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Research Article

Immunomodulatory Efficacy of Hedychium Spicatum Rhizome Powder in Long Term Indoxacarb Intoxicated White Leghorn (WLH) Cockerels

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Abstract

The present investigation was undertaken to ascertain the immunomodulatory efficacy of *Hedychium spicatum* rhizome powder (HSRP) in chronic toxicity produced by indoxacarb in white leghorn (WLH) cockerels by determining the levels of serum immunoglobin (IgY), Interferon-γ (INF-γ), Interleukin -2 (IL-2), and lymphocyte stimulation test (LST), Macrophage function test (MFT) and delayed type hypersensitivity (DTH). 49 white leghorn (WLH), 6-8 weeks old chicks were divided into 07 groups consisting of 7 birds in each gr. Group I was maintained at grower ration whereas groups II to VII were given medicated ration upto 16 weeks. The immunomodulant activity of HSRP was determined using both Cell mediated immune response (CMI) i.e., LST, MFT and DTH and Humoral mediated immune response (HMI) i.e., by evaluating antibody titre against bovine serum albumin (BSA) used as antigen. Quantification of chicken IL-2 and INF-γ were carried out by ELISA method as per the manufacture instructions using standard curve method. The result of present finding indicates that the indoxacarb producing the immunosuppression in long term intoxication in WLH cockerels and simultaneous feeding of rhizome powder of *H spicatum* (HSRP) @ 2000ppm and 4000ppm produced immunomodulatory effects in dose dependent manner by elevation of IgY, INF-γ and IL-2 titres. The decreases in these parameters by indoxacarb reveal the immunosuppression and simultaneous treatment with HSRP improves these values indicating the immunomodulatory properties of *H spicatum* and can be use Ayurvedic medicines alone.

Keywords: *Hedychium Spicatum;* Immunomodulation; Interleukin-2; Interferon-Y; Indoxacarb; White Leghorn Cockerel

Introduction

Immunity has great concerns with the health status of any living organisms. The poultry industry is growing continuously though out the globe including India. The minor health problem in poultry may lead to a significant economic loss in terms of production and productivity. To maintain the health status of poultry and prevent these losses there is tremendous use of chemotherapeutic agent which makes this business not much profitable as it looks like. Medicinal plants having many significant roles in health management system. Many plants having immunostimulant activities and used as traditional medicine in India, China and other European countries for treatment of various chronic diseases [1-4]. India is the richest sources of herb from the ancient time. The use of these herbs in place of the chemotherapeutic agent is cost effective and also produced the better growth and production. One of the important medicinal plants is *Hedychium spicatum* belongs to family Zingiberaceae. The *H spicatum* is known as spiked ginger lily in

english, Kuchri in Hindi, Shati in Sanskrit and Kapurakachari is trade name [5], and grown around the Himalayan region of Uttrakhand, Assam and Arunachal Pradesh. The plants, *H spicatum,* is very known for its traditional medication and it commonly called as an anti-diabetic plant [6]. The plants were used as hepatoprotective agent [7] and produced immunomodulatory [8,9] and antioxidant effects [10]. These plants contain glycosides, alkaloids, falavonoids, phenolics, resins, saponins, tannin, tritepenoids, phytosterols and other phytoconstituents [11-13] which produces the effects on liver, GIT, CVS, Nervous system [14] etc.

Indoxacarb is new oxadiazine group of pesticides used as acaricides by inhibiting the sodium channels [15]. Many time animals are also exposed to this indoxacarb through ingestion of treated feed and fodders and resulting into serious health issues [16]. Indoxacarb and its metabolites leads to immunosuppression by reducing the cell count and bone marrow suppression, atopy of lymphoid organs [17,18]. Lethal dose of indoxacarb is 98 mg/kg body weight in birds but little information is available in white leghorn cockerels.

There is paucity of literature dealing with the immunomodulatory effects of *H.spicatum* in poultry in India. Therefore, the present study was undertaken to explore the immunomodulatory properties of *H spicatum* in indoxacarb intoxicated WLH cockerels.

Materials and Methods

Plant material

Rhizome sample of *H. spicatum* was collected and authenticated from Medicinal Plants Research and Development Centre (MRDC), G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Experimental animals

Forty-nine (49) white leghorn (WLH) cockerels, 6 to 8 weeks old, weighing 300 to 400 g were procured from Instructional Poultry Farm, G.B. Pant University of Agriculture and Technology (GBPUAT), Pantnagar and maintained under deep liter system following standard management practices. Experimental chicks were randomly divided into 07 groups of 7 birds each and were kept for fifteen days for adaptation. Feeding and drinking (tap) water were given *ad libitum* during the entire study period.

Extract preparation

The rhizome was gathered from the plants, chopped like slice, air dried for 15-20 days in shade. Later the chopped rhizomes were placed in incubator at 37°C for 2-4 h and grinded in electric grinder. A fine homogenous powder of *H spicatum* rhizome (HSRP) was obtained and stored in sealed plastic container in dry place at room temperature (RT) till further use. The powder was used to feed the animals under study with the steamed standard computed ration.

Experimental design

Group I was given the grower ration whereas experimental groups from II to VII were given medicated ration upto 16 weeks. At 8- and 16-weeks interval the blood was collected for lymphocyte separation and macrophage function test.

Groups	Treatment	Duration
I	Control (Normal feed)	16 Weeks
II	Indoxacarb @ 250 ppm in feed	16 Weeks
III	Silymarin @ 250 ppm in feed	16 Weeks
IV	Indoxacarb @ 250 ppm+ Silymarin @ 250 ppm in feed	16 Weeks
V	*Hedychium spicatum* rhizome powder (HSRP) @ 4000 ppm	16 Weeks
VI	Indoxacarb @ 250 ppm+ *Hedychium spicatum* rhizome powder (HSRP) @ 2000 ppm in feed	16 Weeks
VII	Indoxacarb @ 250 ppm+ *Hedychium spicatum* rhizome powder (HSRP) @ 4000 ppm	16 Weeks

Table a

After 16 weeks the birds were challenged with antigen and immunological parameters were recorded at 7th and 14th days post administration of antigen. Plan of work was undertaken after approval of the Institutional Ethical Committee (IAEC) vides approval number IAEC/VPT/CVASc/193 dated. 27. 02. 2015.

Immunomodulant activity

The immunomodulant activity of *H spicatum* rhizome was determined using both Cell mediated immune response (CMI) and Humoral mediated immune response (HMI). The cell-mediated immune response of the chicken was estimated by lymphocyte

stimulation test (LST), macrophage function test (MFT) and delayed type hypersensitivity reaction (DTH). Humoral immune response was examined by evaluating antibody titre against bovine serum albumin (BSA, Fraction V) used as antigen. Antibody titres were determined at 7th and 14th days after administration of antigen by employing ELISA.

