Immune Potentiating Effect of Bee Venom on Humoral Parameters of Innate Immunity in Broiler Chickens

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ABSTRACT

To date the medicinal therapeutic use of honeybee products, consisting of honey, propolis, royal jelly, pollen, beeswax and, in particular, bee venom has grown, so the aims of this work were to monitoring and determination it immune-modulatory effect mainly innate immunity parameters in broilers chickens. In the current study cytokines determination as IL6 and INF-γ after injection of BV in different concentration 2%, 3%, and 4%, the IL6 show fair value and long durations in 2nd gp which injected with 3% BV were 23.4, 22.6 and 21.8; pg/ml respectively and also INF-γ values were 289,294 and 277 at 12,19 and 26 days of age. confirming our trials by measuring of nitric oxide and lysozyme which also show the following value 18.4, 20.3 and 22.3 µmol/ml for nitric oxide and 4.5, 4.4 and 4.3 µg/ml for lysozyme In the 2nd gp with 3% BV, however the 4th gp that not injected showed low value of measured cytokines, nitric oxide and lysozyme, that mean excellent immune-potentiating concentration of bee venom in broilers chickens was 3% conc.

Key words: Bee venom, Cytokines, IL6, INF-γ, Nitric oxide, Lysozyme, Broilers chickens

INTRODUCTION

Apitherapy is the medicinal therapeutic use honey bee products, consisting of honey, propolis, royal jelly, pollen, bee wax and other bee products can be also traced back thousands of years and healing properties are included in many religious texts including the Veda, Bible and Quran (Lee et al., 2005; Adewole et al., 2015). Bee Venom (BV) therapy which utilizes the application of BV to treat various diseases has been used since ancient times in traditional medicine (Liu and Tong, 2003; Silva et al., 2015). It based on the fact that these crude extracts exhibit a wide variety of pharmacologically active molecules “biogenic amine enzymes; Phospholipase A2, basic peptides and proteins; melittin and apamin, and mixture of water soluble and nitrogen containing substance (Santos et al., 2011). Bee Venom contains a variety of different peptides including melittin, phospholipase A2, apamin, adolapin and Mast Cell Degranulation Peptide “MCDP” (Park et al., 2004). Pathogens can evolve complex strategies to evade immune detection. The innate immune system is fully developed at birth to fight infection even in the absence of prior exposure to a pathogen; it is complex comprises biochemical and cellular pathways whose function is to recognize and actively remove invading pathogens and to activate the adaptive response. It detects markers on pathogens and acts quickly to control infection, also without stimulation of innate cells; there would be no highly specific, long lasting adaptive immune response. Interferons are cytokines that mediate the response against intracellular pathogens, Type 2 interferon produced by activated TH1 cells, NK cells and T-cytotoxic cells regulates development of adaptive T-cells and activated phagocytes. Cytokines are intracellular signaling proteins that coordinate an immune response and produced in response to stimuli that signal infection. Type 2 nitric oxide synthase (INOS or NOS²) was originally described as an enzyme that is expressed in activated macrophages, generates nitric oxide (NO) from the amino acid L-arginine and there by contributes to the control of replication or killing of intracellular microbial pathogens. Since INF-γ is the key cytokines for induction of NOS² in macrophages and the proteolytic products of TH1 cells, high level of NOS² has been regarded to be mostly restricted to the adaptive phase of immune response (Bogdam et al., 2012). Chickens interleukin-6(IL6) has been confirmed to have a role in pro-inflammatory response (Kaiser et al., 2000). So, the aim

of this study is to measure some humoral factors of innate immunity and its durations in broilers chickens injected with bee venom at different concentrations.

MATERIALS AND METHODS

Experimental design

Two hundred Hubbard broilers were used and All vaccination program was regarded. The chickens were divided in to four groups, 1st group: was injected intramuscular with bee venom at concentrations 2% at 7 and 14 days of age; 2nd group was injected intramuscular with bee venom at concentrations 3% at 7 and 14 days of age; 3rd group was injected intramuscular with bee venom at concentrations 4% at 7 and 14 days of age; 4th group kept as control non injected. Serum samples were collected at 12, 19, 26 days of age from all groups for measuring immunological parameters.

Determination of cytokines

Chicken INF-γ and IL6 were measured using manufactured Sandwich ELISA kits according to (Kaiser et al., 2000 and Kalyuzhny, 2005) on collected serum samples.

Determination of nitric oxide and lysozyme

Lysoplates were prepared by using uniform suspension of M.lysodeikticus for measuring lysozyme conc. in serum samples, while 100 µl serum were used to measure NO conc. by using Griess reagent according to (Schulze et al., 2012).

Statistical analysis

Using the one way ANOVA (Start Soft INC.), the results were presented as means ± SD. Differences were considered significant at P≤0.05.

