



Original Research Article

<https://doi.org/10.20546/ijcmas.2018.707.415>

Cell Mediated Immunity Dysfunction Retrieval Using the Extracts of the Plant *Cassytha capillaries* (Meissen)

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ABSTRACT

Keywords

Immunity, Immunity booster, Natural products, DTH, *Cassaytha capillaries*

Article Info

Accepted:
26 June 2018
Available Online:
10 July 2018

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 a commonly used organophosphorus pesticide Quinolphos was administered to Swiss albino mice and the functioning of cell mediated immunity was measured using 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 *Cassaytha capillaries*. The remediating agents were found to retrieve the lost immunity to a significant level.

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 (Dean *et al.*, 1994). Bannerjee *et al.*, (1996) and Ranjitsingh and Sujatha (2012) and Ranjitsingh and Dhasarathan (2011).

As pesticides are extensively used in farming and insect control, it has every chance to get into the human body through food, water, air

and food chain (Ranjitsingh and Sreekumaran Nair, 2004). In the human system pesticide molecules interferes with immunity (Ranjitsingh and Dhasarathan, 2011). 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 retrieval 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 *Cassaytha capillaries* (Meissen) was collected from trees in Western Ghats 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 Soxhlet apparatus. The extract was dried, purified and stored.

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.

Selection of pesticide

For the present study an organophosphorous pesticide, Quinolphos was chosen. Using Standard methods, LC 50 value was fixed. From this LC 50 value, a sub lethal dose of 0.1 ppm 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 200 mg/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 0.1 ppm) was used as a immune – suppressant drug. Proimmu (Envin Bioceuticals, Shorapur, India) was used as standard immune potentiating drug).

Antigen (SRBC) preparation

To prepare cellular antigens, sheep erythrocytes were obtained from fresh blood of sheep sacrificed in a 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 3000 rpm for 10 minutes. Packed volume of SRBC is resuspended to get a concentration of 0.1 ml containing 1×10^8 cells for immunization and challenge.

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.*, (1999).

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 - Plant extract given (dose levels of 50mg/kg, 100mg/kg and 200mg/kg).
- Group V - Standard immune potentiating drug given Proimmu (30 mg/kg).
- Group VI - Quinolphos (Organophosphorous pesticide) $Cl_2 H_{15}N_2O_3$ immune suppressant
- Group VII - *C. capillaries* extract (200 mg/kg), and Quinolphos (0.1ppm) treated group.
- Group VIII - Proimmu (30 mg) and Quinolphos (0.1 ppm) treated group

Mice were sensitized by injecting 0.1 ml of SRBCs suspension containing 1×10^8 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 0.02 ml of SRBCs (1×10^8 cells/ml) in right hind foot pad. The left hind foot pad was given normal saline (0.02ml). Paw thickness was measured after 24, 48, 72 and 96 hrs using Vernier 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* (200 mg/kg) was given along with Quinolphos (0.1 ppm) for 3 days prior to sensitization with SRBC.

For group VIII Standard immunopotentiating drug proimmu and Quinolphos (0.1 ppm) 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 72 hrs. A positive reaction to SRBC was indicated by edema and induration at the site, measuring 2-5mm diameter. Positive responses were graded as follows,

1. Erythema (+)
2. Erythema with induration (++)
3. Erythema with induration and small blisters (+++)
4. 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 assess immunomodulatory mechanism in animals. The DTH assay is a simple and inexpensive method to assess immune response (Doherty, 1981). Immunosuppressive chemicals elevate DTH response by eliminating the population of T-suppressor cells (Turke *et al.*, 1976 and Gill and Liew, 1978). 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 (Khurana *et al.*, 2013). The most commonly used in vivo assay to determine DTH response is anti-inflammatory cutaneous reaction. Okoli *et al.*, (2003) 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 an experimental mice was assessed after administering the *C.capillaries* extracts and immunosuppressive pesticides (Table 1 and 2).

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 and 2). The percentage decrease in paw edema in Quinolphos treated cases was 58.33% but in *C.capillaries* treated group (200 mg/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 immunopotentiating effect like the standard immunostimulant drug Proimmu.

According to Tiwari *et al.*, (2004) the active principle, sesquiterpene 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.*, (1999) 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.*, 1999) on the inflammatory site showed perivascular cuffing with mononuclear cells followed by a more extensive exudation of mono and poly morphonuclear cells. 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 (Gokule *et al.*, 2003) also observed an elevation in DTH response in mice treated with the plant extract, *Argyrea 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 (Luster *et al.*, 1982). According to (Hirschman *et al.*, 2001) 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 (Gorbachev *et al.*, 2001).

Animals treated with Quinolphos had a suppressed foot pad thickness indicating the suppressive act on cell mediated immunity. According to (Osario and Goldman., 2017), 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 proinflammatroy cytokines (Argus and Woo., 1995). Tiwari *et al.*, (2004) 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_{DTH} cells 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- γ , macrophage migration inhibition factor and tumor necrosis factor β .

Table.1 Effect of Extracts of *C.capillaries* and standard immunomodulating 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.	<i>C.capillaries</i> (50mg/kg)	+++ (3 ⁺)
4	<i>C.capillaries</i> (100mg/kg)	++ (2 ⁺)
5	<i>C.capillaries</i> (200 mg/kg)	+ (1)

Table.2 Effect of the extracts of *C.capillaries* on delayed hypersensitivity (DTH) reactions. (Foot pad thickness - NS- non significant, a=p<0.01,b=p<0.001)

Group	Substance	Dose mg/kg	Mean diameter of foot pad thickness (mm) ± SD			Test of singnificance (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 II ^a
II	<i>C.capillaries</i> extract	50	0.42 ± 0.04 (+ 7.69)	0.31 ± 0.04	0.15 ± 0.02	Group I Vs III ^a
III	<i>C.capillaries</i> extract	100	0.46 ± 0.03 (+ 17.95)	0.26 ± 0.04	0.18 ± 0.02	Group I Vs IV ^b
IV	<i>C.capillaries</i> extract	200	0.50 ± 0.07 (+28.21)	0.30 ± 0.07	0.2 ± 0.02	Group I Vs V ^b
V	Quinolphos	30	0.18 ± 0.4 (- 58.33)	0.23 ± 0.02	0.8 ± 0.10	Group I Vs V ^b
VI	Proimmu	30	0.58 ± 0.06 (+48.32)	0.34 ± 0.14	0.20 ± 0.12	Group I Vs VI ^b
VII	<i>C.capillaries</i> 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 VIII ^a

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 and Woo., 1995) 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.*, (2002) 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.

Acknowledgement

Dr.A.J.A.Ranjitsingh is thankful to UGC for providing Emeritus fellowship to continue this work.

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How to cite this article:

Ranjitsingh, A.J.A., C. Padmalatha, G. Athinarayanan and Dhasarathan, P. 2018. Cell Mediated Immunity Dysfunction Retrieval Using the Extracts of the Plant *Cassythia capillaries* (Meissen). *Int.J.Curr.Microbiol.App.Sci.* 7(07): 3568-3574.
doi: <https://doi.org/10.20546/ijcmas.2018.707.415>