling water bath to inactivate the complement fraction of the serum and antibody production in response to the immunization was assessed by micro-haemagglutination test. The reciprocal of the highest dilution of serum that caused complete haemagglutination was recorded as HA titre and expressed as $\log_2$ values (Thaxton *et al*., 1974).

Organ weight factor

Weight of spleen, bursa of fabricius and thymus were recorded at the end of 6th week study from the sacrificed birds of each group to calculate the organ weight factor as per the following formula (Aniagu *et al.,* 2005 with minor modification).

$$\text{Organ weight factor} = \frac{\text{Organ weight (g)}}{\text{Whole body weight (g)}} \times 1000$$

Haematological parameters

At the end of 6th week study, blood was collected from the jugular vein of the birds in sterile heparinized vaccutainer tubes for hematological study (Jain, 1986)

Statistical analysis

Data obtained were expressed as mean ± SE. The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) test. Duncan's Multiple Range Test was applied to determine significant difference ($p \leq 0.05$) if any, among the treatment groups (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Limit test

Neither mortality nor the behavioral abnormality in the form of signs and symptoms of toxicity were recorded in limit test of CSE in rats. Therefore, oral LD_{50} of the CSE in rats was recorded above 2000 mg/kg bwt.

Cell mediated immune response: It was evaluated by measuring the skin thickness of reactive skin lesion after application of 2,4-Dinitro-fluorobenzene (DNFB) at 24 and 48 hrs. The skin thickness in the birds of T3 (3.53±0.20 mm) was highest among all the treatments followed by T2 (3.17±0.22 mm) and T1 (2.32±0.33 mm) at 24 hrs post sensitization as shown in table 1. Lincomycin, apart from its antibacterial action, is also capable of stimulating cell mediated immunological functions indispensable to the organism's defense against invasive action by micro organisms (Fraschini *et al.,* 1987). Therefore birds of standard group showed significant increase in CMI as compared to control group. The skin thickness of CSE treated T3 birds (3.41±0.20 mm) was significantly ($p \leq 0.05$) greater than lincomycin treated T2 birds (2.92±0.17 mm) followed by control T1 birds (2.20± 0.23 mm) at 48 hrs post sensitization. The results indicated that the highest CMI response in birds of CSE treated group compared to other groups. Significant enhancement in skin thickness in DNCB sensitized albino rats was reported due to *C. fistula* (Laxmi *et al*., 2015).

Several studies have demonstrated mechanisms of increased skin thickness due to application of DNFB in broiler birds. Firstly, Saponins present in *Cassia tora* are implicated in the modulation of immune system by serving as adjuvant (saponins-cholesterol-phospholipid complexes) at low concentrations that stimulate cell mediated immune system by inducing the production of interleukins, especially by antigen-presenting mast cells (Zahid *et al*., 2007). Secondly, significant activation of macrophages, infiltration of polymorph nuclear cells, increased vascular permeability and oedema probably induces T-cell mediated response in *Cajanus* treatment in broiler birds, herb of same family (Datta *et al*., 1999). These reports corroborates with the observation that, contact hypersensitivity in mice was driven mainly by CD8+T cells whereas CD4+T cells were thought to mainly regulate the response via regulatory T-cells. Still, neutrophils were reported as abundant in the inflamed

Table 1: Effect of supplementation of CSE on cell mediated immune response (skin thickness on DNFB swabbing) in broiler birds (n=6)

Duration of DNFB application	Abdominal side	Skin thickness (mm) of birds of group			Level of Significance
		Control (T1)	Standard (T2)	Test (T3)	
Before sensitization	Left	0.57±0.02	0.57±0.02	0.55±0.02	NS
	Right	0.56±0.02	0.55±0.02	0.57±0.02	NS
24 hrs after sensitization	Left	0.57±0.03	0.57±0.02	0.56±0.02	NS
	Right	2.32 ± 0.33^{c}	3.17 ± 0.22^{b}	3.53 ± 0.20^{ab}	S
48 hrs after sensitization	Left	0.57±0.02	0.56±0.03	0.56±0.02	NS
	Right	2.20 ± 0.23^{c}	2.92 ± 0.17^{b}	3.41 ± 0.20^{a}	S

S= Significant, NS=Non significant difference ($p \leq 0.05$)

Mean±SE values having different superscript in row differs significantly ($p \leq 0.05$)

tissue and constitute the majority of the infiltrating cells supporting their important role as effector cells in the tissue (Christensen *et al*., 2014).