Lymphocyte stimulation test (LST)

The evaluation of the activity of T and B lymphocytes was performed according to the method described earlier [19-21]. In brief, Con-A (5 mg/ml in RPMI-1640) and lipopolysaccharide-LPS (4 mg/ml in RPMI-1640) from *E. coli* were used as mitogen for stimulation of T-Cell and B-Cell blastogenesis, respectively. For the estimation of lymphocyte blastogenesis, 96 well, flat bottom tissue culture plates (Cellstar, Grenier bio-one) were used. Each sample was used in triplicate whose average was used as the final reading for the given sample. Each sample had its own control, also in triplicate for comparison. For the triplicate wells, 100 ml of cell suspension, 50 ml of media and 50 ml of CON-A (5mg/ml) and LPS from *E. coli* (4 mg/ml) as mitogen for B and T lymphocytes, respectively, was added. For blank 100 ml of media alone was used. After loading, the plates were sealed with parafilm (Pechiney plastic packaging) and incubated at 37^{0}C for 68 h in CO_2 incubator (Forma Scientific) with 5% CO_2 pressure. After the completion of the incubation period, 50 ml of MTT (4 mg/ml, Sigma) in RPMI-1640 medium was added to each of the well and incubated for 04 h. After the completion of 72 h of incubation, liquid contents of the wells were discarded followed by addition of 50 ml of DMSO in each well. Each well was mixed thoroughly by pipetting to solubilize the formazan crystals and to stop the reaction of cells with MTT. Finally, absorbance, optical density (OD) of each well was measured at 570 nm of wavelength as per the method described by Mertens, 1997 [18]. Triplicate sample wells were averaged to get the final OD of a sample well. Average of the control wells was taken as the final OD of control wells. The difference of the sample and control wells was expressed as the final reading in terms of ΔOD.

Macrophage function test (MFT)

The macrophage functions test (MFT) was employed to determine the phagocytic and bactericidal activity of macrophages. For this purpose, macrophages and polymorphonuclear cells are collected from peripheral blood. The metabolic activity of macrophage was measured employing nitro blue tetrazolium (NBT) dye reduction [21,22] at the commencement of 8 and 16 weeks of the study. In brief, 3-4 ml blood were collected in heparinized vials. Equal volume of RPMI-1640 medium were added in 15 ml conical centrifuge tube followed by addition of 5 ml Histopaque-1077 and were centrifuge the tube at 400x g for 10-15 min. Upper opaque layer was collected in sterile cell culture medium (RPMI-1640) and the cell viability was examined using trypan blue dye exclusion test and final concentration was adjusted to 1 x 10^{7}cells/ml by washing and centrifuging thrice in RPMI-1640 [22]. For NBT Test, 0.2 ml cell suspension (1 x 10^7 cells/ml), 0.1 ml plasma and 0.3 ml of NBT (0.2% in PBS) were added. The reaction mixture was incubated at 37^{0}C for 30 min and then reaction was stopped by adding cold PBS. Mixture was centrifuged at 500 x g for 5 min. Supernatant was discarded and cells were resuspended in a drop of PBS to prepare the cells smear on a clean glass slide. The slides were air dried and fixed in methanol for 2 min and stained with Giemsa's stain for 20 min. The smear was then washed, dried and mounted in DPX mounting media. The numbers of NBT positive cells (black/blue granules of NBT dye in the cytoplasm) were counted under oil immersion and were expressed in percentage.

Delayed type hypersensitivity reaction (DTH)

Delayed type hypersensitivity (DTH) reaction to dinitrochlorobenzene (DNCB) was determined by method [20,23]. In brief, each bird of all groups, featherless areas of about 10 cm^2 was outlined on left and right lateral abdomen for DNCB application. Two weeks prior to completion of the study, outlined areas of each bird were cleaned and 0.25 ml of DNCB (10mg/ml in acetone) was applied on right side while on left side 0.25 ml of acetone was applied to serve as control. Sensitized birds were challenged on 14th day of first application of DNCB (1 mg/ml in acetone) on right side and 0.25 ml acetone on left side at the same site of application. The assessment of reaction was determined by the measuring the skin thickness using vernier Calipers at 0, 12, 24, 48 and 72h of post challenge. The challenge area was also observed for erythema, induration, ulceration and scab formation. The average thickness at 12, 24, 48 and 72h of treated and control group was compared to evaluate DTH.

Quantification of antibody (IgY) titre

Goat anti-chicken alkaline phosphatase (ALP) conjugates used in this study were obtained from (Bethyl Laboratories, Inc., 25043 West FM 1097, Montgomery, TX 77356) and chicken antibody Y

(IgY) (Genxbio Health Sciences Pvt. Ltd) were used in the study. The standard curve of IgY was prepared using different concentration of purified IgY ranging between 125ng/ml to 4000 ng/ml to determine the concentration of IgY in sera of birds challenged with BSA (Figure 1).

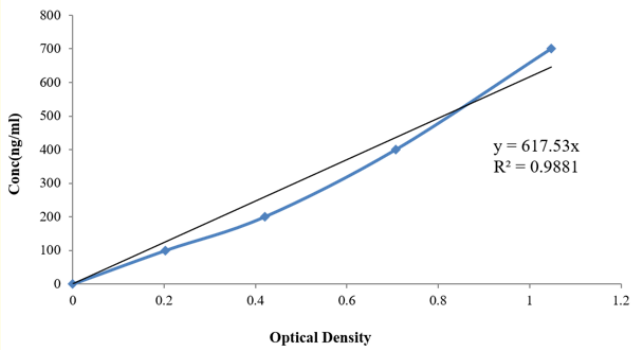

Figure 1: Showing standard curve of IgY at different concentrations for determination of IgY concentration in bird serum challenged with Bovine serum albumin (BSA).

Development of antibodies against BSA and preparation of antisera

The antigen, prepared in sterilized normal saline, (40mg/ml) was administered at the dose of 40 mg/kg body weight, intravenously in each bird two weeks prior to completion of the study. At 7th and 14th day post challenge with BSA, 3 ml of blood was collected from each bird in non-heparinized blood vial and antiserum were collected followed by storage at -20°C till further use. The antibody reactivity with BSA was determined by using indirect ELISA and western blotting.

Indirect ELISA for detection of IgY against BSA

IgY was recorded using the ELISA method as described by Engvall and Perlmann, 1971 [24] with minor modification. In brief, micro-titre plates were coated with BSA in coating buffer (100 ml/well). The plates were incubated at RT for 1h followed by overnight incubation at 4°C. Next day, plates were washed with PBS containing tween-20 (0.05%). Blocking buffer (5% skimmed milk in PBS) were added in each well (full) and incubated at RT for 2 h followed by washing (PBS + Tween-20 (0.05%) + 0.5% skimmed milk). The 100 ml of primary antibody (1: 2000 dilutions) was added and incubated at RT for 2 h followed by triple washing with PBS + Tween-20 (0.05%). 100 ml of alkaline phosphatase conjugated secondary antibody (1:1000 dilutions) were added and incubated at RT for 1h followed by triple washing with PBS + Tween-20 (0.05%). Alkaline phosphatase activity was assayed with the substrate solution (p-nitrophenyl phosphate sodium salt dissolved in di-ethanolamine buffer, 1.0 mg/ml). The plates were incubated for 30 min in dark and the reaction was stopped with 100 ml of 1.5 M NaOH solution. Reading was taken in ELISA reader at 405 nm.

