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Cell mediated immunity dysfunction retrival using the extracts of the plant *Cassytha capillaries* (meissen)

Abstract

The growing incidence of infectious diseases, autoimmune problems and tumour formation are due to the weakness/dysfunction of immune system. Many xenobiotic components that enter human system through food, air and food chain interferes with the functioning of cells and secretions that offer immunity. Pesticides that are being indiscriminately applied get into the human system had been reported to affect the functioning of immune system. To validate this information, in the present study of commonly used organophosphorus pesticide quinolphos was administered to Swiss albino mice and the functioning of cell mediated immunity was measured using the parameter Delayed Type Hyper Sensitivity Reaction (DTH) using sheep red blood cells as a challenging antigen. The pesticide induced a reduction in DTH response. So to improve the DTH dysfunction remedial measures were tried using Standard immune boosting drugs and the extract of a plant *Cassytha capillaries*. The remediating agents were found to retrieve the lost immunity to a significant level.

Keywords: immunity, immunity booster, natural products, DTH, *cassaytha capillaries*

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Ranjitsingh AJA,[1] Padmalatha C,[1] Athinarayanan G,[2] Dhasarathan P[3]

[1]UGC Emeritus Fellow, India

[2]Department of Microbiology, Sri Ram Nallamani Yadava College of Arts and Science, India

[3]Department of Biotechnology, Prathyusha Engineering College, India

Correspondence: Ranjitsingh AJA, UGC Emeritus Fellow El-Shaddai, 1/24 B, Second Street, Gandhi nagar Tirunelveli-627 008, India, Tel 0462-2339169, 9443451076, Email ranjitspkc@gmail.com, ranjitspkc@gmail.com

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Introduction

Cell mediated and antibody mediated defensive mechanism in the immune system protects the body against the invasion of infectious and opportunistic microorganisms and spontaneously arising Neoplasm.[1–4]

As pesticides are extensively used in farming and insect control, it has every chance to get in to the human body through food, water, air and food chain.[5] In the human system pesticide molecules interferes with immunity.[4] To protect the immune system and its functioning, although immunity booster drugs are available, less information is available on safe immunity protective natural products. Hence in the present study an investigation is made to find out the effect of an organophosphorous pesticide on Delayed Type Hyper Sensitivity reaction (DTH) an expression of cell mediated (CMI) immunity and retrival of immunity dysfunction using the extract of the plant *Cassaytha capillaries*. The outcome of the study will help to develop immunity enhancing natural products.

Materials and methods

Extraction of phytochemicals from the plant *c. capillaries*

For the present study a parasitic twinner plant *Cassytha capillaries (*Meissen) was collected from trees in Western Ghat region of Tamil Nadu. The whole plants were shade dried and powdered when dried. The dried powder was used to take extract using the solvent methanol in Soxhelet apparatus. The extract was dried, purified and stored.

Selection of pesticide

Organophosphorous pesticide Quinolphos was chosen. Using Standard methods LC 50 value was fixed. From this LC 50 value a sub lethal dose of 0.1ppm was chosen for the immune suppression studies. The sub lethal dose was dissolved in water and given to the animal through oral route. Weighed quantity of the extract was dissolved in sterilized distilled water, and three concentrations were prepared, viz., 50, 100 and 200mg/kg/day. The plant extract dissolved in water was fed to the mice along with drinking water using a special feeding bottle Quinolphos (Organophosphorous pesticide 0.1ppm) was used as immune–suppressant drug. Proimmu (Envin Bioceuticals, Shorapur, India was used as standard immune potentiating drug).

Antigen (SRBC) preparation

Cellular antigens such as sheep erythrocytes were obtained from fresh blood of sheep sacrificed in the local slaughter house. Sheep blood was collected into Alsevier's solution and stored. Sheep red blood cells (SRBC) were prepared by washing sheep blood in Alsevier's solution thrice by centrifuging at 3000rpm for 10minutes. Packed volume of SRBC is re-suspended to get a concentration of 0.1ml containing $1x10^8$cells for immunization and challenge.

Selection of experiment animal

For the experiments, Swiss albino mice (age 45–60 days) were selected. The mice were fed regularly with water and pellet feed.

Delayed hyper sensitivity response (DTH) assay

SRBC challenge: DTH assay was carried out using Sheep Red Blood Cells (SRBC) as challenging antigen.DTH response in control, standard immune booster drug administered, immunity, suppressed and natural product given mice was determined using the method described by Agarwal et al.

Mice were divided in to eight groups each group containing six mice. Drugs were given to various groups i.e.

