

Assessment of Humoral Immune Response in Rabbits Fed with *Cassia Occidentalis*

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Abstract:

Cassia occidentalis was tested for its humoral immuno modulatory effects by comparing it with a proprietary immunostimulant, levamisole HCL using dexamethasone induced immuno suppression model in rabbits. Thirty six male Newzealand White rabbits were divided into six groups (I, II, III, IV, V, and VI) of six animals in each. Group I was the untreated control.

Group II was dexamethasone sodium (Immunosuppressive) treated. Group III was Levamisole Hcl (Immuno stimulant) treated. Group IV was *Cassia occidentalis* treated. Group V was levamisole and dexamethasone treated and Group VI was dexamethasone and *Cassia occidentalis* treated group. Levamisole Hcl was given at 2.5 mg/kg subcutaneously thrice a week, dexamethasone sodium was given at the rate of 2mg/kg intra muscularly for seven days and *Cassia occidentalis* was given @ 1000 mg/kg orally for 10 days.

To assess the humoral immune response, estimations of antibody titre, total immunoglobulin concentration and haematology were carried out. *Cassia occidentalis*, Dexamethasone + *Cassia occidentalis* and control groups showed no significant differences in the estimation of antibody titre where as dexamethasone group showed significant decrease in the titre as compared to other groups tested. There were no significant changes noticed between *Cassia occidentalis*, dexamethasone, dexamethasone + *Cassia occidentalis* groups in total immunoglobulin concentration. The values of

haematology among groups tested were insignificant. The estimation of antibody titre in the study suggests that the methanolic extract of *Cassia occidentalis* has the potential in reversing the dexamethasone induced immunosuppression in rabbits.

Key words: Dexamethasone, Levamisole, Rabbits, Immunomodulation

Introduction

Several herbs have been used traditionally to prevent and treat human and animal diseases. Recently, scientific evaluation of plants and preparations of plant origin medications have received more attention. *Cassia occidentalis* had been reported to be used as antidote of poison, blood purifier, expectorant, anti inflammatory agent and a remedy for the treatment of liver diseases (Ghani, 1920; Kabiruddin, 1951). *Cassia occidentalis* was tested for antifeedant and insecticidal properties. It was studied successfully for its anti-parasitic (Tona *et al.*, 1999), hepatoprotective activity (Jafri *et al.*, 1999). Stimulation of the immune system is highly desirable for the treatment of immunodeficiency and infectious diseases. The anti-inflammatory and hepatoprotective effects of *Cassia occidentalis* may relate to an influence of plant compounds on immunocompetent cells. The present study was undertaken to find out the effect of methanolic extract of *Cassia occidentalis* leaves on the humoral immune response in rabbits.

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Materials and Methods

Extraction

The leaves of the plant was air dried and 150 grams of air dried powder was extracted with 400 ml of 80% methanol (v/v) using soxhlet apparatus and the extract was subjected to rotary evaporator for removal of the solvent. The paste form of extract was obtained using hot air oven and kept at room temperature for use.

Experimental animals and chemicals used

Thirty six Newzealand white crossbred male rabbits weighing between 1000 and 1500 g obtained from the Department of Laboratory animal Medicine, TANUVAS, Madhavaram, Chennai-51, were used for the study.

All the animals were given a balanced diet and maintained under identical environmental conditions. Immunostimulatory drug levamisole hydrochloride (Alved, Chennai) and immunosuppressive drug dexamethasone sodium (Merind, Mumbai) were used for this study.

Experimental design

Thirty six male Newzealand White rabbits were divided into six groups (I, II, III, IV, V, and VI) of six animals in each. Group I was the untreated control.

Group II was dexamethasone sodium (Immunosuppressive) treated. Group III was Levamisole Hcl (Immuno stimulant) treated. Group IV was *Cassia occidentalis* treated. Group V was levamisole and dexamethasone treated and Group VI was dexamethasone and *Cassia occidentalis* treated group.

Levamisole Hcl was given at 2.5 mg/kg subcutaneously thrice a week, dexamethasone sodium was given at the rate of 2mg/kg intra muscularly for seven days and *Cassia occidentalis* was given @ 1000 mg/kg orally for 10 days. To assess the humoral immune response, estimations of antibody titre, total immunoglobulin concentration and haematology were carried out.

Assays

Estimation of antibody titre

All the eight groups of rabbits were immunized with typhoid-H antigen (King Institute, Guindy, Chennai) at the dose of one ml subcutaneously on 1st day and 15th day. Widal tube agglutination test was performed using widal test kits (Span Diagnostics Ltd.,) on days 0,7,14 and 21 for antibody titre.

Estimation of total immunoglobulin concentration

Total immunoglobulin concentration was assessed by ammonium sulphate precipitation and dialysis method (Talwar and Gupta, 1983). Five ml of saturated ammonium sulphate solution was added slowly to 5ml serum in a test tube to get half saturation of ammonium sulphate in the sample. Contents were mixed continuously by swirling the tube without agitating excessively. The mixture was allowed to stand for 5 minutes so that globulin precipitates. The material was centrifuges for 10 minutes at high speed. The precipitate was allowed to settle down at the bottom of the tube. The supernatant fluid (containing albumin) was poured off and the precipitate was redissolved in 1 ml

distilled water. The dissolved precipitate was put in a semi permeable cellophane tube, and each end of tube was tied in a distilled water beaker. The distilled water was frequently changed to dialyse out ammonium sulphate ions completely. The contents of the cellophane tube were dissolved in 5 ml of normal saline. The globulin concentration was measured in spectrophotometer and the O.D. values obtained was multiplied by 0.70 (conversion factor to get total immunoglobulin concentration). Total erythrocyte count (RBC) was done by Hayem's dilution method. Haemoglobin (Hb) estimation was done by Sahil's acid haematin method and total leukocyte count (WBC) was done by standard dilution method. The results were analysed as per Snedecor and Cochran (1994).

