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Research Article

Immunomodulatory and Protective Effects of Sugar Cane Juice in Chickens against *Eimeria* Infection

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Abstract: Present paper reports the effects of sugar cane juice (SCJ) on immune responses and its protection against *Eimeria* (mixed species) infections in broiler chickens. Experiment 1 was conducted to test the dose efficacy of SCJ and its effects on chickens against coccidiosis. Maximum weight gain per day was recorded in chickens given SCJ @ 400 mg/kg body weight. Significantly ($P < 0.01$) low mortality and increased body weight gain per day were recorded in chickens administered SCJ orally and challenged with mixed species of genus Eimeria. Furthermore, mild hemorrhages, lower lesion scores (+1), and significantly lower number of oocysts shed in faeces were recorded in immunized chickens compared to control. Experiment 2 was carried out to evaluate the immunomodulatory effects of SCJ on cellular and humoral responses. Highest antibody titres (1:1024-1:16; GMT 367.28) were recorded in chickens administered SCJ @ 400 mg/kg body weight in comparison with control (1:16-1:2; GMT 2.82). To demonstrate the cell mediated immunity (CMI), amplitude of toe-web swelling 72 h post avian tuberculin injection was recorded in the experimental and control groups. Maximum swelling (8.13 ± 0.21 mm) was recorded in the experimental group as compared to control (1.04 ± 0.10); indicating higher cell mediated immune response. From these results it was concluded that SCJ elicit a significantly higher cellular and humoral responses compared to control, which may provide protection against Eimeria infections (mixed species) in chickens.

Key Words: Sugar cane juice, immunomodulation, protection, chicken, coccidiosis

Introduction

Coccidiosis, an important disease of poultry, causes huge economic losses because of mortality and morbidity throughout the world. The disease has immunosuppressive effect; cases of Newcastle disease, infectious bursal disease and hydropericardium syndrome have been reported during disease outbreaks in the vaccinated flocks (Personal communication). In the United States, $1.5 million annual losses due to coccidiosis have been reported (1). Chemoprophylaxis and anticoccidial feed additives are routinely used to control the disease but that has resulted in the emergence of drug resistance (2-5). New anticoccidial drugs have been developed to avoid the drug resistance and administered on a rotational basis with existing drugs. However, this has resulted in the increased cost of poultry rearing and they are potentially dangerous to the human health due to their residual effects. Although some commercial vaccines are used in certain countries, they are not used worldwide due to disease occurrence in spite of vaccination.

Different types of immunomodulators have been used and evaluated on the basis of their protective and therapeutic effects on infectious and non-infectious diseases. Sugar cane extract has been reported to enhance the immune responses, immune function, and growth in chickens (6). The objective of the present study was to investigate the immunomodulatory and protective effects of sugar cane juice (SCJ) on chickens against *Eimeria* (mixed species) infections.

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Materials and Methods

Infective material

Mixed species of genus *Eimeria* (local isolates; mainly *E. tenella*, *E. acervulina*, and *E. necatrix*) maintained at the Immunoparasitology Laboratory, Department of Veterinary Parasitology, University of Agriculture, Faisalabad-Pakistan were used in the present study.

Sugarcane extracts

Sugarcane juice (contains water 70%, molasses 3%, filter mud 2%, sugar 10%, and bagasse 15%) produced from sugar cane (*Saccharum officinarum*) obtained from local cane crusher was subjected to filtration (0.25 µm). The amount of glucose in SCJ was measured to calculate the concentration of SCJ and adjusted at 100 mg/mL in phosphate buffered saline (PBS; pH 7.2).

Experimental chicks

Three hundred broiler chicks (Hubbard) reared at the Experimental Station, Department of Veterinary Parasitology, University of Agriculture, Faisalabad-Pakistan were used in the present study. The chickens were fed commercial feed (Table 1) and water ad libitum.

Experiment 1

This experiment was conducted to test the dose efficacy of SCJ in chickens against coccidiosis. For this purpose, at 12 days of age, 200 chickens (average weight 260 g per chick) were divided in to 4 equal groups and were inoculated with SCJ with a pipette into the crop of the chickens at the following dosages:

Table 1. Chemical composition of the commercial feed.

