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Study of immunomodulatory activity of Health' Boost-Herbal poultry feed supplement in rats

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ABSTRACT

In the present study, an attempt has been made to explore the Immunomodulatory activity of Health' Boost (HB) – a poultry feed supplement in Cellular and Humoral immune response rat model. Two doses of the trial drug (600 & 800 mg/kg) were administered in rats. The assessment of Humoral immunity was carried out by HA titre and was found to be significantly increased (P<0.0001) when compared with HB control and Immunosuppressed CP control group. Whereas, the DTH response was evaluated by % increase in paw edema and the results were found to be increased significantly (P<0.0005) in HB treated rats. Increase in the DTH response indicates that Health Boost has a stimulatory effect on lymphocytes and accessory cell types required for the expression of the reaction. Administration of HB at both the levels showed statistically significant (P<0.0002) increase in WBC count when compared to Cyclophosphamide treated and control treated mice. To understand the mechanism of lymphoid organs and other tissues for maintaining the strong immune system, so in the present study we also studied the effect of Health' Boost on weight of various organs and it revealed a significant increase in Spleen (P<0.0033) and Thymus weight (P<0.0027) respectively. Further clinical studies have to be conducted to extend these results and understand the exact mechanism of bioactive molecules present in the formulation, thereby confirm its traditional use as an Immunomodulator.

Keywords: Health' Boost (HB), Immunomodulation, Sheep red blood (SRBC) cells, HA titre, DTH.

INTRODUCTION

Herbal medicine has become an integral part of standard Health care based on combination of time honoured traditional usage and ongoing scientific research. Some of the medicinal plants are believed to enhance the natural resistance of the body to infections.[1] Herbal drugs possess immunomodulatory property and generally act by stimulating both specific and nonspecific immunity. Immunology is a branch of microbiology which defines the study of defence mechanism of the body against harmful invading causes and it is one of the most developing and crucial area of biomedical research.[2] The immune system evolved to discriminate self from non self. Innate or natural immunity is broadly reactive, does not require priming, and is of relatively low affinity.

Adaptive immunity is antigen-specific, depends upon antigen exposure or priming, and can be of very high affinity. The two arms of immunity work closely together, with the innate immune system being more active early in an immune response and adaptive immunity becoming progressively dominant over time. The major effectors of immunity are granulocytes, monocytes/macrophages, natural killer cells, mast cells, and basophils, B and T-lymphocytes. The impact of the immune system in human disease is enormous. Immunological diseases (e.g., rheumatoid arthritis, type 1 diabetes mellitus, and asthma; solid tumors and hematologic malignancies) are growing at epidemic proportions that require aggressive and innovative approaches to develop new treatments.[3] Immunomodulators are natural or synthetic substances that help to regulate or normalize the immune system, which

are recommended for people with autoimmune diseases and they are widely used in chronic illness to restore health in people who have been on lengthy courses of antibiotics or anti-viral therapies. Plant sterols and sterolins are excellent immunomodulators found in waxy fruits and vegetables although they are lost when vegetables and fruits are cooked.

Synthetic immunomodulator such as azathioprine, 6-mecaptopurine and methotrexate work by suppressing the immune system and decreasing inflammation in the digestive tract in people with inflammatory bowel disease, ulcerative colitis, and Crohn's disease.[4] The various organs of the immune system (lymph nodes and spleen) are essential for the production and development of the various classes of Lymphocytes.[5] Hypersensitivity is an immunologic state of an individual to particular antigens interactions of which with specific antibodies or sensitized cells lead to harmful or occasionally fatal results. These reactions are called hypersensitivity reactions.[6] Cyclophosphamide acts on both cyclic and intermitotic cells, resulting in general depletion of immune competent cells. Cyclophosphamide (CP) is an alkylating agent widely used in anti-neoplastic therapy. It is effective against a variety of cancers such as lymphoma, myeloma and chronic lymphocytic leukaemia. CP induced immunosuppression is reported to prompt various types of infection.[7,8] The present study was done to investigate the Immunomodulatory activity of Health Boost-a poultry feed supplement in rats.