To determine the antibody sensitivity range, the micro-titre plates were coated with optimal amount of antigen (1µg of BSA) per well that falls within the detection range of indirect ELISA. Antibody dilutions for anti-BSA antibody raised on bird challenged with BSA was used ranging from 1: 250 to 1: 32,000. The binding curve of antibody indicated that the reactivity of antibody raised against BSA was as high as 1.48 at optical density (OD) at 405 nm (Figure 2). However, the saturation of reactivity of BSA with anti-BSA was observed at dilution of 1:16000. The straight line was obtained in the antibody dilution range from 1:8000 to 1: 32000. The titre of antibodies is defined as maximal dilution of antibodies at which reactivity was visible. It was found to be 1:8000 with anti-BSA and optimum antibody dilutions is found to be 1: 2000.

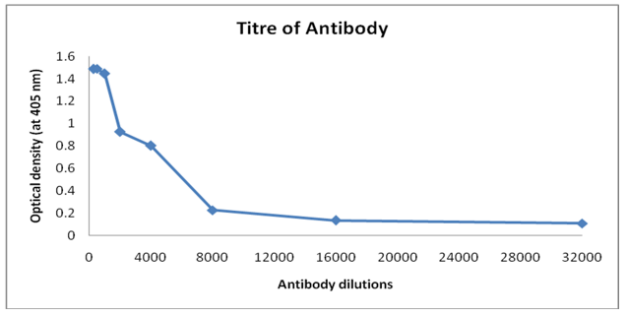

Figure 2: The binding curve of antibody indicated that the reactivity of antibody raised against BSA was as high as 1.48 at optical density (OD) at 405 nm in different antibody dilution i. e. optimization curve for antibody titre.

Western blotting

Western blotting combines the resolution of gel electrophoresis with the specificity of immunochemical detection. Western blotting can be used to validate the generation of Immunoglobulins against BSA. Protein sample (BSA) was dissolved in PBS buffer. The

unstained Protein sample (BSA) along with pre-stained protein mol. wt marker was resolved by SDS-PAGE and the proteins were transferred to a nitrocellulose (NC) membrane (0.45 mm) using semi-dried system a transfer apparatus (SCIE-PLAS, Cambridge, UK) at 2 mA/square cm for 90 min. The NC membrane was kept in blocking solution (5% skimmed milk in PBS) at 37°C for two hours. The blocked membrane was probed with chicken anti-BSA antibody diluted 1:250 in PBS for one hour at 37°C with constant shaking. The washing was performed three times with PBS containing 0.05% Tween-20, allowing 10 minutes per wash. The NC membrane was incubated in goat anti-chicken- ALP conjugated for 45 minutes at 37°C with constant shaking, followed by washing performed thrice with 10 minutes at each wash, after which 5-bromo-4-chloro-3′-indolyphosphate/nitro-blue tetrazolium chloride (BCIP/NBT) substrate was added for colour development. Thereafter, the reaction was stopped by washing the membrane in double distilled water and the results were observed.

Quantitative determination of chicken cytokines interleukin-2 (IL-2) and interferon-γ (INF-γ)

Quantification of chicken interleukin-2 (IL-2) and chicken interferon -γ (INF-γ) were carried out by ELISA method (Blue Gene Biotech, China) as per the manufacture instructions using standard curve method. A standard curve was constructed by plotting the average O.D. for each standard on X-axis against the cytokine concentration on the Y- axis, and a best fit curve was drawn by excel sheet (Figure 3 and 4). Concentrations of samples corresponding to the mean absorbance were calculated from the standard curve.

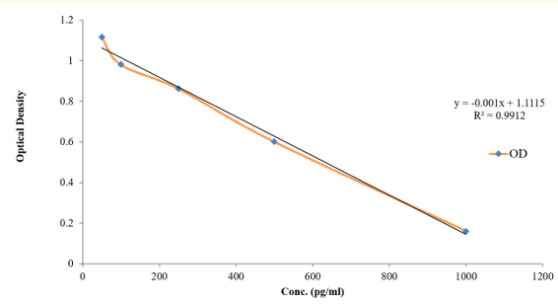

Figure 3: Showing standard curve of interleukin-2 (IL-2), average O.D. for each standard on X-axis against the IL-2 concentration (pg/ml) on the Y- axis, and concentrations of samples corresponding to the mean absorbance were calculated using the standard curve.

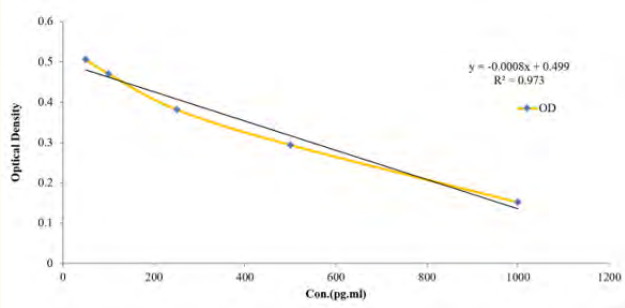

Figure 4: Showing standard curve of interferon-γ (INF-γ), average O.D. for each standard on X-axis against the INF-γ concentration (pg/ml) on the Y- axis, and concentrations of samples corresponding to the mean absorbance were calculated using the standard curve.

Results

To explore the immunomodulatory efficacy of *H spicatum* in indoxacarb intoxicated WLH cockerels, both HMI and CMI were carried out in present study. HMI is a form of Immunity mediated by circulating antibodies (immunoglobulins IgA, IgM and IgB), which coat the antigens and target them for destruction by polymorphonuclear neutrophils. Circulating antibodies are produced by the plasma cells of the reticuloendothelial (RE) system. Antibodies produced by plasma B cells are found mainly in the blood, spleen and lymph nodes, neutralize or destroy antigens in several ways. In order to confirm the generation of antibody against immunized bird with BSA. The SDS-PAGE followed by Western blotting was performed. The SDS PAGE of BSA protein showed a blue prominent band of 66kd molecular weight stained with CBB (Figure 5) and similar finding were also recorded through Western blotting, a prominent band of 66 kDa using sera of BSA challenged birds (Figure 6). These findings indicate that antibody was raised in sera of birds in response to BSA and that was also quantified later by indirect ELISA.

Significant ($P < 0.05$) reduction in serum IgY (µg/ml) level in indoxacarb treated birds on 7th and 14th days post challenged with BSA in comparison to control group I which was 17% and 20% less than control at 7th and 14th days post challenged with BSA (Figure 7). Simultaneous treatment with HSRP and silymarin in indoxacarb treated groups IV, VI and VII showed significant ($P < 0.05$)

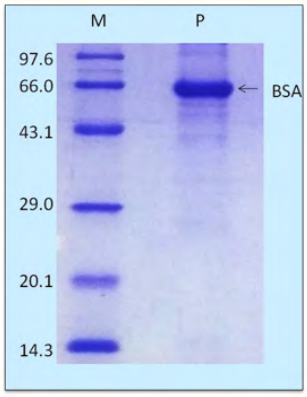

Figure 5: The SDS PAGE of BSA protein showing a blue prominent band of 66kd molecular weight stained with commasaie brilliant blue (CBB). Lane M: Marker; Lane P: BSA protein.