Humoral immune response

It was judged by estimating haemagglutination (HA) titers of birds against sheep RBC at the 6th week. The HA titers were significantly (p≤0.05) higher in *Cassia* treated broiler birds (4.33±0.21) as compared to birds of lincomycin treated T2 (3.5±0.22) and control group T1 (2.17 ± 0.17) as showed in table 2. Similarly, studies have demonstrated cellular and humoral immune system modulation activities of *C. tora* (Tiwari *et al*., 2011) and *C. fistula* (Laxmi *et al*., 2015) in rats.

Several days after antigen exposure, antigen-specific antibodies, predominantly of the IgM isotype, are generated and released into the general circulation by B-cells and plasma cells (terminally differentiated B-cells). This results into increased HA titre of immunized bird. Further, the presence of quercetin dihydrate in extracts of *Cassia* might be responsible for elevated humoral immune response as quercetin has been reported to up regulate IFN-γ & Th-2 gene expression and production, which in turn modulate NK cell function and act as immunostimulant (Nair *et al*., 2002). Similarly, Bhattacharya *et al*. (2016) reported that total immunoglobulins and mercaptoethanol sensitive (IgM) antibody titer (log 2) values in response to sheep red blood cells (SRBC) was significantly higher (p<0.05) in the birds fed Azolla diet compared to the other dietary groups at 6 weeks of age. Therefore, replacement of 5.5% of basal diet with *Azolla pinnata* meal on dry matter basis may elicit higher immunity in commercial broilers.

Organ weight factor

As shown in table 3, the organ weight factor of spleen of CSE treated group (1.45 ± 0.10) decreased non-significantly (p≤0.05) when compared to standard (1.52 ± 0.16) and control group (1.54±0.11). Bursa of fabricius varied non-significantly (p≤0.05) among the birds of control, standard and test groups (T1, T2 and T3). The organ weight factor for thymus gland was recorded higher non-significantly (p≤0.05) in broiler birds of *Cassia* treated group (1.69 ± 0.13) than standard (1.59 ± 0.17) and control group (1.48 ± 0.14). The difference in weight of thymus, bursa and spleen obtained were in corroboration with the findings of Soltan *et al.* (2008). The weights of thymus, bursa of fabricius and spleen can be used to assess the relative immune status in poultry (Rivas and Fabricants, 1988).

Haematological parameters

The effect of supplementing CSE on haematological parameters of broiler birds has been depicted in table

Table 2: Effect of supplementation of CSE on humoral immune response against SRBC in broiler birds (n=6)

Particular	Birds of group			Level of Significance
	Control (T1)	Standard (T2)	Test (T3)	
HA titre Log_2 value	2.17±0.17^{c}	3.5±0.22^{b}	4.33±0.21^{a}	S

S= Significant difference (p≤0.05)

Means having different superscript in row differs significantly (p≤ 0.05)

Table 3: Effect of supplementing CSE on organ weight factor in broiler birds (n = 6)

Organ weight factor	Birds of group			Level of Significance
	Control (T1)	Standard (T2)	Test (T3)	
Spleen	1.54±0.11	1.52 ± 0.16	1.45 ± 0.10	NS
Bursa of fabricius	0.63± 0.04	0.74 ± 0.06	0.78 ± 0.08	NS
Thymus	1.48 ± 0.14	1.59 ± 0.17	1.69 ± 0.13	NS

