Saccharum officinarum Derived High Molecular Mass Glycoproteins as Native Biological Response Modifiers in Chickens

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ABSTRACT
Present study reports the biological response modifying (BRM) activities of sugar cane (Saccharum officinarum L.) derived high molecular mass glycoproteins (HMMGs) in chickens. HMMGs were recovered from sugar cane juice by size exclusion chromatography. The electrophoretic analysis of HMMGs sample revealed the presence of eight different glycoprotein fractions with molecular weights ranging from 82.81 to 255.56 kDa. These HMMGs were evaluated for their BRM activities in terms of cellular and humoral immune responses and production performance. Results showed that HMMGs significantly enhanced (P<0.05) the in vivo and in vitro lymphoproliferative responses to phytohaemagglutinin-P and Concanavalin-A, respectively in chickens. Significantly elevated B-cell mediated immune response in terms of antibody titres to sheep red blood cells, Newcastle disease and Infectious Bursal disease vaccines (P<0.05) in HMMGs administered chickens were also detected. Production performance was determined in terms of feed conversion ratios that were significantly improved (P<0.05) in chickens administered with HMMGs as compared to those of control group. On the whole, results suggested that HMMGs have immunostimulatory potential and can be used as native BRM agents in chickens to boost up their immunity and production performance.

INTRODUCTION
Immune system constitutes a remarkably sophisticated defense system in vertebrates that virtually participates in all processes of life from birth to death and protects them from various infectious and non-infectious diseases (Silin et al., 2009). In healthy individuals, it is capable to neutralize the invading pathogens successfully. However, infection develops only when the immune system is compromised or the load of pathogenic organisms is extremely high. Resultantly, the overwhelmed immune system leads to local or generalized colonization of infectious agents that interrupt the balanced physiology of the host (Baron, 1996).

There are various exogenous and endogenous factors which impair the activity of immune system leading to immunosuppression and mostly their elimination becomes impossible (Qureshi et al., 1998). These may include heat stress, mycotoxin exposure and certain immunosuppressive diseases (Awais et al., 2013). Under the circumstances, it is imperative to devise new ways and means to protect the immune balance of the animals in general and birds in particular to achieve maximum production potential. In this regard, a number of prophylactic approaches are being used to protect the immune competency including irrational use of chemicals/antibiotics that resulted in the emergence of drug resistant strains, environmental pollution and residual effects in animal products being utilized by the human beings (Sahoo et al., 2010). In such circumstances, biological response modifiers (BRMs) from plant origin with immunostimulatory activities have been proven to be suitable and promising alternatives to conventional chemotherapeutic agents (Yoon et al., 2013). Now a days, natural products are becoming more imperative as efficient sources of pharmaconutrients, either as herbal...
remedies for treatment of chronic ailments or as raw materials from which active ingredients having particular BRM activities can be isolated. Due to the reason, more than 60% of newly approved anti-cancerous and anti-infective drugs are derived from natural sources (Harvey, 2008). In this regard, a number of bioactive molecules including polysaccharides, glycoproteins, peptides, lectins and flavonoids extracted from different botanical sources have been identified for their immunomodulatory activities in different animal models (Cui et al., 2011; Thakur et al., 2011; Svaiger et al., 2011).

Earlier, biological and immunological activities of crude sugar cane extracts have been exhibited in different animal studies. These include anti-thrombotic, anti-inflammatory, anti-oxidant and immunomodulatory activities viz. adjuvant effects on the immune responses (El-Abasy et al., 2002); protective efficacy against avian coccidiosis (Awais et al., 2011), endotoxic shock in mice (Motobu et al., 2006); radiation induced injury (Amer et al., 2005); and reconstituting effects on B-cells in immunosuppressed chickens (El-Abasy et al., 2004). In our previous studies, aqueous and ethanolic extracts from sugar cane juice and bagasse, respectively were evaluated to demonstrate their immunopotentiating and immunotherapeutic activities present in sugar cane, responsible for such biological activities. Keeping in view, the current study was designed to evaluate the BRM activities of sugar cane derived glycoproteins in chickens.

MATERIALS AND METHODS

Procurement and processing of sugarcane: Sugarcane plants used in this study were procured from local market. After botanical authentication, plant specimen was kept in the Ethno-Veterinary Research and Development Centre, University of Agriculture Faisalabad-Pakistan (voucher No. 0171). Sugarcane stalks were crushed immediately after harvesting and juice was centrifuged (5000 g for 15 minutes at 4°C). Supernatant was filtered (0.22 µm membranes) and stored at −20°C till further use.