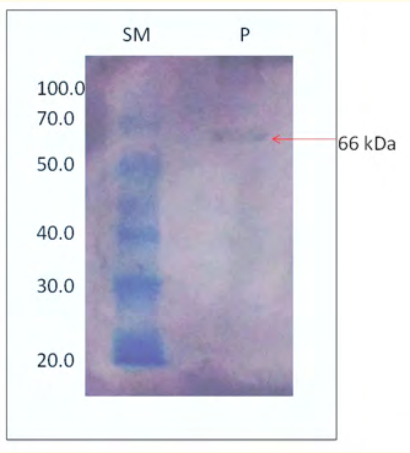

Figure 6: Western blot of BSA protein using anti-sera of bird challenged with BSA. Lane SM: Prestained Marker; Lane P: BSA protein.

elevation upto 18% and 19% in serum IgY levels in comparison to indoxacarb group II at both 7th and 14th days post challenged with BSA. However, the serum level of IgY was similar in HSRP alone but significantly higher in silymarin treated group in comparison to untreated groups. Cell mediated immune response involves of the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.

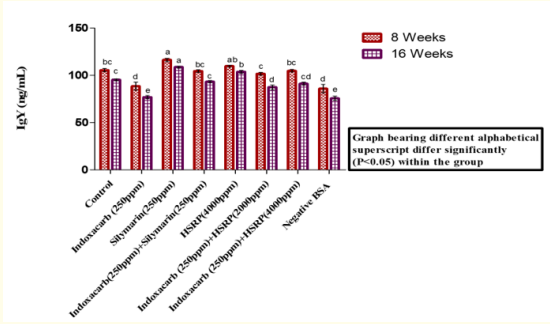

Figure 7: Effect on serum IgY (ng/ml) concentration following administration of Hedychium spicatum root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

The serum IL-2 (pg/ml) and INF-γ (pg/ml) concentration depicted in figure 8 and 9. A significant ($P < 0.05$) reduction by 38% and 36% was observed in indoxacarb treated group II at 7th and 14th day post challenged with BSA in comparison to control group I. Treatment with HSRP in groups VI and VII showed a significant ($P < 0.05$) amelioration upto 30% and 45% in comparison to indoxacarb treated group II and was similar to group IV treated with silymarin+ indoxacarb and was restored towards normal as level as in control group I. In addition, group VIII was added for immunological studies, and was neither given any medication nor BSA also has lowered IL-2 levels at par with group II. However, the silymarin and HSRP alone showed significant higher levels of serum IL-2 in comparison to control group I (Figure 8).

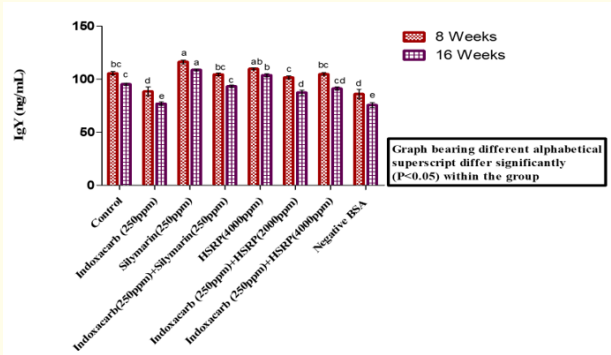

Figure 8: Effect on serum IL-2 (pg/ ml) concentration following administration of Hedychium spicatum root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

A significant (P < 0.05) reduction in serum INF-γ levels by 27% and 30% was observed in indoxacarb treated group II on 7th and 14th day post challenge with BSA in comparison to control group I. Simultaneous treatment with HSRP showed significant amelioration by 19% and 34% at low dose and 24% and 47% at high dose in the INF-γ levels in indoxacarb treated group VI and VII in comparison to group II on 7th and 14th days post challenge with BSA in dose dependent manner and was at par with silymarin groups (Figure 9).

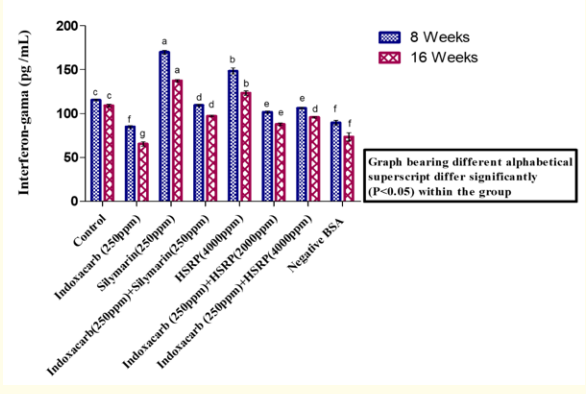

Figure 9: Effect on serum INF-γ (pg /ml) concentration following administration of Hedychium spicatum root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

Significant (P < 0.05) reduction of Δ O.D. value of T-lymphocyte and B-lymphocyte in indoxacarb treated group II in comparison to control group I at 8- and 16-weeks intervals respectively (Figure 10 and 11). In groups VI and VII treated with indoxacarb+ HSRP showed significant (P < 0.05) elevation of Δ O. D to at 8- and 16-weeks interval in comparison to group II which was close to control. In group IV treated with indoxacarb+ silymarin showed significant (P < 0.05) elevation in comparison to group II.

A significant (P < 0.05) reduction in% NBT positive cells was record to be in indoxacarb treated groups II in comparison to control at 8- and 16-weeks interval, respectively (Figure 12). In groups VI and VII treated with indoxacarb+ HSRP showed significantly (P < 0.05) increased MFT which was close silymarin+ indoxacarb treated group IV at 8- and 16-weeks interval.

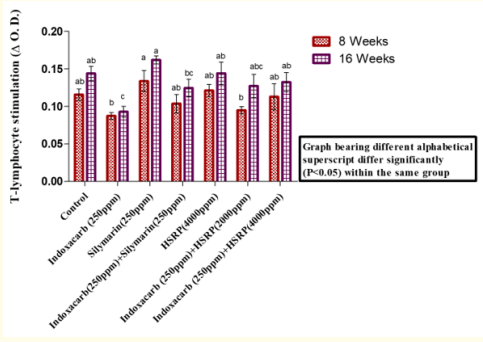

Figure 10: Effect on T-lymphocyte stimulation (Δ O. D.) following administration of Hedychium spicatum root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

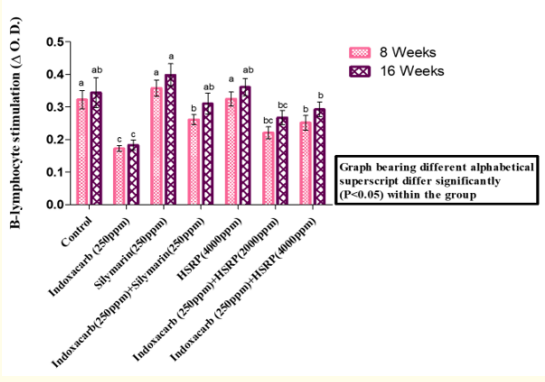

Figure 11: Effect on B-lymphocyte stimulation (Δ O. D.) following administration of Hedychium spicatum root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

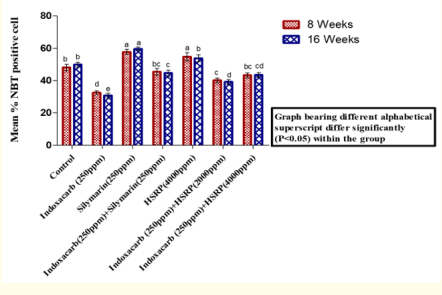

Figure 12: Effect on macrophage function test (MFT) following administration of Hedychium spicatum root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

Delayed type hypersensitivity reaction was represented by the thickness of skin (mm) as it was measured (Figure 13). Maximum DTH response was observed at 24hr followed by subsequent decrease by 48hr and negligible reaction at 72hr post challenge with DNCB. The skin thickness in indoxacarb treated group II was significantly ($P < 0.05$) lowered after 12hr time interval in comparison to control group I. Groups IV, VI and VII treated showed the significant elevation of skin thickness in comparison II and was near to group I (Figure 13).