NS-Non significant difference (p≤0.05)

Table 4: Effect of supplementation of CSE on hematological parameters in broiler birds (n=6)

Haematological parameter (unit)	Birds of group			Level of Significance
	Control (T1)	Standard (T2)	Test (T3)	
Hb (g/dl)	9.20± 0.32^{c}	9.80 ± 0.36^{bc}	10.57 ± 0.23^{ab}	S
PCV (%)	29.67 ± 0.95	30.17 ± 0.87	31.50 ± 0.67	NS
TEC (million/mm^3)	2.83 ± 0.12^{b}	2.96± 0.10^{b}	3.31 ± 0.11^{a}	S
TLC (thousand/ mm^3)	26.17 ± 0.98^{b}	28.00 ± 1.52^{b}	32.83 ± 0.83^{a}	S
Heterophil (%)	24.66 ± 0.61	25.50 ± 0.22	26.00 ± 0.45	NS
Lymphocyte (%)	72 ± 0.80	73.00± 0.37	72.67± 0.33	NS
Monocyte (%)	1.83± 0.40	0.83 ± 0.40	0.83 ± 0.40	NS
Eosinophil (%)	1.00 ± 0.63	0.50 ± 0.22	0.33 ± 0.21	NS
Basophil (%)	0.33 ± 0.21	0.17 ± 0.17	0.17 ± 0.17	NS

S= Significant, NS=Non significant difference ($p \leq 0.05$)

Means having different superscript in row differs significantly ($p \leq 0.05$)

4. Haemoglobin content of broiler birds of CSE treated group (10.57 ± 0.23 g/dl) was recorded significantly ($p \leq 0.05$) higher than control group (9.20± 0.32 g/dl). Similarly, birds of test group (3.31 ± 0.11 million/mm^3) showed greater TEC than control group (2.83 ± 0.12 million/mm^3). PCV findings showed non-significant changes among the treatment groups. The mean TLC in blood samples of birds of *Cassia* extract treated group (32.83 ± 0.83 thousand/ mm^3) was significantly 25.45% higher ($p \leq 0.05$) than birds of control group (26.17 ± 0.98 thousand/ mm^3). Heterophil count of birds of test group (26.00 ± 0.45%) was non-significantly higher than control group (24.66 ± 0.61%). Similarly, Sahu *et al*. (2015) also reported significant increase in TLC, heterophil and eosinophil count in taurine and methionine supplemented treatments in broiler chicken.

Similarly, increased total WBC and neutrophil count in *Tridex procumbens* treated rats was reported in a study (Oladunmoye, 2006). T-lymphocytes and other cells of immune system are known to activate production of antibodies. Increased heterophil count (polymorph nuclear cells in the birds) was observed in response to delayed type hypersensitivity reaction mediated by T cells. Neutrophils play an important role in many autoimmune diseases and that they interact with the adaptive immune response in several ways (Christensen *et al*., 2014).

Flavonoids content in herb were reported to decrease the immobilization and adhesion of leukocytes to endothelial wall and degranulation of neutrophils without affecting superoxide production, thereby regulating inflammatory responses in tissue injury and immune responses (Ferrandiz *et al*., 1996). CSE treated birds showed eosinopenia (0.33 ± 0.21%) when compared to untreated birds (1.00 ± 0.63%). Similarly, Gokhale and Saraf (2000) also reported significant reduction in eosinophil count on administering ethanolic extract of *Tephrosia purpurea* to rats.

Thus, this study indicated that broiler birds supplemented with methanol extract of *Cassia tora* significantly stimulated both cellular and humoral immune responses in conjunction with improvement in blood parameters. Further, its use as an alternative to antibiotic growth promoter may be advocated in poultry ration based on specific studies on mechanism of action of phytochemicals.

ACKNOWLEDGEMENTS

This research work is a part of ICAR funded project "Outreach program on ethnoveterinary medicine" and the financial assistance received from ICAR, New Delhi is duly acknowledged.

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