Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Evaluation of Some Sugarcane (*Saccharum officinarum* L.) Extracts for Immunostimulatory and Growth Promoting Effects in Industrial Broiler Chickens

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ARTICLE HISTORY

Received: November 05, 2011
Revised: January 16, 2012
Accepted: February 29, 2012

Key words:
Chickens
Growth promoter
Immunostimulation
Saccharum officinarum

ABSTRACT

Present paper describes the immunostimulatory and growth promoting effects of some sugar cane extracts (SCEs) in broiler chickens. Aqueous extract (AE) from sugar cane (*Saccharum officinarum*) juice and ethanolic extract (EE) from bagasse were used to demonstrate their effects on lymphoproliferative responses to Phytohemagglutinin-P (PHA-P) and Concanavalin-A (Con-A); antibody response to sheep red blood cells (SRBCs); growth rate and feed conversion ratio (FCR) in experimental chickens as compared to control. Results showed significantly higher ($P<0.05$) *in vitro* and *in vivo* lymphoproliferative responses to Con-A and PHA-P, respectively in chickens administered with SCEs as compared to those in control group. Further, significantly higher ($P<0.05$) lymphoproliferative responses were detected in chickens administered with EE as compared to chickens administered with AE. Anti-SRBC total Igs, IgG and IgM titers were significantly higher ($P<0.05$) in chickens of experimental groups administered with SCEs as compared to those of control group; whereas titers were comparable among the experimental groups. The organ-body weight ratios of lymphoid organs were statistically similar in experimental and control groups. Both the experimental groups administered with SCEs showed better FCR and significantly higher ($P<0.05$) weight gains as compared to control. In conclusion, oral administration of SCEs showed immunostimulatory effects in broiler chickens and resulted in improved feed utilization and decreased amount of food needed for unit gain in body weight.

To Cite This Article: Awais MM and M Akhtar, 2012. Evaluation of some sugarcane (*Saccharum officinarum* L.) extracts for immunostimulatory and growth promoting effects in industrial broiler chickens. Pak Vet J, 32(3): 398-402.

INTRODUCTION

Immune system is the main regulatory system controlling homeostasis of the body and has a key role in the smooth progression of life from birth to death. Incompetency of the immune system paves way to infectious, malignant, auto-immune and inflammatory disorders (Silin *et al*., 2009). One of the practical ways to protect the immune balance is by the use of pharmaceutical immunostimulators. Although vaccines and antibiotics have contributed a lot in controlling many infectious diseases but frequent consumption of various chemicals and antibiotics has resulted in some problems such as development of antibiotic resistant strains and environmental pollution (Farzana *et al*., 2009).

In such circumstances, it becomes imperative to introduce a novel production system for economically important food animals based on the consideration of safe food and less polluted environment (El-Abasy *et al*., 2002). For this purpose, a variety of immunostimulators has been developed and is being commonly used for the control of infectious diseases. Although certain synthetic immunomodulators like vitamin E, levamisole and selenium are routinely used in poultry but there are several disadvantages related to their use (Szeleszczuk *et al*., 2003). In the current scenario, native bioactive substances, which have immunopotentiating effects in animals, considered to be promising candidates (Patwardhan and Gautum, 2005).

There are number of native biological response modifiers from botanical origin such as *Aloe vera*, *Astragalus membranaceous*, *Azadirachta indica*, *Allium sativum* and *Andrographis peniculata* (Kumar *et al*., 2011; Akhtar *et al*., 2012) which have immunostimulatory effects in human beings and animals making them less susceptible to certain infectious insults by boosting their intrinsic potential to perform better immunogenically. In this regard, sugar cane extract (SCE) has been shown to

have biological and immunological properties including adjuvant effects on the immune responses (El-Abasy *et al*., 2002, 2003a), protective effects against *Eimeria* infection (Awais *et al*., 2011), endotoxic shock in mice (Motobu *et al*., 2006), radioprotective effects (Amer *et al*., 2005), reconstituting effects on the B-cells in cyclophosphamide induced immunosuppression in chickens (El-Abasy *et al*., 2004) and immunopotentiating effects in chickens (Akhtar *et al*., 2008).