Isolation and characterization of glycoproteins: High molecular mass glycoproteins (HMMGs) were isolated from sugar cane juice by size exclusion chromatography (Legaz et al., 1998). Briefly, filtered sugar cane juice was adjusted to a pH 8.0 by adding saturated solution of Na2CO3 followed by centrifugation at 20000×g for 15 minutes at 4°C. Clarified juice was chromatographed through a Sephadex G-10 column (Sigma®USA) (15×2.5 cm), pre-equilibrated with saturated Na2CO3. Elutions were carried out with DH2O and analyzed for glycoproteins by taking optical density profile at 280nm on ELISA reader (BioTek-MQX200, USA). Fractions at first two peaks obtained in profile were pooled together and considered as a mixture of glycoproteins. After separation of a sufficient quantity of required fractions, their mixture was chromatographed through Sephadex G-50 column (Sigma®, USA) (30×2.5 cm), pre-equilibrated as described above. Fractions (1 mL) were subjected to ELISA reader in a 96 well microtitration plates (Flow Labs., UK) and their absorbance was taken at 280nm. The fractions in first peak were considered for having HMMGs, lyophilized separately and stored in vacuum dessicators till further use.

The HMMGs were characterized by using Experion™ automated electrophoresis station (BioRad®, USA) for their quantification and determination of molecular weight according to the manufacturer’s instructions.

Experimental design: One-day-old broiler chicks (n=100; Hubbard) were purchased from local hatchery and reared under standard management conditions at Experimental shed, Department of Parasitology, UAF. All the birds were vaccinated according to local vaccination schedule. After 5 days of acclimatization, chicks were randomly divided into 4 equal groups viz. D1 to D4. Groups D1, D2 and D3 were administered HMMGs @ 50, 100 and 150 mg/kg BW on 5th-7th days of age, respectively while Group D4 was kept on phosphate buffered saline.

Evaluation of BRM activities: The BRM activities of HMMGs were investigated in terms of cellular and humoral immune responses. Cell mediated immune (CMI) responses were evaluated by in vivo and in vitro lymphoproliferative responses to Phytohemagglutinin-P (PHA-P) and Concanavalin-A (Con-A), respectively (Corrier, 1990; Qureshi et al., 2000). Humoral immunity was assessed by antibody responses to sheep red blood cells (SRBC), New Castle disease (ND) vaccine and Infectious bursal disease (IBD) vaccine. Antibody response to SRBCs was detected by using the methodology described by Qureshi and Havenstein (1994); whereas, antibody titers against ND vaccine were detected by indirect hemagglutination assay (Alexander, 1988). The titers against IBD vaccine were detected by using ProFLOK® IBD ELISA Kit (Synbiotics Corporation, USA).

Relative weights of lymphoid organs and Feed conversion ratios (FCRs): Chickens from all the groups were individually weighed and killed humanely at day 35th post administration of HMMGs. Lymphoid organs were incised and weighed; and per cent organ-body weight ratios were calculated. Body weights and Feed consumption by each group were recorded on weekly basis to calculate weekly FCRs.

Statistical analysis: Data obtained were analyzed using ANOVA and LSD for the determination of statistical significance between experimental and control groups (P<0.05). Statistical differences for humoral responses against SRBCs and ND vaccine were determined by comparing geometric mean titers.

RESULTS

Automated electrophoretic analysis of HMMGs: The electrophoretic analysis of HMMGs isolated through molecular sieving technique revealed the presence of eight different glycoproteins in the sample with molecular...
weights ranging from 82.81 to 255.56 kDa. The molecular weights of constituent glycoproteins and their concentrations (ng/µL) are described in Table 1.

### Biological response modifying activities

#### Cellular immune responses:

Maximum in vivo lymphoproliferative response (mm±SE) to PHA-P in terms of toe web swelling (0.91±0.025) was recorded in group D2 (HMMGs 100mg/kg of BW) after 24 hours followed by group D1 (0.91±0.031), D1 (0.86±0.031) and control group (0.63±0.040), respectively; whereas, statistical difference was similar (P>0.05) among all the experimental groups administered with HMMGs. The difference in response was statistically higher (P<0.05) in HMMGs administered groups, when compared with control group. A similar response was recorded at 48 and 72 hours post PHA-P injection (Fig. 1a). On both day 7th and 14th post administration of HMMGs, statistically higher (P<0.05) in vitro lymphoproliferative response to Con-A was detected in HMMGs administered chickens irrespective of the dose rate, when compared with those of control group (Fig. 1b).