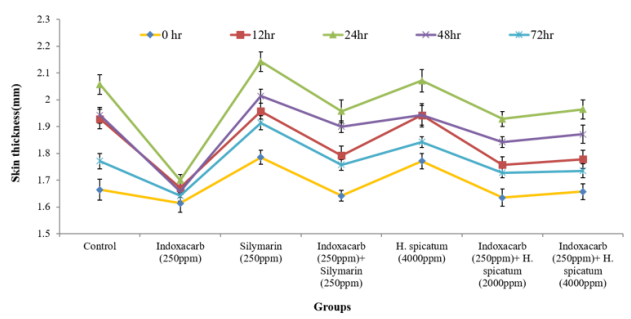

Figure 13: Effect on delayed type hypersensitivity (DTH) reaction following administration of Hedychium spicatum root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

Discussion

The major avian immunoglobin class IgY present in the serum and egg yolk shows fundamental structural differences in comparison to mammalian IgG as it has higher molecular weight with presence of an additional C_H domain and two carbohydrate side chain [25].

The present findings revealed that the prolonged feeding of indoxacarb for 16 weeks depressed antibody titre in WLH cockerels which indicated the immunosuppressive potential of indoxacarb on humoral immune response in WLH cockerels. The reduction in the antibody titre could be attributed to hampered proliferation and activation of B- lymphocytes, responsible for biosynthesis of immunoglobulins. This was also supported by the evidence of leukocytopenia and lymphocytopenia with lowered serum globulin level in indoxacarb intoxicated cockerels in the present investigation (Table 1). The lowered the serum globulin level with leukopenia in indoxacarb treated broiler was also reported earlier in albino mice treated with indoxacarb [26,27]. Indoxacarb causes thymic necrosis, splenic lymphoid depletion and bone marrow atrophy in rats [28] and hemosiderosis in spleen, kidney and liver [29]. The hemosiderosis of various tissue and reticulocytosis is considered as the sign of immunosuppression [30]. The hydroethanolic and methanolic extract of *Hedychium spicatum* revealed immunomodulatory property in cyclophosphamide treated albino mice [8,9]. It is, thus, concluded from this study that HSRP has immunomodulatory efficacy which might be attributed to the amelioration in serum IgY levels by HSRP in indoxacarb intoxicated cockerels.

Group	Treatment	ALCx10^3/µl	
		8 Weeks	16 Weeks
I	Control	10.79 ± 0.47^{abA}	11.02 ± 0.09^{aA}
II	Indoxacarb (250 ppm)	7.81 ± 0.31^{dA}	7.66 ± 0.38^{cA}
III	Silymarin (250 ppm)	11.17 ± 0.28^{aA}	11.11 ± 0.46^{aA}
IV	Indoxacarb (250 ppm) + Silymarin (250 ppm)	9.81 ± 0.42^{bcA}	10.08 ± 0.48^{abA}
V	*H. spicatum* (4000 ppm)	10.74 ± 0.27^{abA}	10.77 ± 0.32^{aA}
VI	Indoxacarb (250 ppm) + *H. spicatum* (2000 ppm)	9.19 ± 0.60^{cA}	9.22 ± 0.52^{bA}
VII	Indoxacarb (250 ppm) + *H. spicatum* (4000 ppm)	9.49 ± 0.31^{cA}	9.94 ± 0.25^{abA}

Table 1: Effect on absolute lymphocyte count (X 10^3/µL) levels of *Hedychium spicatum* root powder (HSRP) in feed for 16 weeks in indoxacarb treated WLH cockerels (Mean ± SEM, n = 7).

Note: Mean values in column bearing different alphabetic superscript a, b, c, d and e differ significantly ($p < 0.05$) when compared vertically; Mean values in row bearing alphabetical superscript A and B differ significantly ($p < 0.05$) when compared horizontally.

IL-2 is a α-helical cytokine produced predominantly by activated dendritic cells (DCs), natural killer (NK) cells and NKT cells [31]. It involves in T-cells immunity through the $CD8^+$ and memory T- cells [32]. IL-2 controls the homeostasis of T regulating cells in peripheral immune tissue. IL-2 is synthesized and released from spleen and lymph nodes. IL-2 is presented in soluble form or remains bound to dendritic cells and the extracellular matrix. Use

of IL-2 either alone or as complex with particular neutralizing IL-2 specific antibodies to activate $CD8^+$ T cells responses or induces the expansion of the T_{Reg} cell population, thus causing either immune stimulation or suppression [33]. The immunosuppressive effect produced by indoxacarb was mitigated by the HSRP. This mitigation might be due to the immunomodulatory effect produced by the HSRP [8,9]. The cytokine IFN-γ belongs to the family of interferon which is divided further into two distinct classes, type I IFN that includes the IFN-α and IFN-β molecules, which are considered as the classical interferon induced in response to viral infections whereas INF-γ belongs to type II of INF. It has a lower specific antiviral activity but presents more immunomodulatory properties than the type I interferon [34]. The main function of IFN-γ is macrophage activation and exerts microbicidal functions [35]. Indoxacarb inhibited the production of T-cell derived cytokines (IL-2 and INF-γ) in our study which indicated the immunosuppressant potential of indoxacarb. Indoxacarb might have produced immunosuppressant by its detrimental effect on lymphoid organs specially thymus and lymph nodes [29]. Thus, amelioration by HSRP could attributed to its ability to managed the toxic effect as on lymphoid organs due to presence of antioxidant phytoconstituents improving histological changes in spleen by HSRP in our study also supported its immunomodulatory property. There is no report on effect of indoxacarb on INF-γ, however, the suppression in expression of IL-2, INF-γ, IL-4 and IL-10 by other class of insecticide like chlorpyriphos, cypermethrin and captan in *in vitro* cell line model [25,26]. The significant ($P < 0.05$) elevation of IgY, IL-2 and INF-γin group VI and VII might be due to antioxidant and immunomodulatory potential of HSRP.

Both T and B-lymphocytes are stimulated by the mitogens concanavalin (Con-A) and LPS, respectively, independent of their antigen specificity. This proliferative response is considered to reflect clonal expansion after the antigen sensitization *"in vivo".* Significant depression in proliferation of B-lymphocytes indicates the lower capacity of B-lymphocyte to form clones and convert into plasma cells. Plasma cells are ultimately responsible for the synthesis and secretion of antibodies and have less responsive to antigen leading to suppression in humoral response. Immunosuppression with other class of insecticide in which significant reduction in mean delta OD after sensitization with DNCB in broiler chicks [36]. Thus, it is likely that indoxacarb produced immunosuppression by interference in lymphocyte proliferation in cockerels and simultaneously feeding of HSRP reverse this effect to ameliorate and produce the immunomodulatory responses. Similar finding was also recorded by Uttara and Mishra, 2009 [37].