However, it remained to be defined which major component(s) of sugar cane were involved in the development of such immunological activities. Present paper reports the immunostimulatory and growth promoting effects of aqueous and ethanolic extracts of sugar cane juice and bagasse, respectively in industrial broiler chickens.

MATERIALS AND METHODS

Preparation of aqueous and ethanolic extracts: Stalks of fresh sugar cane (*Saccharum officinarum*) plants were purchased from the local market of Faisalabad, Pakistan and their authenticity was confirmed by the concerned botanist of University of agriculture, Faisalabad (UAF), Pakistan. The plant specimen was kept in the Ethno-veterinary Research and Development Centre, Department of Parasitology, UAF as voucher No. 0171.

Aqueous extract (AE) from sugar cane juice and ethanolic extract (EE) from sugar cane bagasse were prepared by following the methodology (Awais *et al*., 2011). The final freeze dried extracts (both AE and EE) were subjected to proximate analysis (AOAC, 1990) and results are shown in Table 1. The final concentration (100 mg/ml) for each extract was constituted in 0.1 M phosphate buffered saline (PBS; pH 7.2).

Table 1: Composition of aqueous and ethanolic extracts based on proximate analysis

Constituent (%)	Aqueous extract	Ethanolic extract
Crude Protein	2.5	3.5
Crude Fat	0.4	49.2
Ash	16.1	27.1
Nitrogen free extract	81.0	20.3

Experimental design: Day old broiler chicks (Hubbard) (n=150) were purchased from local hatchery and reared under standard management conditions at Animal House, Institute of Microbiology, UAF, Pakistan. All the chickens were provided feed and water *ad libitum*; and inoculated with the routine vaccination. After the 5 days period of acclimatization, chickens were randomly divided into three groups namely A, B and C, each comprising of 50 chickens and administered orally with sugar cane extracts (SCEs) for three consecutive days (5^{th}, 6^{th} and 7^{th} days of age) with the help of an oral gavage as per schedule.

Group A: AE (4mL/kg of body weight/day)
Group B: EE (4 mL/kg of body weight/day)
Group C: PBS (4mL/kg of body weight/day) and served as control.

Immunological evaluation: On day 14^{th} post-administration of SCEs, chickens from all the groups were evaluated for their immune profile. Lymphoproliferative response to Phytohemagglutinin-P (PHA-P) and concanavalin A (Con-A) and antibody response to sheep red blood cells (SRBCs) were used to detect the cell-mediated and humoral immune responses, respectively.

Classical toe-web assay was used to assess the *in vivo* lymphoblastogenic response as described by Corrier (1990). Briefly, on day 14^{th} post-administration of SCEs, experimental and control chickens were injected PHA-P (Sigma®, USA) (100µg/100µl/ chicken) intradermally between the third and fourth digits of the right foot. The left foot injected with PBS (100µl) served as control. The thickness of the interdigital skin was measured with a pressure sensitive micrometer screw gauge at 24, 48 and 72 hours post injection. Lymphoproliferative response to PHA-P was calculated by the formula:

Lymphoproliferative response = (PHA-P response, right foot) – (PBS response, left foot)

Peripheral blood lymphocytes blastogenesis assay was used to evaluate the *in vitro* lymphoproliferative response in both treated and non treated chickens according to the method described by Qureshi *et al*. (2000). On day 7^{th} and 14^{th} post administration of SCEs, five birds from each group were randomly assigned for evaluation of *in vitro* lymphoblastogenic response to Con-A. The optical density (OD) was read on a plate reader (BioTek-MQX200, USA) at 540 nm wavelength. The mean OD values from each group were used to calculate the stimulation indices by using the formula:

$$\text{Stimulation Index} = \frac{\text{Con A stimulated} - \text{unstimulated}}{\text{Unstimulated}}$$