MATERIALS AND METHODS

Sample collection

The drug sample Health Boost was obtained from Suguna Foods Pvt Ltd, Herbal Division, Suguna Lifeherbs, Coimbatore, Tamil Nadu- 641018.

Drugs & Chemicals

Cyclophosphamide tablets (Endoxan, Cadila Heathcare Ltd.), Total Protein estimation kit (Erba Mannheim, Baddi, Himachal pradesh, india). All other chemicals and reagents used are of laboratory grade, purchased from S D fine-chem Ltd, Mumbai, India.

Preliminary Phytochemical Investigation

The drug sample was used for preliminary phytochemical screening with a series of chemical tests viz., Molisch's, Fehling's, Benedicts and Barfoed's test for carbohydrates; Biuret and Millon's tests for proteins; Salkowski and Libermann-Burchard's reactions for steroids; Legal's and Baljet's test for glycosides; Hager's test for alkaloids; and ferric chloride, Lead acetate tests for tannins and phenols.[9]

Animals

Wistar rats (150-200 g) of either sex were used in the study. The animals were kept under standard conditions of light and dark cycle with food and water. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize (if any) non-specific stress. The experimental protocols were approved by the Institutional Animal Ethics Committee (PESCP / IAEC/ 15 /2015) and conducted according to CPCSEA guidelines, Govt. of India.

Preparation of antigen

Sheep red blood cells (SRBC) were collected in Alsever's solution and were washed 3-4 times with large quantity of sterile and pyrogen free saline. Then, the cells are adjusted to a concentration of 0.1 mL containing 1×10^8 cells for immunization.[10,2]

Humoral antibody (HA) and delayed type hypersensitivity (DTH) response

Antigen challenge

On 0^{th} day, all groups were sensitized with 0.1 ml of SRBC containing 1×10^8 cells, i.p.

Experimental design

Animals were divided into different groups each containing 6 animals
Group I - vehicle (normal saline)
Group II – Health' Boost (HB) control, 400 mg/kg, p.o (1-14 days)
Group III - Positive control, Cyclophosphamide (CP), 50 mg/kg, p.o (12^{th}, 13^{th} & 14^{th} day)
Group IV & V – CP (50 mg/kg, p.o, 12^{th}, 13^{th} & 14^{th} day) and HB (600 & 800 mg/kg), p.o, (1-14 days)

Haemagglutination antibody (HA) titre

On 15^{th} day, blood was withdrawn from retro-orbital plexus of each animal under anaesthesia. Blood was centrifuged, and serum was separated. Antibody levels were determined by haemagglutination technique using 96

wells (12x8) flat bottomed titre plates. 25 µl of serum was serially diluted with 25 µl of phosphate-buffered saline. Sheep RBC (1 x 10^8 cells) was added to each of plates and was incubated at 37ºC for 1 hr. Highest dilution that has shown visible agglutination was considered as haemagglutination antibody titre.[11, 12]

Delayed Type Hypersensitivity (DTH) response

On 14th day prior to injection, right hind footpad thickness was measured with Digital vernier calliper (Bliss classic, Yamayo). Then animals were challenged by injecting 1% SRBC (20 µl) into the right hind footpad. On 15th day footpad thickness was again measured. Difference between prior and post challenge footpad thickness was reported as DTH response.[11]

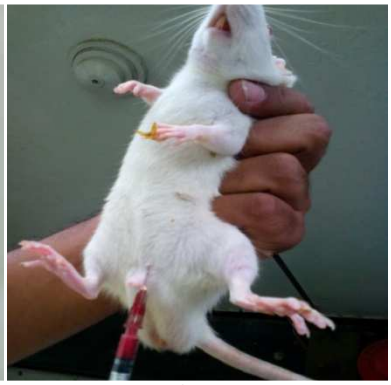
Haematological analysis

The fresh whole blood samples were used for the estimation of WBC.[13]