The significant elevation by HSRP in MFT which indicates immunomodulatory effect might be due to the increased activity of macrophage by improved metabolic profile of these cells. The decrease in NBT positive cells indicates the reduced functional status of phagocytic cells/macrophages. The macrophages are considered as important component of the defence systems as they neutralize the external invasion. The macrophages not only phagocytize and remove foreign invading agents from the body but also present antigen to other immune cells involved in the active immunity [36].

The interaction with specific antigen, the cells release lymphokines and cytokines. In DTH reaction, the primary lymphocytes response leads to accumulation of mononuclear cells and their interaction results in increased vascular permeability at adjacent site of stimulus [38]**.** Reduction in DTH response in present investigation may also be associated with the findings of leukopenia in indoxacarb treated cockerels in this study. Lymphocytopenia and leukopenia were also observed following immunosuppression by indoxacarb in mice 18. No reports are available on immunotoxicity study of indoxacarb; however, several reports are available on other insecticide like chlorpyriphos [35] and carbaryl [36] in chickens. Indoxacarb lowered the cell mediated immune response as shown by significant reduction in DTH reaction to DNCB. This finding of the present investigation indicates functional impairment of another subpopulation of T- cells i.e., T-affector cells [39]. Reduction in DTH response was also observed following exposure to other class of insecticides like carbaryl [35] and chlorpyriphos [40] in broiler chick.

Group	Treatment	IgY (µg/ml)	
		7th days	14th days
I	Control	105.37 ± 1.76^{bcA}	95.32 ± 1.18^{cB}
II	Indoxacarb (250 ppm)	88.47 ± 4.21^{dA}	76.89 ± 1.78^{eB}
III	Silymarin (250 ppm)	116.53 ± 1.80^{aA}	109.25 ± 0.96^{aB}
IV	Indoxacarb (250 ppm) + Silymarin (250 ppm)	104.34 ± 1.50^{bcA}	93.92 ± 0.99^{cB}

V	*H. spicatum* (4000 ppm)	109.99 ± 1.03^{abA}	104.09 ± 1.46^{bB}
VI	Indoxacarb (250 ppm) + *H. spicatum* (2000 ppm)	101.74 ± 1.60^{cA}	87.61 ± 2.32^{dB}
VII	Indoxacarb (250 ppm) + *H. spicatum* (4000 ppm)	104.98 ± 1.35^{bcA}	91.62 ± 1.45^{cdB}
VIII	Negative BSA	85.97 ± 4.21^{dA}	75.74 ± 2.09^{eA}

Supplementary Table

Table 2: Effect on serum IgY (ng/ml) concentration following administration of *Hedychium spicatum* root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n=7).

Note: Mean values in column bearing different alphabetic superscript a, b, c, d and e differ significantly ($p < 0.05$) when compared vertically; Mean values in row bearing alphabetical superscript A and B differ significantly ($p < 0.05$) when compared horizontally.

Group	Treatment	IL-2 (pg/ ml)	
		7th days	14th days
I	Control	538.16 ± 16.70^{cA}	507.70 ± 22.74^{bcA}
II	Indoxacarb (250 ppm)	335.47 ± 6.24^{fA}	326.65 ± 13.52^{efA}
III	Silymarin (250 ppm)	676.85 ± 22.38^{aA}	597.07 ± 14.19^{aB}
IV	Indoxacarb (250 ppm) + Silymarin (250 ppm)	493.42 ± 7.24^{dA}	449.17 ± 15.80^{cdB}
V	*H. spicatum* (4000 ppm)	599.93 ± 5.30^{bA}	561.13 ± 25.15^{abA}
VI	Indoxacarb (250 ppm) + *H. spicatum* (2000 ppm)	436.79 ± 18.71^{eA}	380.38 ± 27.92^{deA}
VII	Indoxacarb (250 ppm) + *H. spicatum* (4000 ppm)	489.31 ± 5.84^{dA}	430.72 ± 42.29^{dA}
VIII	Negative BSA	313.48 ± 5.96^{fA}	306.52 ± 9.54^{fA}

Table 3: Effect on serum IL-2 (pg/ ml) concentration following administration of *Hedychium spicatum* root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

Note: Mean values in column bearing different alphabetic superscript a, b, c, d and e differ significantly ($p < 0.05$) when compared vertically; Mean values in row bearing alphabetical superscript A and B differ significantly ($p < 0.05$) when compared horizontally.

Group	Treatment	INF-γ (pg/ ml)	
		7th days	14th days
I	Control	538.16 ± 16.70^{cA}	507.70 ± 22.74^{bcA}
II	Indoxacarb (250 ppm)	335.47 ± 6.24^{fA}	326.65 ± 13.52^{efA}
III	Silymarin (250 ppm)	676.85 ± 22.38^{aA}	597.07 ± 14.19^{aB}
IV	Indoxacarb (250 ppm) + Silymarin (250 ppm)	493.42 ± 7.24^{dA}	449.17 ± 15.80^{cdB}
V	*H. spicatum* (4000 ppm)	599.93 ± 5.30^{bA}	561.13 ± 25.15^{abA}
VI	Indoxacarb (250 ppm) + *H. spicatum* (2000 ppm)	436.79 ± 18.71^{eA}	380.38 ± 27.92^{deA}
VII	Indoxacarb (250 ppm) + *H. spicatum* (4000 ppm)	489.31 ± 5.84^{dA}	430.72 ± 42.29^{dA}
VIII	Negative BSA	313.48 ± 5.96^{fA}	306.52 ± 9.54^{fA}

Table 4: Effect on serum INF-γ (pg /ml) concentration following administration of *Hedychium spicatum* root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

Note: Mean values in column bearing different alphabetic superscript a, b, c, d and e differ significantly ($p < 0.05$) when compared vertically; Mean values in row bearing alphabetical superscript A and B differ significantly ($p < 0.05$) when compared horizontally.

Group	Treatment	Mean of Δ OD ± SE	
		8 Weeks	16 Weeks
I	Control	0.115 ± 0.0071^{abB}	0.145 ± 0.0086^{abA}
II	Indoxacarb (250 ppm)	0.088 ± 0.0040^{bA}	0.092 ± 0.0066^{cA}
III	Silymarin (250 ppm)	0.134 ± 0.0129^{aA}	0.161 ± 0.0049^{aA}
IV	Indoxacarb (250 ppm) + Silymarin (250 ppm)	0.106 ± 0.0113^{abA}	0.129 ± 0.0111^{bcA}
V	*H. spicatum* (4000 ppm)	0.120 ± 0.0077^{abA}	0.152 ± 0.0137^{abA}
VI	Indoxacarb (250 ppm) + *H. spicatum* (2000 ppm)	0.097 ± 0.0043^{bA}	0.118 ± 0.0144^{abcA}

VII	Indoxacarb (250 ppm) + *H. spicatum* (4000 ppm)	0.101 ± 0.0160^{abA}	0.122 ± 0.0117^{abA}

Table 5: Effect on T-lymphocyte stimulation (Δ O. D.) following administration of *Hedychium spicatum* root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

Note: Mean values in column bearing different alphabetic superscript a, b, c, d and e differ significantly ($p < 0.05$) when compared vertically; Mean values in row bearing alphabetical superscript A and B differ significantly ($p < 0.05$) when compared horizontally.