SRBCs were used as non-pathogenic T-dependent antigen to demonstrate the antibody response and antibody titers were detected by using microplate haemagglutination test according to the method described by Yamamoto and Glick (1982) with minor modifications (Qureshi and Havenstein, 1994). On day 14^{th} post administration of SCEs, chickens were injected SRBCs (5%) via intramuscular route (1 ml/chicken) followed by a booster at day 14^{th} post primary injection. Blood was collected at day 7^{th} and 14^{th} post primary and secondary injections to separate the serum. All the samples were analyzed for total Ig, IgM (mercaptoethanol-sensitive) and IgG (mercaptoethanol-resistant) anti-SRBCs antibodies. The titer of the well containing 50% agglutination and 50% reticulum settling (clumping) was considered as the total anti-SRBC antibody titer of the test sera. To detect IgG titer, 0.01M mercaptoethanol (50 µl) in PBS was added instead of using PBS alone, followed by the pervious mentioned procedure. IgM titers were calculated by subtracting the IgG titers from total antibody titers of the respective samples.

Weekly weight gain, feed conversion ratio and relative weight of lymphoid organs: Chickens from all the groups were weighed individually and feed consumption by each group was recorded on weekly basis post administration of SCEs. Lymphoid organs including bursa of Fabricius, thymus, spleen and cecal tonsils were removed and weighed on day 35^{th} post administration of SCEs.

Statistical Analysis: Data thus obtained were analyzed using ANOVA and LSD for the determination of statistical significance between experimental and control groups. Value of P<0.05 was considered to be statistical significant for weekly weight gain, lymphoproliferative responses and antibody titers; whereas, for relative organ weight ratio of lymphoid organs value of P was considered to be <0.01.

RESULTS

Immunological evaluation: Cell mediated immunity in terms of lymphoproliferative response was assessed by measuring amplitude of toe-web swelling in chickens of experimental and control groups post PHA-P injection. Results revealed significantly higher (P<0.05) *in vivo* lymphoproliferative responses in chickens administered with SCEs (either AE or EE) as compared to chickens of control group. Moreover, there were significantly higher (P<0.05) lymphoproliferative responses in chickens administered with EE as compared to those administered with AE (Fig. 1). These results indicated the highest cellular immune response against PHA-P injection in chickens administered with EE followed by those administered with AE as compared to chickens of control group.

In vitro lymphoproliferative response of PBLs against Con-A is shown in Figure 2. When PBLs were incubated with Con-A, a significantly higher (P<0.05) lymphoproliferative response was observed on both day 7th and 14th post administration of SCEs in treated chickens when compared with the PBLs obtained from the control chickens (P=0.0021). However, among the treatment groups, chickens administered with EE showed significantly higher (P<0.05) response as compared to those administered with AE. Further, results obtained from *in vitro* studies were consistent to those obtained from *in vivo* studies for the demonstration of lymphoproliferative response.

Results of microplate haemagglutination test revealed that oral administration of any of the SCEs resulted in significantly higher (P<0.05) total Igs, IgG and IgM titers against SRBCs at day 7th and 14th post primary injection of SRBCs as compared to control group. However, the difference among the experimental groups was statistically similar. On day 7th and 14th post secondary injection, chickens of experimental groups showed again significantly higher (P<0.05) total Ig, IgG and IgM antibody titers, when compared with chickens of control group. However, among the experimental groups, chickens administered with EE showed significantly higher (P<0.05) titers for total Ig, IgG and IgM anti-SRBC antibodies as compared to chickens administered with AE (Table 2).

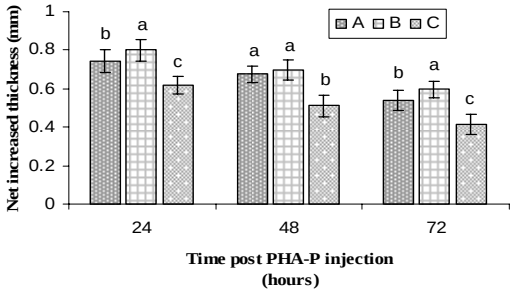

Fig. 1: Lymphoproliferative response to PHA-P in experimental and control chickens. Bars sharing similar letters on a particular time interval are statistically non-significant (P>0.05). A = Aqueous extract of sugar cane juice; B= Ethanolic extract of sugar cane bagasse; C= Control.