Chemical Analysis	g/kg
Protein	200.0
Fat	45.0
Carbohydrate	420.0
Fiber	50.0
Lysine	12.0
Ash	55.0
Calcium	10.0
Phosphorus	5.0
Sodium	1.5
Methionine + Cystine	7 .0
Methionine	4.0

Note: ME = 11.995 MJ/kg

Group A : 300 mg/kg body weight/day for 3 consecutive days
Group B : 400 mg/kg body weight/day for 3 consecutive days
Group C : 500 mg/kg body weight/day for 3 consecutive days
Group D : PBS for 3 consecutive days (served as control)

On day 7 post administration of SCJ, the chicks in all groups were challenged with mixed species of genus *Eimeria* (60,000-70,000 sporulated oocysts/chick). Mortality was recorded and body weight gain per day (from day 1 to 7) was calculated. Droppings were examined (25 random samples from each group) from day 3 to 12 post challenge for determination of oocysts per gram of droppings (7). On day 12 post challenge, survived chickens in all the groups were slaughtered and secured for gross caecal and intestinal lesions, classified on a scale of 0-4 (8) to evaluate the effects of primary infection as well as re-infection in a contaminated environment. A sore of "0" indicated no lesions, "1" stood for a few scattered lesions, "2" for marked lesions with bleeding, "3" exhibited extensively developed lesions with thickening of caecal and intestinal mucosa and ballooning, and "4" was assigned for bloody intestinal contents and large caecal scores.

Experiment 2

This experiment was carried out to evaluate the immunomodulatory effect of SCJ on cellular and humoral responses. For this purpose, 100 chickens (average weight 260 g per chick) at 12 days of age were divided into 3 equal groups (33 chicks in each), namely A, B, and C.

Chickens in group A (given SCJ 400 mg per kg body weight for 3 consecutive days) and group B (given PBS for 3 consecutive days) were injected with 3% saline suspension of sheep red blood cells (SRBC) via intravenous route. Blood samples were collected at day 15 post SRBC inoculation to monitor the level of serum antibodies by indirect haemagglutination test (9) and results were expressed in terms of geomean titre (GMT) (10).

Chickens in group C were given a single injection of 0.2 ml avian tuberculin (Tubercolina PPd Aviaria, Italy), in the left toe-web, while right toe-web was inoculated with PBS as control. The toe-web swelling was measured at 72 h post injection (11).

Data analysis

Data on oocyst count was analyzed using the general linear model (GLM) procedure in Analysis of Variance and means were compared using Dunnett's test to compare the treatments with the control group. Data on mortality, lesion scores, and body weight were analyzed using the Chi square test (12).

Results

Experiment 1 was conducted to test the dose efficacy of SCJ and its effects on chickens against coccidiosis in comparison with control. Chickens in the control group after challenge showed gross lesions including ballooning of intestine, caeca filled with blood, and severe hemorrhages on the intestine (+4) and caeca (+3). Anorexia and depression were obvious signs in control chickens. Survived chickens showed retarded growth with decreased body weight gain per day. In contrast, significantly ($P < 0.01$) low mortality and increased body weight gain per day were recorded in chickens administered SCJ orally and challenged with mixed species of genus *Eimeria* (Table 2, Figure). Mild hemorrhages, lower lesion scores (+1), and significantly lower number of oocysts shed in faeces were recorded in experimental chickens compared to control (Tables 2 and 3).

Table 2. Per cent mortality and lesion score in chickens orally administered SCJ and infected with *Eimeria.*

Group	Mortality (%)	Mean lesion score		Hemorrhagic faeces
		Intestine	Caeca	
A	22	+1	+2	+
B	7	0	+1	+
C	5	0	+1	+
D	80	+4	+3	+++

$X^2 = 91.01$
$P = 0.01$

\+ Transit hemorrhages
+++ Continuous hemorrhages

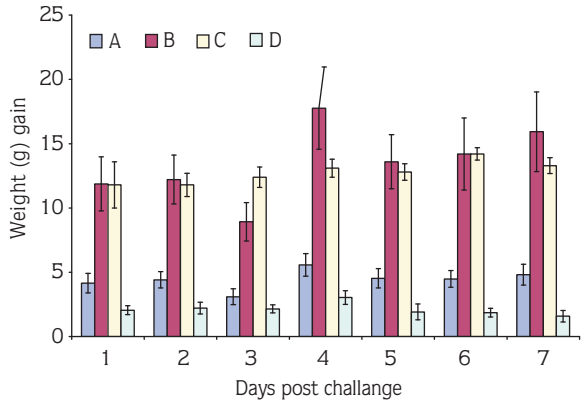

Figure. Per day increase in body weight after challange in SCJ administered chickens.

Maximum weight gain per day was recorded in the chickens of group B given SCJ @ 400 mg/kg body weight (Figure), although there was no significant difference in weight gain between groups B and C.

In the present study (experiment 2), highest antibody titres (1:1024-1:16; GMT 367.28) were recorded in chickens administered SCJ @ 400 mg/kg body weight in comparison with control (1:16-1:2; GMT 2.82). Maximum swelling (8.13 ± 0.21 mm) was recorded in the experimental group after 72 h compared to the control group (1.04 ± 0.10).