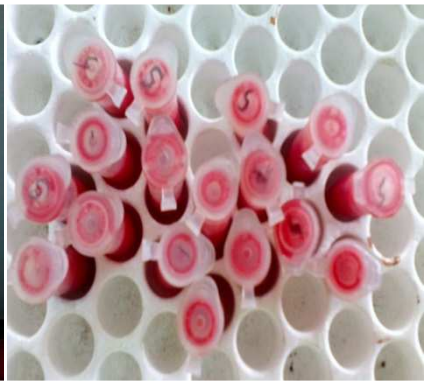
Statistical analysis

The values were calculated as mean ± SEM. The significance of the difference of the mean value with respect to control group was analysed by one way ANOVA followed by Tukey's post test. P<0.05 was considered significant.

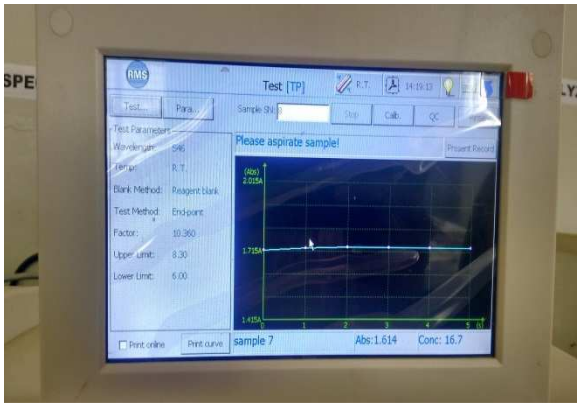
SRBC



Admin of SRBC



PBS Diluted SRBC



Biochemical parameters estimation using auto analyser

RESULTS AND DISCUSSION

In the present study, an effort was made to explore the Immunomodulatory activity of Health' Boost- a poultry feed supplement, using various *in vivo* screening models in experimental animals. To study the humoral and cellular immune response, Sheep red blood cells (SRBC) suspension used for antigen challenge and immunization against cyclophosphamide in rats / mice. When the animals are sensitized with SRBC, it will get diffused in the extra vascular space and enters the lymph node via the lymphatics. Particulate antigens are taken up by macrophages lining the sinuses or disperse in the lymphoid tissues and are processed. Table 1 & Figure 1 shows the assessment of humoral immunity which was carried out by Haemagglutination antibody (H.A) titre to SRBC, a T-dependent antigen. The HA titre in HB control group (400 mg/kg) was significantly (P<0.0001) increased when compared with normal control group. Whereas, in CP control group it was decreased when compared to normal rats. The rats treated with HB (600 & 800 mg/kg) + CP were shown increase in HA (P<0.0001) titre when compared with the immunosuppressed CP control group.

Table 1: Effect of Health' Boost on Humoral immune response

Groups (n=6)	HA titre
Control (normal saline)	15.26 ± 1.358
HB control, 400 mg/kg	22.50 ± 2.00***
Negative control, CP 50 mg/kg	8.00 ± 0.843***
CP + HB, 600 mg/kg	30.58 ± 1.334***
CP + HB, 800 mg/kg	33.93 ± 2.253***

*Values are expressed as Mean ±SEM (n=6). Statistical analysis was carried out by One way Anova followed by Tukey-Kramar multiple comparison test, ***P<0.0001.*