Group	Treatment	Mean of Δ OD ± SE	
		8 Weeks	16 Weeks
I	Control	0.322 ± 0.0259^{aA}	0.362 ± 0.0424^{abA}
II	Indoxacarb (250 ppm)	0.179 ± 0.0079^{cA}	0.195 ± 0.0141^{cA}
III	Silymarin (250 ppm)	0.379 ± 0.0233^{aA}	0.420 ± 0.0324^{aA}
IV	Indoxacarb (250 ppm) + Silymarin (250 ppm)	0.272 ± 0.0146^{bA}	0.329 ± 0.030^{abA}
V	*H. spicatum* (4000 ppm)	0.341 ± 0.0198^{aA}	0.380 ± 0.0251^{abA}
VI	Indoxacarb (250 ppm) + *H. spicatum* (2000 ppm)	0.227 ± 0.0168^{bcA}	0.281 ± 0.0204^{bcA}
VII	Indoxacarb (250 ppm) + *H. spicatum* (4000 ppm)	0.263 ± 0.0208^{bA}	0.309 ± 0.0208^{bA}

Table 6: Effect on B-lymphocyte stimulation (Δ O. D.) following administration of *Hedychium spicatum* root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7.

Note: Mean values in column bearing different alphabetic superscript a, b, c, d and e differ significantly ($p < 0.05$) when compared vertically; Mean values in row bearing alphabetical superscript A and B differ significantly ($p < 0.05$) when compared horizontally.

Group	Treatment	Mean % NBT positive cell ± SE	
		8 Weeks	16 Weeks
I	Control	48.084 ± 1.866^{bA}	49.875 ± 1.305^{bA}
II	Indoxacarb (250 ppm)	32.645 ± 1.025^{dA}	30.94 ± 1.254^{eA}
III	Silymarin (250 ppm)	57.748 ± 1.465^{aA}	59.586 ± 1.069^{aA}
IV	Indoxacarb (250 ppm) + Silymarin (250 ppm)	45.593 ± 1.941b^{cA}	43.955 ± 1.658^{cA}
V	*H. spicatum* (4000 ppm)	54.883 ± 2.394^{aA}	53.641 ± 1.927^{bA}
VI	Indoxacarb (250 ppm) + *H. spicatum* (2000 ppm)	40.23 ± 1.346^{cA}	39.261 ± 1.387^{dA}
VII	Indoxacarb (250 ppm) + *H. spicatum* (4000 ppm)	43.113 ± 1.316^{bcA}	43.576 ± 1.314^{cdA}

Table 7: Effect on macrophage function test (MFT) following administration of *Hedychium spicatum* root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

Note: Mean values in column bearing different alphabetic superscript a, b, c, d and e differ significantly ($p < 0.05$) when compared vertically; Mean values in row bearing alphabetical superscript A and B differ significantly ($p < 0.05$) when compared horizontally.

Groups	Treatments	0hr	12hr	24hr	48hr	72hr
I	Control	1.664 ± 0.038^{bD}	1.928 ± 0.035^{aB}	2.057 ± 0.036^{abA}	1.942 ± 0.029^{abB}	1.771 ± 0.028^{cC}
II	Indoxacarb (250 ppm)	1.614 ± 0.034^{bB}	1.671 ± 0.018^{cAB}	1.7 ± 0.021^{dA}	1.657 ± 0.02^{dAB}	1.642 ± 0.023^{dAB}
III	Silymarin (250 ppm)	1.785 ± 0.026^{aD}	1.957 ± 0.029^{aBC}	2.142 ± 0.036^{aA}	2.014 ± 0.026^{aB}	1.914 ± 0.026^{aC}

IV	Indoxacarb (250 ppm) + Silymarin (250 ppm)	1.642 ± 0.022^{bC}	1.792 ± 0.035^{bB}	1.97 ± 0.042^{bcA}	1.9 ± 0.021^{bcA}	1.757 ± 0.021^{cB}
V	*H. spicatum* (4000 ppm)	1.771 ± 0.028^{aC}	1.942 ± 0.036^{aB}	2.071 ± 0.042^{aA}	1.942 ± 0.042^{abB}	1.842 ± 0.022^{bBC}
VI	Indoxacarb (250 ppm) + *H. spicatum* (2000 ppm)	1.635 ± 0.032^{bD}	1.757 ± 0.029^{bcC}	1.928 ± 0.028^{cA}	1.842 ± 0.021^{cB}	1.728 ± 0.018^{cC}
VII	Indoxacarb (250 ppm) + *H. spicatum* (4000 ppm)	1.657 ± 0.029^{bD}	1.778 ± 0.034^{bBC}	1.964 ± 0.035^{bcA}	1.871 ± 0.034^{bcAB}	1.735 ± 0.026^{cBC}

Table 8: Effect on delayed type hypersensitivity (DTH) reaction following administration of *Hedychium spicatum* root powder (HSRP) in feed (different dietary levels) for 16 weeks in indoxacarb treated white leg horn (WLH) cockerels (Mean ± SEM, n = 7).

Note: Mean values in column bearing different alphabetic superscript a, b, c, d and e differ significantly ($p < 0.05$) when compared vertically; Mean values in row bearing alphabetical superscript A and B differ significantly ($p < 0.05$) when compared horizontally

Conclusion

The present study is carried out to find the immunomodulatory properties of *H spicatum* root powder (HSRP). The result of present finding indicates that the indoxacarb producing the immunosuppression in long term intoxication in white leg horn (WLH) cockerels and simultaneous feeding of rhizome powder of *H spicatum* (HSRP) @ 2000ppm and 4000ppm produced immunomodulatory effects in dose dependent manner by elevation of IgY, INF-γ and IL-2 titres and also correcting the lymphocyte stimulation test (LST), Macrophage function test (MFT) and measuring skin thickness (mm) by delayed type hypersensitivity (DTH) reaction with DCNB.

Declaration of Competing Interest

The authors declare that they have no financial and commercial conflicts of interest. This research received no external funding and the expenditure was covered by internal funding of the institute.

Ethical Approval

This study was approved by the Institutional Ethical Committee (IAEC) of G.B. Pant University of Agriculture and Technology, Pantnagar, 263145, Uttarakhand (India) vides approval number IAEC/VPT/CVASc/193 dated. 27. 02. 2015.

