

Evaluation of cell mediated immune response in rabbits fed with *Cucurbita maxima* seeds

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ABSTRACT

Cucurbita maxima seed was tested for its immunomodulatory effect by comparing with immunostimulant, Levamisole HCL using dexamethasone induced immuno suppression model in rabbits in terms of assessing cell mediated immune response. Thirty six rabbits were randomly divided into six groups of six animals in each. Group I was the untreated control. Group II was dexamethasone sodium treated. Group III was levamisole HCL treated. Group IV was treated with *Cucurbita maxima* seeds. Group V was levamisole and dexamethasone treated and Group VI was treated with dexamethasone and *Cucurbita maxima*. Levamisole HCL was given at the rate of 2.5 mg/kg subcutaneously thrice a week, dexamethasone sodium was given at the rate of 2 mg/kg intramuscularly for seven days and *Cucurbita maxima* was given at the rate of 1000 mg/kg orally for 10 days. To assess the cell mediated immune response, leukocyte migration immigration test, delayed type hypersensitivity test and haematological indices were carried out. The body weight and spleen weight were also recorded. The results indicated that there were significant increase in % leukocyte migration inhibition in *Cucurbita maxima* and dexamethasone + *Cucurbita maxima* treated groups as compared to control. In tuberculin sensitivity, *Cucurbita maxima*, dexamethasone + *Cucurbita maxima*, levamisole, dexamethasone + levamisole showed significant increase in skin thickness as compared to control. This study suggested that the *Cucurbita maxima* seed has the potential to reverse dexamethasone induced cell mediated immunosuppression in rabbits.

Key words: *Cucurbita maxima*, Dexamethasone, Immunity, Levamisole, Rabbits

INTRODUCTION

Modulation of immune responses to alleviate infectious diseases has been of interest for many years. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment for various diseases has been on the rise in the last few decades (Fong, 2002). *Cucurbita maxima* is commonly used as antidiabetic (Xia, 2007) and internally as well as externally for management of worms and parasites. In the last few decades, research has been focused on the antihypertension, antitumor, antibacteria, antifungal, anti hypercholesterolemia and anti-inflammatory effects of this plant commonly termed as pumpkin (Cheong *et al.*, 1997, Vassiliou *et al.*, 1998, Fahim *et al.*, 1995). Most of the therapeutic effects of medicinal plants in the treatment of infectious diseases are mainly due to their effects on the immune system (Jafarian *et al.*, 2012). Hence, the present study was undertaken to assess the effect of *Cucurbita maxima* seed on cell mediated immune response in rabbits.

MATERIALS AND METHODS

Thirty six male Newzealand White rabbits weighing 1000-1500g were randomly divided into six groups with six animals in each. Group I was the untreated control. Group II was treated with dexamethasone sodium, an immunosuppressing agent at the rate of 2mg/kg intramuscularly for seven days and group III was treated with proprietary immunostimulant, Levamisole hydrochloride at the rate of 2.5 mg/kg subcutaneously thrice a week. Group IV was fed with *Cucurbita maxima* (shade dried powder preparation) at the rate of 1000 mg/kg PO for 10 days. Group V was treated with levamisole and dexamethasone sodium and group VI was treated with dexamethasone sodium and *Cucurbita maxima* seed (Shade dried crude powder preparation). This study was approved by Institutional Animal Ethical Committee. Every week, body weight of the animals was recorded. The animals were slaughtered on day 21 and the spleen weight was recorded and the blood samples were collected for analysis.

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Cell mediated immunity response was assessed by Leukocyte Migration Inhibition (LMI) Test (Hudson and Hay, 1980). Briefly, 3 ml of Histopaque was taken in a test tube. Four ml of heparinised blood collected from rabbits was layered above histopaque with care taken to avoid mixing of the two materials. This test tube was spun at 4000 rpm for 30 min and the blood separated into plasma layer at the top and creamy buffy coat. This buffy coat was removed carefully and put in to fresh eppendorf tubes.

Approximately double the volume of HBSS with EDTA was added to buffy coat and the suspension was spun at 3000 rpm. The supernatant was discarded and the pellet obtained was washed twice using HBSS without EDTA and then with PBS, pH 7.2. Each time the suspension was spun at 3000 rpm. After final wash, the pellet was suspended in 0.5 ml of PBS with a pH of 7.2.

The dissolved pellet was placed in another eppendorf tubes and capillary tubes were placed over it. Three fourth of the capillary tubes were filled and one end of the tubes was sealed with colourless plasticine. The capillary tubes were placed in fresh test tubes and spun at 3000 rpm for 15 minutes for packing the cells and then it was cut with a fine cutter at the cell fluid interphase. The cell packed capillaries end was placed within the well and held in place by means of non-toxic grease. RPMI medium was added to all the wells and bovine serum antigen (BSA) was added to certain wells. Antigen containing wells were test wells, antigen lacking were control wells. The wells were sealed with circular coverslips using non-toxic grease and placed in CO₂ incubator (Forma Scientific Inc., Ohio, USA) at 37°C for 20 hrs under five percent CO₂ tension. The plates were then observed for migration of leukocytes in the treatment and control groups. Migration zone were drawn in a graph using camera lucida and the area of migration was measured.

$$\text{Per cent area of migration inhibition} = 100 - \frac{\text{Area of migration of cells with antigen}}{\text{Area of migration of cells without antigen}} \times 100$$

A migration inhibition of more than 20% was taken as positive test.