Results and Discussion

Antibody titre

Dexamethasone caused significant decrease in the antibody titre (14.17 ± 2.17) when compared to all other groups (Table-1). This is in accordance with the findings of Pruett *et al.*, (1987). Levamisole produced significant increase in titre when compared to control and was able to overcome the dexamethasone suppressed antibody titre. Similar results were obtained by Babiuk and Mishra (1981). The control, *Cassia occidentalis* and Dexamethasone + *Cassia occidentalis* groups showed relatively similar values in antibody titre (56.17 ± 0.03 , 55.00 ± 7.10 , 45.83 ± 6.71 respectively). Dexamethasone + Levamisole groups showed significant increase in antibody titre (183.33 ± 29.02).

Table-1 Effect of immunomodulators on serum antibody titre

Groups	7th day	14th day	21st day	Group means \pm
Control	52.54 ± 0.02	76.06 ± 0.06	96.07 ± 0.06	$56.17c \pm 0.03$
Dexamethasone	10.00 ± 4.47	20.00 ± 0.00	26.67 ± 4.22	$14.17d \pm 2.17$
Levamisole	73.33 ± 6.67	133.33 ± 16.87	213.33 ± 33.73	$105.00b \pm 61.27$
<i>Cassia occidentalis</i>	53.33 ± 8.43	73.33 ± 6.67	93.33 ± 13.33	$55.00c \pm 7.10$
Dexamethasone + Levamisole	120.00 ± 17.89	293.33 ± 26.67	320.00 ± 71.55	$183.33a \pm 29.02$
Dexamethasone + <i>Cassia Occidentalis</i>	50.00 ± 10.00	66.67 ± 8.43	66.67 ± 8.43	$45.83c \pm 6.71$

($P \leq 0.05$)

Total serum immunoglobulin concentration

There were no significant differences between dexamethasone, *Cassia occidentalis*, dexamethasone + *Cassia occidentalis* groups ($32.39 \pm$

0.19 mg/ml, 32.69 ± 0.13 mg/ml, 32.76 ± 0.42 mg/ml respectively). The dexamethasone group showed significant decrease in total serum immunoglobulin level when compared to control group

(Table-2). The inhibitory effect produced by dexamethasone is in accordance with the findings of Anderson et al., (1999). Dexamethasone + Levamisole showed significant increase in the total serum

immunoglobulin levels (41.40 ± 0.21 mg/ml). *Cassia occidentalis* caused a decline in immunoglobulin level and it could not restore the immunoglobulin level in immune suppressed animals.

Table-2 Effect of immunomodulators on serum immunoglobulin concentration (mg/ml)

Groups	7thday	14th day	21st day	Group means \pm SE
Control	33.01 ± 0.023	33.25 ± 0.02	33.27 ± 0.004	$32.94c \pm 0.01$
Dexamethasone	31.78 ± 0.16	32.92 ± 0.05	32.50 ± 0.28	$32.39cd \pm 0.19$
Levamisole	35.63 ± 0.19	35.74 ± 0.17	36.56 ± 0.53	$35.25b \pm 0.28$
<i>Cassia occidentalis</i>	33.56 ± 0.19	33.08 ± 0.04	32.89 ± 0.26	$32.69c \pm 0.13$
Dexamethasone + Levamisole	39.97 ± 0.13	43.63 ± 0.16	48.94 ± 0.09	$41.40a \pm 0.21$
Dexamethasone + <i>Cassia Occidentalis</i>	40.41 ± 0.20	44.39 ± 0.16	47.70 ± 0.17	$32.76c \pm 0.42$

($P \leq 0.05$)

Hematology

Dexamethasone produced decrease in WBC levels (Table-3). This is in accordance with the findings of Wilkie et al., (1979). There were no changes in the

RBC and Hb levels between groups. It is obvious that the body haem factors were not stimulated like immune factors by the drugs.

Table-3 Effect of immunomodulators on haematological parameters

Groups	Total RBC(X106)cmm	Hb (g/dl)	Total WBC(cmm)
Control	5.88 ± 0.02	12.29 ± 0.06	$7873.79a \pm 22.65$
Dexamethasone	5.82 ± 0.03	12.15 ± 0.10	$7354.42b \pm 31.79$
Levamisole	5.89 ± 0.03	12.32 ± 0.12	$7902.96a \pm 53.15$
<i>Cassia occidentalis</i>	5.84 ± 0.07	12.19 ± 0.15	$7767.50a \pm 16.99$
Dexamethasone + Levamisole	5.90 ± 0.04	12.30 ± 0.10	$8034.67a \pm 49.46$
Dexamethasone + <i>Cassia Occidentalis</i>	5.84 ± 0.05	12.27 ± 0.15	$7728.79a \pm 109.46$

($P \leq 0.05$)

Conclusion

The estimation of antibody titre in the study suggests that the methanolic extract of *Cassia occidentalis* has the

potential to improve immunity in rabbits whereas the levels of immunoglobulin could not be reversed by *Cassia*

occidentalis in the presence of dexamethasone in rabbits.

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