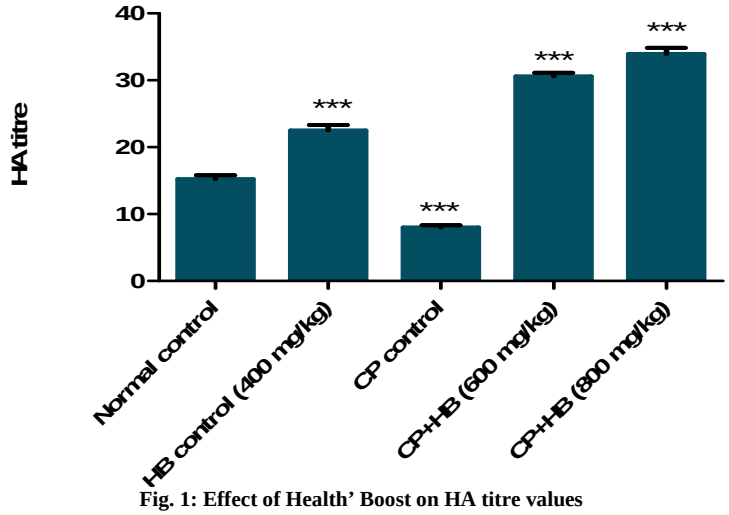


Fig. 1: Effect of Health' Boost on HA titre values

Delayed type hypersensitivity (DTH) reaction represents cell mediated immune response that exerts important immunoprotective or immunopathological effect. When it activates TH cells encounter certain antigens such as SRBC. They secrete cytokines that induce a localized inflammatory reaction called DTH. SRBC-induced delayed type of hypersensitivity was used to assess the effect of Health' Boost on cell mediated immunity (CMI) by measuring the severity of footpad edema in cyclophosphamide induced immunosuppressed rats. The DTH reaction was significantly increased (P<0.0005) in HB + CP treated group when compared with the CP treated control group. Whereas, the HB control was not significant when compared with normal control. Increase in the DTH response indicates that Health' Boost has a stimulatory effect on lymphocytes and accessory cell types required for the expression of the reaction (Table 2 & Figure 2).

Table 2: Effect of Health' Boost on Cellular immune response

Groups (n=6)	% Increase in paw oedema
Control (normal saline)	15.48
HB control, 400 mg/kg	17.78
Negative control, CP 50 mg/kg	8.64***
CP + HB, 600 mg/kg	20.43*
CP + HB, 800 mg/kg	27.28***

*Values are expressed as Mean ±SEM (n=6). Statistical analysis was carried out by One way Anova followed by Tukey-Kramar multiple comparison test, ***P<0.0005.*

We also carried out the Total WBC count (Table 3 & Figure 3), which showed a significant result (P<0.0001) in rats treated with HB (600 & 800 mg/kg) + CP when compared to CP control group. The levels of serum total protein in HB treated rats were also found to be significant (P<0.0002) when compared with CP control group (Table 4).

Table 3: Effect of Health' Boost on Total Leucocyte count

Groups (n=6)	WBC count (1000/mm^3)
Control (normal saline)	8.99 ± 0.338
HB control, 400 mg/kg	10.08 ± 0.556***
Negative control, CP 50 mg/kg	3.16 ± 0.260***
CP + HB, 600 mg/kg	6.45 ± 0.481***
CP + HB, 800 mg/kg	7.75 ± 0.197***

*Values are expressed as Mean ±SEM (n=6). Statistical analysis was carried out by One way Anova followed by Tukey-Kramar multiple comparison test, ***P<0.0001.*