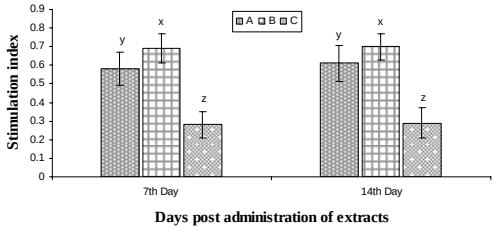

Fig 2: Lymphoblastogenic response of peripheral blood leukocytes against Concanavalin A on day 7th and 14th post administration of sugar cane extracts in experimental and control chickens. Bars sharing similar letters on a particular time interval are statistically non-significant (P>0.05). A=Aqueous extract of sugar cane juice; B=Ethanolic extract of sugar cane bagasse; C=Control.

Effect on the development of lymphoid organs, weekly weight gains and FCR: The results revealed apparently higher per cent organ-body weight ratios of all the lymphoid organs in both the experimental groups as compared to control group; but the difference was statistically non-significant (data not shown).

Live body weight gains in both the experimental groups (A & B) were significantly higher (P<0.05) as compared to control group. Moreover, among the

Table 2: Antibody response to sheep red blood cells (SRBCs) in experimental and control chickens

Group	Day 7 PPI	Day 14 PPI	Day 7 PSI	Day 14 PSI
Total anti-SRBCs antibody titer				
A	45.3[a]	36.8[a]	48.5[b]	45.3[b]
B	52.0[a]	42.2[a]	55.7[a]	59.7[a]
C	27.9[b]	22.6[b]	42.2[c]	29.9[c]
Immunoglobulin-M				
A	26.9[b]	20.8[a]	25.9[a]	11.0[b]
B	32.3[a]	26.2[a]	25.8[a]	14.4[a]
C	16.6[c]	12.8[b]	22.5[b]	7.3[c]
Immunoglobulin-G				
A	18.4[a]	16.0[a]	22.6[b]	34.3[b]
B	19.7[a]	16.0[a]	29.9[a]	45.3[a]
C	11.3[b]	9.8[b]	19.7[c]	22.6[c]

Means sharing similar letters in a column are statistically non-significant (P>0.05); A = Aqueous extract of sugar cane juice; B= Ethanolic extract of sugar cane bagasse; C= Control; PPI= Post-primary injection; PSI= Post-secondary injection

Table 3: Weekly weight gains and feed conversion ratios post administration of sugar cane extracts in experimental and control chickens

	Week 1	Week 2	Week 3	Week 4	Week 5
Weekly weight gains (Mean±SE)					
A	422±25.3[a]	676±32.3[a]	1028±32.8[a]	1456±38.4[a]	1886±42.7[a]
B	409±24.8[b]	673±30.8[a]	1024±31.4[a]	1452±39.5[a]	1878±43.0[a]
C	370±28.2[c]	612±32.1[b]	918±35.2[b]	1329±42.0[b]	1687±51.9[b]
Weekly feed conversion ratios					
A	1.98	2.04	2.03	2.06	2.09
B	2.00	2.04	2.04	2.07	2.10
C	2.19	2.25	2.19	2.16	2.20

For weekly weight gains, means sharing similar letters in a column are statistically non-significant (P>0.05); A=Aqueous extract of sugar cane juice; B= Ethanolic extract of sugar cane bagasse; C= Control

experimental groups the live body weights were higher in group A as compared to group B; but the difference was statistically non-significant (Fig. 3).

At week 5th post administration of SCEs (42nd day of age), group A administered with AE was observed with the highest feeding efficiency having the best FCR value 2.09 (as feeding efficiency is inversely proportional to FCR) followed by group B (2.10) administered with EE and control group (2.20) (Fig. 4).

DISCUSSION

A wide range of biological effects of sugar cane have been reported such as immunostimulatory, anti-thrombosis, anti-inflammatory, vaccine adjuvant, anti-oxidant, modulation of acetylcholine release and anti-stress activities (Takara *et al*., 2002; El-Abasy *et al*., 2003a; Ledon *et al*., 2007; Akhtar *et al*., 2008). Such findings demand for further investigations on sugar cane to identify the component(s) responsible for such activities. Moreover, prophylactic activities of sugar cane (extracts/components) against infectious diseases may be exploited to minimize the use of antibiotics and/or anthelmintics in poultry birds.