#### Humoral immune responses:

At day 5th post-primary injection (PPI), highest geometric mean anti-SRBC antibody titer (55.72) was detected in chickens administered with HMMGs (D3; 150 mg/kg of BW) followed by groups D2 (48.50), D1 (27.86) and control (15.50) respectively. Similar results were observed on day 24.25. Moreover, at day 5th and 10th post-secondary injection (PSI), total anti-SRBCs IgG titers again showed same pattern as that on day 5th PPI. Similar trend was detected for anti-SRBCs IgM titers on days 5th and 10th PPI and PSI of SRBCs. At day 5th PPI, the highest IgG anti-SRBC antibody titers were detected in chickens of group D2 and D3 (12.12, each) followed by D1 (6.96) and control (3.03), respectively. At day 10th PPI, chickens of group D2 showed the highest response followed by those of group D3 (32.00), D1 (21.11) and control (16.00) (Table 2).

On the other hand, higher geometric titers against ND vaccine were detected in HMMGs administered chickens as compared to control group. Maximum geometric titer (256.00) was recorded in chickens of group D1 (HMMG @ 150 mg/kg of body weight) followed by D2 (256), D3 (194.01) and control group D4 (168.90). Further, chickens administered with HMMGs (150 mg/kg of BW) showed statistically higher (P<0.05) titers against IBD vaccine as compared to those of group D3 and control group (Figure 2a-b).

#### Relative weight of lymphoid organs and FCRs:

A statistically non-significant difference (P>0.05) was observed for the organ-body weight ratios in both HMMGs administered and control chickens (data not shown). Weekly FCR values were statistically improved/better (P<0.05) in chickens administered with HMMGs (150 mg/kg of BW) as compared to all other groups including control (Fig. 3).

### DISCUSSION

In current study, in vivo cellular immune responses were evaluated by classical toe-web assay and results
Fig. 2: (a-b): Antibody titers against ND and IBD vaccines. a. GMT against ND vaccine. Bars sharing similar letters are statistically non-significant (P>0.05); D1, D2 and D3= High molecular mass glycoproteins @ 50, 100 and 150mg/kg BW, respectively. D4=Control

Fig. 3: Weekly feed conversion ratios (Mean±SE) post-administration of HMMGs. Bars sharing similar letters are statistically non-significant (P>0.05); D1, D2 and D3= High molecular mass glycoproteins @ 50, 100 and 150mg/kg BW, respectively. D4=Control

Results of humoral immune responses demonstrated that HMMGs administration resulted in higher total Igs, IgG and IgM titers against SRBC post primary and secondary injection of SRBC. Higher titers in experimental groups as compared to control indicated the higher humoral response that might be due to sugar cane factor (Prype et al., 1990). Immunostimulatory effects of sugar cane derived polysaccharides on classical complement pathway in human serum by their interaction with antibodies had also been reported previously (Awais et al., 2011). Results of present study are consistent to the previous findings of similar studies that oral administration of sugar cane extracts resulted in higher antibody titers to SRBCs in chickens artificially inoculated with Eimeria spp. (El-Abasy et al., 2003); elevated antibody response to SRBCs in immunocompromised chickens (Amer et al., 2005); stimulatory activity of sugar cane juice on immunoglobulin production (Akhtar et al., 2008) and stimulatory effect of aqueous and ethanolic extracts of sugar cane on immunoglobulin production (Awais and Akhtar, 2012). Antibody responses to ND and IBD vaccines revealed that all the groups administered with HMMGs showed higher titres against ND and IBD vaccines as compared to control groups. Similar to our findings, enhanced immune performance in terms of increased antibody titers to different vaccines by the use of medicinal plants and their components had also been reported in previous studies (Akhtar et al., 2012); whereas, Waihenya et al. (2002) reported a contradictory finding after the administration of crude extract of Aloe secundiflora in chickens in an immunological study on Aloe.

Weekly FCRs were almost similar in all HMMGs administered groups but observed better than control chickens; indicating improved feed utilization in HMMGs administered groups and a decrease in the amount of food needed for unit gain in body weight. Awais and Akhtar (2012) and El-Abasy et al. (2004) also revealed similar findings that oral administration of sugar cane extract resulted in higher body weight gain and feeding efficiency as compared to chickens of control group.
Conclusion: Results suggested that sugar cane derived HMMGs had biological properties of stimulating the both cellular and humoral arms of immunity as well as growth rates in industrial broiler chickens.

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