Group I - Control

Group II - IV Plan extract given (dose levels of 50mg/kg, 100mg/kg and 200mg/kg)

Group V - Standard immune potentiating drug given Proimmu (30mg/kg)

Group VI- Quinolphos (Organophosphorous pesticide) $Cl_2H_{15}N_2O_3$ immune suppressant

Group VII- *C.capillaries* extract (200mg/kg), and Quinolphos (30mg/kg) treated group

Group VIII- Proimmu and Quinolphos (0.1ppm) treated group

Mice were sensitized by injecting 0.1ml of SRBCs suspension containing $1X10^8$cells in the nape of the neck on day 0.1ml of SRBCs suspension containing $1X10^8$cells in the nape of the neck on day 0. Plant extracts were given to the mice from three days prior to the sensitization and continued till day 7. On day, 7, mice were challenged by sub cutaneous injection of 0.02ml of SRBCs ($1x10^8$cells/ml) in right hind foot pad. The left hind foot pad was given normal saline (0.02ml). Paw thickens was measured after 24, 48, 72 and 96hrs using vermier calipers (Mitotoya digital meter). The difference between the left and right foot thickness, expressed in mm was taken as a measure of DTH.

Quinolphos and Proimmu were administered to groups V and VI along with feed for 3 days prior to sensitization with SRBC. For group VII, the extract of *C. capillaries* (200mg/kg) was given along with Quinolphos (0.1ppm) for 3days prior to sensitization with SRBC.

For group VIII Standard immuno potentiating drug proimmu and Quinolphos (0.1ppm) were given 3 days prior to sensitization with RBC.

Plant extracts and proimmu were given through drinking water from day-3 until 7th day. Skin reactions at the site of SRBC injection were carefully monitored for 72hrs. A positive reaction to SRBC was indicated by edema and induration at the site, measuring 2-5mm diameter. Positive responses were graded as follows,

A. Erythema (+)

B. Erythema with induration (++)

C. Erythema with induration and small blisters (+++)

D. Erythema with induration and large blisters (++++)

The responses 2+, 3+ and 4+ were accepted as evidence for positive reactivity.

Results and discussion

Delayed type hypersensitivity reaction assay

Delayed Type Hypersensitivity (DTH), an expression of cell mediated immune response has been used to asses immunomodulatory mechanism in animals. The DTH assay is a simple and inexpensive method to assess immune response.[6] Immunosuppressive chemicals elevate DTH response by eliminating the population of T-suppressor cells.[7,8] The DTH response to antigenic challenge (SRBC–Sheep Red Blood Cell) provides a useful system for identification of compounds with selective effect on the immune response.[9] The most commonly used *in vivo* assay to determine DTH response is anti-inflammatory cutaneous reaction. Okoli et al.,[10] have identified some active principles like lupeol, premnazole, usnic acid, pinitol zanhasaponins A and B etc. in several plants to function as anti-inflammatory agents. In the present study the T-cell response (CMI) level in the form of DTH response in experimental mice was assessed after administering the *C. Capillaries* extracts and immunosuppressive pesticides (Table 1) (Table 2).

Table 1 Effect of Extracts of *C. capillaries* and standard immuno modulating drug on SRBC induced DTH responses in swiss albino mice

S. No	Experimental Group	DTH Response to SRBC
1	Control	0
2	Immunosuppressed	++++ (4+)
3	*C.capillaries* (50mg/kg)	+++ (3+)
4	*C.capillaries* (100mg/kg)	++ (2+)
5	*C.capillaries* (200 mg/kg)	1

Table 2 Effect of the extracts of *C. capillaries* delayed hypersensitivity (DTH) reactions. Foot pad thickness

Group	Substance	Dose mg/kg	Mean diameter of foot pad thickness (mm) ± SD			Test of significance (24 hrs)
			24 hr	48 hr	72 hr	
I	Control sterile water		0.39 ± 0.02	0.30 ± 0.06	0.21 ± 0.02	Group I Vs IIa
II	*C.capillaries* extract	50	0.42 ± 0.04 (+ 7.69)	0.31 ± 0.04	0.15 ± 0.02	Group I Vs IIIa
III	*C.capillaries* extract	100	0.46 ± 0.03 (+ 17.95)	0.26 ± 0.04	0.18 ± 0.02	Group I Vs IVb
IV	*C.capillaries* extract	200	0.50 ± 0.07 (+28.21)	0.30 ± 0.07	0.2 ± 0.02	Group I Vs Vb
V	Quinolphos	30	0.18 ± 0.4 (-58.33)	0.23 ± 0.02	0.8 ± 0.10	Group I Vs Vb
VI	Proimmu	30	0.58 ± 0.06 (+48.32)	0.34 ± 0.14	0.20 ± 0.12	Group I Vs VIb
VII	*C.capillaries* extract 200mg/kg with Quinolphos 30mg/kg	230	0.38 ± 0.07 (-2.56)	0.26 ± 0.13	0.19 ± 0.02	Group I Vs VII NS
VIII	Quinolphos with Proimmu (each 30mg/kg)	60	0.43 ± 0.04 (+10.26)	0.36 ± 0.10	0.24 ± 0.06	Group I Vs VIIIa