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Bibliography

1. Atal CK., *et al.* "Immunomodulatory agents of plant origin 1: preliminary screening". *Journal of Ethnopharmacology* 18 (1986): 131-141.
2. Wagner H. "Immunoprophylaxe und-therapie durch pflanzenpraeparate". *Zeitschrift fur Allgemeinmedizin* 24 (1983): 1282-1289.
3. Wagner H. "Phytoproparate zur immunoprophylaxae and immunotherapie". *Biologische Medizin* 1 (1984): 3.
4. Wagner H. "In: Krogsgaard Larsen P, Brogze S, Christensen H (Eds.) Alfred Benzon Sympsoium 20. Koford Munksgaard, Copenhagen (1984).
5. Kumari I., *et al.* "*Hedychium spicatum* buch-ham (Kuchri), A treasure house of essential oils". *International Journal of Current Pharmaceutical Research* 13.4 (2021): 25-31.
6. Pandeya, K.B., *et al.* "A critical review on traditional herbal drugs: An emerging alternative drug for diabetes". *International Journal of Organic Chemistry* 3 (2013): 1-22.
7. Thapliyal S., *et al.* "Pharmacognostic Screening of Hedychium spicatum Rhizomes". *International Journal of Chemistry and Pharmaceutical Sciences* 3.4 (2014): 483-488.
8. Kajaria D., *et al.* "*In-vitro* evaluation of immunomodulatory effect of polyherbal compound - Bharangyadi". *Journal of Drug Delivery and Therapeutics* 3.1 (2013): 36-39.

9. Puri A., *et al*. "Immunostimulant activity of certain plants used in Indian traditional Medicine". *Journal of Medicinal Plants Research* 7 (2013): 3242-3246.

10. Singh KL and Bagh GC. "Phytochemical analysis and determination of total phenolics content in water extracts of three species of Hedychium". *International Journal of Pharm Tech Research CODEN (USA)* 5.4 (2013): 1516-1521.

11. Sravani T and Paarakh PM. "Antioxidant property of Hedychium spicatum Buch-Ham. Rhizomes". *Indian Journal of Natural Products and Resources* 3.3 (2012): 354-358.

12. Divya K., *et al*. "Study of antiasthmatic properties and chemical characterization of indigenous ayurvedic compounds (polyherbal formulations)". *American Journal of Phytomedicine and Clinical Therapeutics* 6 (2013): 457-466.

13. Choudhary GK and Singh SP. "Cytotoxic potential of rhizome extracts of *Hedychium spicatum* L. in HepG2 cell line using MTT". *The Indian Journal of Animal Sciences* 87 (2017): 313-315.

14. Thapliyal S., *et al*. "Hepatoprotective and Antioxidant Activity of Methanol Extract of *Hedychium spicatum* against CCl4-Induced Liver Injury in Rats". *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 5.2 (2014): 1428.

15. Lapied B., *et al*. "Indoxacarb, an oxadiazine insecticide, blocks insect neuronal sodium channels". *British Journal of Pharmacology* 132.2 (2001): 587-595.

16. Haas PJ. "Evaluation of subacute toxicity of carbamate on hematological and serobiochemical changes in rats". *American Journal of Veterinary Research* 44 (1983): 879.

17. Frame SR. "Oncogenicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) eighteen-month feeding study in mice. Unpublished report No. HLR 799-96 from DuPont Haskell Laboratory, Newark, Delaware, USA". Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA (1997).

18. Mertens JJWM. "Subchronic oral toxicity: 90-day study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) feeding study in dogs. Unpublished report No. HLO 494-95, RV3 from WIL Research Laboratories, Inc., Ashland, Ohio, USA". Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA (1997).

19. Boyse EA., *et al*. "Cytotoxic test for demonstration of mouse antibody". *Methods in Medical Research* 10 (1964): 39-47.

20. Rai-el-Balhaa G., *et al*. "Lymphoblastic transformation assay of sheep peripheral blood lymphocytes: a new rapid and easy-to-read technique". *Comparative Immunology, Microbiology and Infectious Diseases* 8.3-4 (1985): 311-318.

21. Chauhan RS. "Lymphocyte stimulation test in- laboratory Manual of Immunology for DBT sponsored training course on "Immunopathology: Modern trends in diagnosis and control" (1998): 29-30.

22. Talwar GP. "A Handbook of Practical Immunology". Vikas Publishing House, Pvt. Ltd., New Delhi (1983).

23. Phanuphak P., *et al*. "Tolerance and contact sensitivity to DNFB in mice". *The Journal of Immunology* 112 (1974): 115-123.

24. Engvall E and Perlmann P. "Enzyme linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G". *IImmunochemistry* 8 (1971): 871-874.

25. Chalghoumi R., *et al*. "Hen egg yolk antibodies (IgY), production and use for passive immunization against bacterial enteric infections in chicken: A review". *Biotechnologie, Agronomie, Société et Environnement* 13 (2009): 295-308.

26. Kumar A., *et al.* "Effect of indoxacarb induced toxicity on biochemical parameters in broilers (*Gallus domesticua*)". *Journal Of Veterinary Pharmacology and Toxicology* 12 (1-2) (2014): 21-23.

27. Kaur S., *et al*. "Indoxacarb interaction alters immunotoxic and genotoxic potential of endotoxin". *Journal of Pesticide Science* 41.3 (2016): 65-70.

28. Frame SR. "Oncogenicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) two-year feeding study in rats". Unpublished report No. HLR 1174-96, RV1 from DuPont Haskell Laboratory, Newark, Delaware (1997).

29. Mertens JWM. "Chronic toxicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX- KN127) one year feeding study in dogs". Unpublished report No. HLO 885-96, RV1 from WIL Research Laboratories, Inc., Ashland, Ohio, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA (1997).

30. Bloom JC and Brandt JT. "Toxic responses of the blood," In Casarett and Doul's Toxicology (C. D. Klaassen, Ed.), Mcgraw-Hill, New York (2001): 389-417.

31. Setoguchi R., *et al*. "Homeostatic maintenance of natural Foxp3+ CD25+ CD4+ regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization". *Journal of Experimental Medicine* 201.5 (2005): 723-735.

32. Taniguchi T., *et al*. "Structure and expression of a cloned cDNA for human interleukin-2". *Nature* 302 (1983): 305-310.

33. Boyman O and Sprent J. "The role of interleukin-2 during homeostasis and activation of the immune system". *Nature Reviews Immunology* 12 (2012): 180-190.

34. Farrar MA and Schreiber RD. "The molecular cell biology of interferon- and its receptor". *Annual Review of Immunology* 11 (1993): 571-611.

35. Oberholzer A., *et al*. "Cytokine signaling-regulation of the immune response in normal and critically ill states". *Critical Care Medicine* 28.4 (2000): N3-N12.

36. Singh BP., *et al*. "Immunotoxicity of carbaryl in chicken". *Indian Journal of Experimental Biology* 45 (2007): 890-895.

37. Uttara J and Mishra SH. "Preliminary evaluation of immunomodulatory and antistress activity of methanol extract of Hedychium spicatum". *Pharmacologyonline* 1 (2009): 1057-1071.

38. Luster MI., *et al*. "Approches and concepts in immunotoxicology, in: Experimental immunotoxicology". Edited by R. J. Smialowicz and M. P. Holsapple, (CRC Press, New York) (1996): 13.

39. Danneberg AM. "Delayed type hypersensitivity and cell mediated immunity in the pathogenesis of tuberculosis". *Immunology Today* 12 (1991): 228.

40. Malik G., *et al*. "Biochemical and immunological alteration induced by chlorpyriphos intoxication in broiler chicks". *The Indian Journal of Animal Sciences* 75.6 (2005): 668-671.