To assess the delayed type hypersensitivity (DTH), tuberculin sensitivity test was performed (Ross *et al.*, 2001). Briefly, 0.1ml of purified protein derivative (PPD) (span diagnostics Ltd., Surat) administered subcutaneously on first day and tuberculin sensitivity was done by again administering

0.1 ml PPD intradermally on 21st day and the grades of induration were measured using vernier callipers after 48 hrs. The neutrophil count was done using Leishman's staining method. The whole blood was tested for absolute lymphocyte count. The data were statistically analysed by ANOVA as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The results of the study are depicted in Table-1 and 2. Dexamethasone group produced a highly significant reduction in percent leukocyte migration inhibition (16.46 ± 0.15) as compared with all other groups. Corticosteroid mediated cell mediated immune suppression was suggested by Jafarian *et al.* (2012). There was significant increase in the % LMI in *Cucurbita maxima* and dexamethasone + *Cucurbita maxima* groups (16.33% and 12.02%, respectively) as compared to control group (19.47 ± 0.03). These effects of LMI might be due to the secretion of macrophage inhibitory factors (MIF) from activated macrophages and T lymphocyte cells induced by *Cucurbita maxima* and levamisole respectively. Dexamethasone + levamisole group showed significant increase in LMI (22.90%) as compared to other groups. This is evident since levamisole is known to restore corticosteroid induced depletion of lymphocytes.

The skin thickness increase was lower in the dexamethasone group ($2.51 \pm 0.03\text{mm}$) when compared to the control ($3.25 \pm 0.01\text{mm}$). When compared to control *Cucurbita maxima*, Dexamethasone + *Cucurbita maxima*, levamisole, Dexamethasone + levamisole showed significant increase in skin thickness (31.69%, 48%, 7.08% and 41.54%, respectively). DTH response could be due to sensitized T lymphocytes, when challenged by antigen, they were converted into lymphoblast and secrete a variety of molecules including proinflammatory lymphokines attracting more scavenger cells to the site of reaction (Delves and Roitt, 1998). Increased DTH of this plant might be due to the effect on the lymphocytes and accessory cell types required for the expression of the reaction (Mitra *et al.*, 1999). The reduction in the spleen weight by dexamethasone and the reversal by levamisole and *Cucurbita maxima* clearly indicate the role of spleen in immunity as secondary lymphoid immune organ (Tizzard, 1996). In this study, haematological parameters showed increased neutrophilic and decreased lymphocytic count in dexamethasone treated group. Anderson *et al.* (1999) showed increase in neutrophilic count with suppression of lymphocytic count by the dexamethasone treatment. Seeds of *Cucurbita maxima* could reverse the decreased levels of absolute lymphocyte count when treated in combination with dexamethasone, significantly and the effect was comparable

TABLE 1: Effect of *Cucurbita maxima* on immunological and hematological parameters

Groups		Mean LMI (%)	Skin thickness increase (mm)	Total neutrophil count(%)	Absolute lymphocyte count (Cumm)
Control	I	19.47 ^c ± 0.03	3.25 ^c ±0.01	46.28 ^b ±0.15	5119.75 ^b ±8.96
Dexamethasone	II	16.46 ^d ± 0.15	2.51 ^d ±0.03	56.06 ^a ±1.85	4675.25 ^c ±15.90
Levamisole	III	19.84 ^c ± 0.03	3.48 ^b ±0.02	46.55 ^b ±0.35	5135.46 ^b ±25.55
Cucurbita maxima	IV	22.65 ^b ± 0.11	4.28 ^b ±0.04	45.24 ^b ±0.26	5166.38 ^a ±15.62
Dexamethasone + Levamisole	V	23.93 ^a ± 0.20	4.60 ^a ±0.05	40.85 ^c ±0.57	5194.46 ^a ±16.05
Dexamethasone + Cucurbita maxima	VI	21.81 ^b ± 0.22	4.81 ^a ±0.03	45.50 ^b ±0.18	5170.17 ^a ±14.01

Values (Mean ± S.E.M., n=6) in the same column bearing no superscript common vary significantly (P≤0.05).

TABLE 2: Effect of *Cucurbita maxima* on spleen and body weight related parameters

Groups		Spleen weight(mg/1000g bwt)	Mean Body weight (grams)
Control	I	448.33 ^c ±1.34	1150.58±30.67
Dexamethasone	II	403.00 ^d ±6.48	1138.25±11.82
Levamisole	III	545.00 ^b ±8.06	1151.17±25.20
Cucurbita maxima	IV	576.83 ^a ±3.41	1144.92±10.90
Dexamethasone + Levamisole	V	605.33 ^a ±7.69	1150.71±19.98
Dexamethasone + Cucurbita maxima	VI	578.00 ^a ±4.16	1151.13±11.72

Values (Mean ± S.E.M., n=6) in the same column bearing no superscript common vary significantly (P≤0.05).

with the effect produced by levamisole in rabbits immunosuppressed with dexamethasone. *Cucurbita maxima* could also decrease the neutrophilic count significantly in presence of dexamethasone but the effect was not greater than that of levamisole in presence of dexamethasone. This implies the proreitory immunostimulatory effect of levamisole. It is also evident in the results obtained in leukocyte migration inhibition test.

Body weights of rabbits in different groups did not differ indicating the unchanged health status of animals by these treatments during the period of the study. The capacity of *Cucurbita maxima* seed to reverse the dexamethasone suppressed immunity was comparable to that obtained earlier when using *Punica granatum* plant powder in an identical experimental setting (Ross, 2001). Triterpenoids and phenolic

compounds are said to possess immunomodulators (Raphael and Kuttam, 2003, Du *et al.*, 2011). Pumpkin is a good source of phenolic compounds and terpenoids (Tannin-Spitz *et al.*, 2007, Jafarian, 2012).

It is suggested that *Cucurbita maxima* seeds could reverse the depletion of cell mediated immune response in rabbits and the observed immunomodulatory effects produced by *Cucurbita maxima* seeds in immunosuppressed model could be due to the presence of phytochemicals in the seeds.

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