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DTH reactions in mice after SRBCs antigenic challenge was tested for normal, immunity suppressed (Quinolphos given) and immunity stimulated mice (*C.capillaries* extracts and Proimmu). DTH response was high in Proimmu (Standard drug) treated groups the antigenic challenge by SRBC resulted in a significant increase in foot pad thickness in left paw (receiving SRBC), than right paw (receiving normal saline as control) (Table 1) (Table 2). The percentage decrease in paw edema in Quinolphos treated cases was 58.33% but in *C.capillaries* treated group (200mg/kg), it got increased to 28.2%. In Proimmu treated mice also there was a significant increase in DTH response. In the mice treated with *C.capillaries* and Quinolphos, the DTH was significantly improved (+10.26 $P<0.05$). The comparison of these results indicates that the extracts of *C.capillaries* have immuno potentiating effect like the standard immuno stimulant drug Proimmu.

According to Tiwari et al.,[11] the active principle, sesquitepene lactone present in the plant *Tridax procumbens* assisted in cell mediated immune response and enhanced DTH reaction, which was reflected from the increased foot pad thickness due to heightened infiltration of macrophages to the inflammatory site, Datta et al.,[12] reported that the active fraction CI-1-Protein in the plant pigeon pea *Cajanus cajan* enhanced DTH response significantly in mice on SRBC antigenic challenge. Histological investigation by Datta et al.,[12] on the inflammatory site showed perivascular cuffing with mononuclear cells followed by a more extensive exudation of mono and poly morphonuclear cells.[13] The effector cells that promote DTH reactions (T_{DTH} cells) cause the activation of macrophages, infiltration of polymorphonuclear cells, increased vascular permeability and odema, thereby it induced T-cell mediated response[14] also observed an elevation in DTH response in mice treated with the plant extract, *Argyreia speices* and challenged with SRBC and this was due to the effect of plant drug on T-lymphocyte and accessory cell types required for the expression of reaction.[15] According to Hirschman, et al. the extracts of the plant *Cyttaria spp* was able to elevate the DTH response due the activation of CD4 (+) and CD 8 (+) T cells as reported earlier.[16]

Animals treated with Quinolphos had a suppressed foot pad thickness indicating the suppressive act on cell mediated immunity. According to Osario & Goldman[17] the DTH response is associated with T-cells and sensitized T-cells release mediators to promote inflammatory processes. The possible mechanism behind DTH reactions, include, activation of complements, releasing of mediators by activated mast cells; kinin reactive oxygen or nitrogen species by archidonic acid metabolites histamine and pro-inflammatory cytokines.[18] Tiwari et al.[11] stated that the delayed type hypersensitivity reaction is characterized by large influxes of non-specific inflammatory cells, in which macrophage is a major participant. DTH reactions develop when antigen activities sensitized T_{DTH} cells. These cells generally appear to be a T_{H1} subpopulation although sometimes T cytotoxic cells are also involved. Activation of T-delayed type hypersensitive (T_{DTH}) cells by antigen presented through appropriate antigen presenting cells results in the secretion of various cytokines including interleukin-2, interferon-ϒ, macrophage migration inhibition factor and tumor necrosis factor β. The overall effects of these cytokines are to recruit macrophages into the area and activate them, promoting increased phagocytic activity vis-à-vis increased concentration of lytic enzymes for more effective killing. Argus & Woo[18] reported that the immunosuppressive Quinolphos have profound suppressive effect on all forms of DTH and cell mediated immunity. Animals treated with Quinolphos and receiving *C.capillaries* extract showed a significant counteracting effect to Quinolphos induced T-cells suppression. According to Gilberston et al.,[19] the body's immunity has been suppressed in diseases like cancer and AIDS. The chemotherapy and radiation therapy in cancer treatment contribute to depress the immune system. As side effects are associated with these synthetic modulations, plant drugs like *C.capillaries* can be co administered along with chemo therapeutic agents.

In the present study the report on the extracts of *C.capillaries* indicate that the active compound of this plant can be used for chemo-protection against the toxicity induced by pesticide and the combination of the active compounds with receptors of immune system can elevate immune response.

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Conflict of interest

Author declares that there is no conflict of interest.

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