Discussion

Sugar cane juice has been found to have a wide range of biological activities including immunostimulation, anti-thrombogenic, anti-inflammatory, vaccine adjuvant, anti-oxidant activity, modulation of acetylcholine release, and anti stress effects (6,13-19). The present paper reports the immunomodulatory effects of SCJ and its protective effects against coccidiosis (mixed species of genus *Eimeria*) in commercial broiler chickens.

In experiment 1, chickens in the control group after challenge showed gross lesions on the caeca and intestine and severe hemorrhages. Anorexia and depression were obvious signs in control chickens. Retarded growth with decreased body weight gain per day was recorded in survived chickens; probably through inflammatory effects that divert energy from growth and affect the weight gain (20).

In experiment 1, significantly ($P < 0.01$) low mortality and increased body weight gain per day were recorded in chickens administered SCJ orally and challenged with mixed species of genus *Eimeria*. Mild hemorrhages, lower lesion scores, and significantly lower number of oocysts shed in faeces were recorded in experimental chickens compared to control. In the control group, survived chickens showed retarded growth with decreased body weight gain per day; probably through inflammatory effects that divert energy from growth and affect the weight gain (20). Maximum weight gain per day was recorded in chickens of group B given SCJ @ 400 mg/kg body weight, although the difference in weight gain between groups B and C was not significant.

Table 3. Effect of oral administration of SCJ on oocyst production in chickens after challenge with *Eimeria.*

Groups → Days↓	A	B	C	D
3	7402 ± 376.17^{B}	1664 ± 318.09^{C}	1813 ± 361.2^{C}	30402 ± 3828^{A}
4	9471 ± 1014.98^{B}	2704 ± 521.80^{C}	2756 ± 596.4^{C}	51130 ± 5357.8^{A}
5	14689 ± 2926.50^{B}	4215 ± 163.20^{C}	4230 ± 163.7^{C}	77310 ± 1638.7^{A}
6	27210 ± 5119.5^{B}	7260 ± 177.32^{C}	7253.6 ± 163.7^{C}	139610 ± 2989^{A}
7	51735 ± 8985.2^{B}	13910 ± 667.4^{C}	13870 ± 727.3^{C}	191020 ± 6460.7^{A}
8	86200 ± 6205.19^{B}	25960 ± 1029.^{7C}	25300 ± 1434.5^{C}	263160 ± 9331.4^{A}
9	142446 ± 27226.9^{B}	45410 ± 1959.2^{C}	44630 ± 1502.6^{C}	446980 ± 43326.3^{A}
10	128420 ± 2546.9^{B}	29680 ± 1010.4^{C}	27854 ± 1232.5^{C}	415680 ± 45672^{A}
11	86640 ± 6040.20^{B}	16750 ± 640.50^{C}	14680 ± 743.2^{C}	328760 ± 93451^{A}
12	55290 ± 7897.40^{B}	8640 ± 354.20^{C}	7654 ± 258.4^{C}	290680 ± 6463.2^{A}

The beneficial effects of SCJ on body weight gain and decreased mortality in chickens upon challenge with mixed species of genus *Eimeria* compared to control chickens are probably due to the protective effects of SCJ on coccidiosis. It can be assumed that natural antibodies (Bo) against a polysaccharide from sugar cane mediate its complement-activating effect (21,22) that inhibits the invasion/development of the coccidian parasites protecting the chickens from infection. In vivo, sugar cane factors were also reported to induce protective responses against certain bacteria including *Pseudomonas aeruginosa* and *Proteus mirabilis* (23).

It is speculated that SCJ may positively regulate host natural immunity against bacterial, viral, and protozoan infections via effects on macrophages, neutrophils, and natural killer (NK) cells (24). In the present study (experiment 2), the highest antibody titres were recorded in chickens administered SCJ @ 400 mg/kg body weight in comparison with control. The significant increase in the antibody response in the experimental group compared to control suggested the stimulatory effect of SCJ on antibody production. It can also be speculated that SCJ may enhance the number of antibody producing cells, suggesting the SCJ-driven enhanced phagocytosis (25). The enhanced effect of SCJ on antibody producing cells may affect local mucosal immune responses corresponding with the onset of specific immunity to *Eimeria* infection. Activation of the classical complement pathway by a polysaccharide from sugar cane extracts and its interaction with immunoglobulins have earlier been reported (22).

To demonstrate the cell mediated immunity (CMI), the amplitude of swelling was recorded in the experimental and control groups at 72 h post tuberculin injection. Maximum swelling in the experimental group compared to the control group indicated higher cell-mediated immune response.

From these results it was concluded that SCJ elicit a significantly higher cellular and humoral responses compared to control, which may provide protection against *Eimeria* infections (mixed species) in chickens.

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