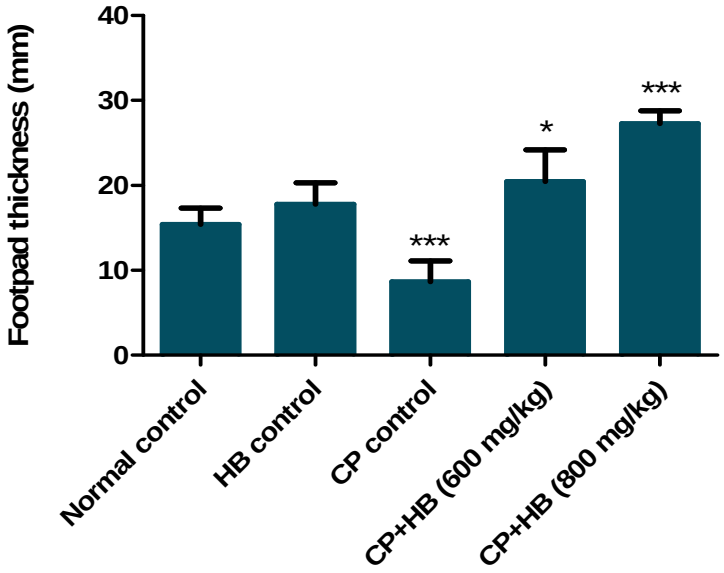


Fig. 2: Effect of Health' Boost on Footpad thickness

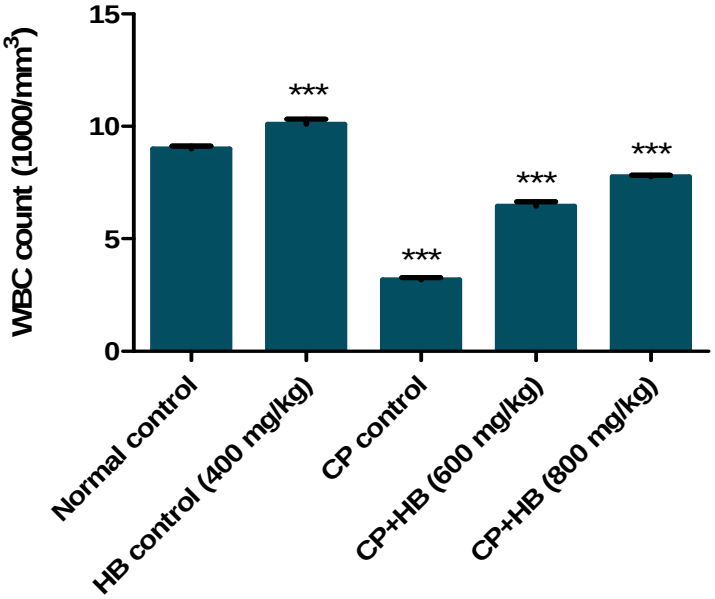


Fig. 3: Effect of Health' Boost on Total Leucocyte count

Table 4: Effect of Health' Boost on Serum Total protein

Groups (n=6)	Total protein (g/dL)
Control (normal saline)	6.24 ± 0.554
HB control, 400 mg/kg	6.42 ± 0.288
Negative control, CP 50 mg/kg	5.34 ± 0.219***
CP + HB, 600 mg/kg	6.25 ± 0.527**
CP + HB, 800 mg/kg	6.67 ± 0.397***

*Values are expressed as Mean ±SEM (n=6). Statistical analysis was carried out by One way Anova followed by Tukey-Kramar multiple comparison test, ***P<0.0002.*

As the body lymphoid organs and other tissues are responsible for maintaining the strong immune system, it is necessary to understand the mechanism involved. So, in the present study we also studied the effect of Health' Boost on weight of various organs and it revealed a significant increase in the weight of Spleen (P<0.0033) and Thymus (P<0.0027) respectively.

Table 5: Effect of Health' Boost on Relative organ weight

Groups (n=6)	Spleen (g)	Thymus (g)
Control (normal saline)	0.30 ± 0.055	0.24 ± 0.0350
HB control, 400 mg/kg	0.39 ± 0.0044*	0.14 ± 0.0204***
Negative control, CP 50 mg/kg	0.28 ± 0.0501**	0.10 ± 0.0187***
CP + HB, 600 mg/kg	0.35 ± 0.0288*	0.12 ± 0.0175
CP + HB, 800 mg/kg	0.37 ± 0.0314**	0.19 ± 0.0187***

*Values are expressed as Mean ±SEM (n=6). Statistical analysis was carried out by One way Anova followed by Tukey-Kramar multiple comparison test, **P<0.0033, **P<0.0027, ***P<0.0001.*

CONCLUSION

The present study concludes that Health' Boost- a poultry feed supplement formulation developed by Suguna Foods Pvt Ltd, Herbal Division, Suguna Lifeherbs, exhibited Immunomodulatory activity. This could be useful to improve or compromise the immune system. However, further studies are necessary to understand the exact mechanism of the bioactive molecules present in this formulation which are responsible for Immunomodulatory activity.

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