Both AE and EE induced up-regulation of humoral and cellular immune responses that illuminated the potential benefits of these SCEs to poultry industry. *In vivo* lymphoproliferation studies in the current experiment demonstrated that chickens administered with any of the SCEs showed higher cellular immune responses as compared to chickens of control group which might be due to stimulatory effects of SCEs on the phagocytic activity of macrophages that led to an increase in the toe web thickness in response to T-cell mitogens (Akhtar *et al*., 2008). Toe web swelling, an indicator of lymphoproliferation/enhanced cell mediated immune (CMI) response might be due to enhanced delayed type hypersensitivity (DTH) which suggested that the magnitude of immune response depends on the function and number of lymphocytes. From the results, it can also be assumed that increase in the function and number of lymphocytes in the lymphoid tissue might be responsible for the development of the improved immune responses (El-Abasy *et al*., 2003a).

Higher i*n vitro* lymphoproliferation in Ae and EE administered chickens canbe correlated with the fact that the mitogen receptors on T- lymphocytes present in the PBLs come in direct contact with the T-cell mitogen (Con-A) and the lymphocytes go through cell division (Qureshi *et al*., 2000). Con-A stimulated the PBLs which produced interleukine-1 by monocytes in PBL fraction, which stimulated the proliferation of lymphocytes (Abbas *et al*., 1991). These results of *in vitro* study were consistent with the findings obtained in the PHA-P experiment.

Significantly higher anti-SRBC antibody titers in experimental groups indicated the higher humoral response suggesting that both AE and EE had immunological properties to enhance the antibody production. This enhanced humoral response might be due to sugar cane factor (Pryce *et al*., 1990). Li *et al*. (1983) showed that sugar cane polysaccharides had immunostimulating effects to activate the classical complement pathway in human serum by interacting with antibodies. Previous studies also revealed that oral administration of SCE resulted in higher antibody response to sheep red blood cells in chickens initially infected with oocysts of *Eimeria tenella* (El-Abasy *et al*., 2003b); in radiation induced immunosuppressed chickens (Amer *et al*., 2004); increased serum antibody responses and number of antibody-producing cells in splenocytes, peripheral blood and intestinal leukocytes of chickens administered with SCE and polyphenol rich fraction of SCE (Hikosaka *et al*., 2007); and stimulatory effects of sugar cane juice on antibody production (Akhtar *et al*., 2008). From the current study, it can be speculated that stimulation of lymphocytes by any of the SCEs facilitated the immune responses.

Non-significant effect of SCEs on the development of lymphoid organs was observed in chickens of experimental groups as compared to control group. Similar findings were reported by Amer *et al*. (2004); whereas, El-Abasy *et al*. (2004) reported the higher relative lymphoid weight of the spleen and bursa in chickens when administered with SCE after cyclophosphamide-induced immunosuppression as compared to negative control.

The growth promoting effects of SCEs were evaluated on weekly basis which showed that weight gains in both the experimental groups were significantly higher ($P<0.05$) as compared to control group. Similarly, FCR were almost similar in both the experimental groups of chickens but better than the chickens of control group; indicating better feed utilization in experimental groups given any of the SCEs and a decrease in the amount of food needed for unit gain in body weight. Studies of El-Abasy *et al*. (2004) also revealed similar findings that oral administration of SCE to cyclophosphamide induced immunosuppressed chickens resulted in a higher body weight gain and feeding efficiency than those of immunosuppressed control chickens, not treated with SCE.

In conclusion, these results suggest that aqueous and ethanolic extracts of sugar cane juice and bagasse, respectively have biological properties of stimulating the both cellular and humoral arms of immunity as well as growth rates in industrial broiler chickens. Further, supplementation of SCEs, especially EE, as an immunostimulant in feed may be used to prevent and/or minimize the infections occurring in poultry industry.

Acknowledgement: The funds for this project were sponsored by Pakistan Science Foundation, Islamabad, Pakistan [Project No. P-AU/Bio (412)].

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