RESULTS

Results of cytokines value

IL6 (pg/ml) in 1st group was 16.5 pg/ml at 12 days of age elevated to 19.3 pg/ml at 19 days of age and slightly reduced to be 18.2 at 26 days of age. The recorded values in 2nd gp were 23.4, 22.6 and 21.8; while in 3rd gp were 23.8, 22.6 and 21.3 µg/ml respectively. However, 4th gp values were 6, 10 and 12 pg/ml. In the other hand INF-γ measured in all groups under study at different ages was as follow: 210 pg/ml, 242 and 245 in 1st gp 289, 294 and 277 in 2nd gp, 295, 305 and 289 in 3rd gp, 25, 45 and 58 pg/ml at 12, 19 and 26 days of age respectively.

Results of nitric oxide and lysozyme value

Recorded nitric oxide values in 1st gp which injected with 2% BV were 12.6, 18.4 and 22.3 µmol/ml at 12, 19, and 26 days of age respectively, while the lysozyme values were 4.7, 4.9 and 4.8 µg/ml at the same ages respectively. In the 2nd gp which injected with 3% BV, the following value 18.4, 20.3 and 22.3 µmol/ml for nitric oxide and 4.5, 4.4 and 4.3 µg/ml for lysozyme, while the 3rd gp which injected with 4% BV nitric oxide values were 24.8, 25.3 and 23.2 µmol/ml and lysozyme values were 4.8, 4.9 and 4.5 µg/ml. Control non injected 4th gp recorded that nitric oxide levels were 10.34, 11.3 and 12.67 µmol/ml and lysozyme levels were 2.4, 2.9 and 3.2 µg/ml at 12, 19 and 26 days of age respectively.

DISCUSSION

Recently, bee venom has become on focus of interest as an alternative and preventive therapy for the treatment of a vast range of clinical conditions, as it contains several biochemically and pharmacoologically active principles were claimed to directly or indirectly express its potency and medical efficacy. Nowadays scientists are performing intensive clinical trials to improve the use of bee venom. Our trials in this scientific paper gives the ability to determine the immuno-stimulant effect of bee venom on humoral parameters of innate immunity in broilers chickens which give a promising for alternative medicine in infectious poultry diseases. (Cui and Lu., 2002). In the present study cytokines determination as IL6 and INF-γ after injection of BV in different concentration, IL6 (pg/ml) in 1st gp which injected with 2% conc. was 16.5 pg/ml, 19.3 pg/ml and 18.2 pg/ml at 12, 19, 26 days of age, while INF-γ values were 210 pg/ml, 242 and 245 in the same ages respectively, otherwise when BV injected in 3% conc. in 2nd gp and 4% in 3rd gp the results of IL6 were 23.4, 22.6 and 21.8; while in 3rd gp were 23.8, 22.6 and 21.3 pg/ml respectively and INF-γ values were 289, 294 and 277 in 2nd gp, 295, 305 and 289 in 3rd gp, however the 4th gp that not injected showed low value of measured cytokines.BV is a complex mixture of proteins, peptides and low molecular components. The composition of fresh and dried BV differs mainly in regards to the volatile components; the overall biological activity is similar (Bogdanov, 2015), as it has anti-oxidants, anti-coagulants, anti-inflammatory properties and bioactive substances like melittin and phospholipase BV mainly used to treat many inflammatory disorders such as arthritis, cancer, diseases of nervous system, heart and blood system abnormalities, skin diseases and others (Castro et al., 2005). Also, in the current study nitric oxide values in 1st gp were 12.6, 18.4 and 22.3 µmol/ml at 12, 19, and 26 days of age respectively, and the lysozyme values were 4.7, 4.9 and 4.8 µg/ml at the same ages respectively. While the 2nd gp recorded 18.4, 20.3 and 22.3 µmol/ml for nitric oxide and 4.5, 4.4 and 4.3 µg/ml for lysozyme and the 3rd gp nitric oxide values were 24.8, 25.3 and 23.2 µmol/ml and lysozyme values were 4.8, 4.9 and 4.5 µg/ml. Control non injected 4th gp recorded that nitric oxide levels were 10.34, 11.3 and 12.67 µmol/ml and lysozyme levels were 2.4, 2.9 and 3.2 µg/ml at 12, 19, and 26 days of age respectively. In addition, BV was shown to induce the synthesis of IL-1 and TNF-α in monocytes (Bomalaski et al., 1995) and to induce, as well as, Th1 lineage development from CD4 (+) T cells by increasing the expression of a Th1-specific cytokine (Nam et al., 2005). Th1 clones have been found earlier to synthesize mRNA for IL-2, IFN-γ and TNF-α (Cherwinski et al., 1987). Further, recently Stuhlmeier (2007) stated that bee and melittin mediate immune-modulating effects and large quantities of oxygen radicals are produced in a dose-dependent manner in leukocytes exposed to BV.

Conclusions
The findings of the current trials provide further evidence that BV boosters the body's immune system in broilers chickens so bee venom therapy by using live injectable venom by different concentrations in broilers chickens has a beneficial immunological value on innate immunity parameters which give a promise to potentiate the broilers immunity against infectious diseases.

REFERENCES