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RESEARCH ARTICLE

Immunomodulatory Activity of *Vetiveria zizanioides* Extract on Peritonial Macrophages of Albino Mice

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ABSTRACT

Vetiveria zizanioides L. (Poaceae) is a medicinal plant which is used as a thirst quencher in southern part of India especially in Kerala. The present study was conducted to scientifically evaluate the effects of extracts of *V. zizanioides* on phagocytic function of macrophages. *In vivo* effect of aqueous, ethanol and hexane extract of the plant at two doses (10mg/kg body weight and 25mg/kg b.w.) were evaluated by oral administration of the extracts on Swiss albino male mice. *In vitro* immunomodulatory potential of the above extracts at different concentrations (10µg/ml, 25µg/ml, 50 µg/ml and 100µg/ml) was studied using peritoneal macrophages from Swiss albino mice. All extracts gave phagocytic modulation *in vivo*. The aqueous extract of *vetiveria zizanoides* at a dose of 25mg/kg b.w. showed significant (p<0.05) increase in phagocytic activity in comparison with the control. An increased phagocytic response was shown by murine peritoneal macrophages after treatment with the extracts *in vitro*. A dose dependent response was observed in all cases. The results of the present study indicate the immunomodulatory effect of *V. zizanioides* extracts on murine peritoneal macrophages, as evidenced by its effect on phagocytosis which is a nonspecific immune mechanism.

KEYWORDS

Vetiveria Zizanioides, Phagocytosis, Imunomodulation, Macrophages, Nonspecific Immune Mechanism

INTRODUCTION

Immunomodulation refers to the suppression or stimulation of immune response. Several plant products and herbal drugs are known to possess immunomodulatory properties. They act by stimulating or suppressing both specific and non specific immunity. Many plants used in traditional medicine have immunomodulating activities.

In South India especially in Kerala, water for drinking purpose is boiled along with pieces of roots, leaves or barks of some plants commonly called as thirst quenchers.

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It is a general belief that the water decoctions of thirst quenchers can purity blood and can improve general health. It is also believed that regular consumption of this can improve body's defense mechanism. These plants are reported to have many Pharmacological effects.

V. zizanioides also called khus –khus, perennial grass of the family Poaceae, native to tropical Asia and also introduced into the tropic of both hemispheres. Its thick fragrant roots contain oil. This is used for ulcer, vomiting nausea, dyspepsia, cough, fever, low back pain.

MATERIAL AND METHODS

Plant Materials

Roots of the *Vetiveria zizanoids* was collected in April 2011from Alapuzha District, Kerala. The

specimen was authenticated by the Dr. V.T. Antony, Post Graduate & Research Department of Botany. St Berchmans College, Changanacherry, Kerala. A voucher specimen (No. RHK 6408) was deposited at Regional Herbarium of Kerala (RHK), St Berchmans College, Changanacherry.

Preparation of the Extracts

Root of the plant was shade dried, powdered and stored in airtight containers. The powder was subjected to successive soxhlet extraction using solvents of varying polarity, n-hexane, ethanol and water. The solvent was removed under reduced pressure and the extract was stored at 4^{o}C till used.

Animals

Study was conducted in Swiss albino mice (20-30g). They were maintained in animal house under standard condition (temp 25± 2^{o}C and light period of 12h) and fed with standard pellet diet and water adlibitum. This study got clearance from institutional animal ethics committee (approval number B21032014-09)

Treatment Protocol

The animals were dividing into eight group consisting six animals each Group I was of normal control which received PB.S, Group II received Levamisole at a concentration of 25 mg/kg body wt (positive control) animals of group III, IV, V, VI, VII & VIII received orally 10 and 25 mg/kg b.w of the three extracts of the plant (water, ethanol and hexane extracts dissolved in PBS) at volume of 0.2 ml /day for 30 days.

Preparation of Peritoneal Mouse Macrophages

One milliliter of 3% Brewer thioglycollate medium (Himedia, India) was injected intraperitoneally into mice as a stimulant to elicit peritoneal macrophages. Four days, later, the peritoneal exudates was collected by peritoneal lavage with 10 ml of RPMI 1640 medium (Himedia, India) Supplemented with L-Alanyal L –Glutamine, HEPES buffer, 60 mg per litre penicillin, 100 mg per litre streptomycin 10% FBS and sodium carbonate. The excudate was centrifuged at 400 g, 4^{0}c to 10 min. Discard the supernatant and re-suspend cell pellet in RPMI 1640 medium. The cell number was determined by counting in a hemocytometer and cell viability was tested by the hypan-blue dye exclusion technique (Zhang et al, 2008).

***In Vitro* Phagocytosis Assay**

Phagocytosis assay was performed according to Hay and West Wood (2002) with slight modification. Macrophages (2x 10^{6} cells/ml) were seeded in 24-well plates with a sterile glass cover slip and incubated for 2h in 5% CO_2 humidified incubator; then non-adherent cells were removed by washing in RPMI – 1640 medium.

The remaining adherent cells were cultured in RPMI – 1640 medium supplemented with 10% FBS and incubated for 24h with different concentration of the plant extracts dissolved in 0.1% DMSO in PBS (5µg/ml, 10µg/ml, 20µg/ml and 40µg/ml) Lipopolysaccharide (LPS) at 5µg/ml was used as mitogen and 0.1% DMSO in PBS was used as a control.

After incubation, the culture medium was removed and the wells were washed with fresh medium. One milliliter RPMI – 1640 medium and 100µg/ml yeast suspension (10^{8}particles/ml) were added to each well and incubated further for 1hr at 37^{0}c in a 5% CO_2 humidified incubator. The wells were washed twice gently with culture medium after incubation.

After washing, cover slips were fixed with methanol and then stained with Giemsar dye. The cover slips were removed for the well and invested on microscope slides and observed under oil immersion (x100) objective. Phagocyte index was calculated by the following equation.

Phagocyte Index (PI) = number of yeast cells phagocytosed by macrophages/no. of macrophages.

***In Vivo* Phagocytosis Assay**

The peritoneal macrophages were collected from treated mice. The remaining produce was same as that for the in vitro phagocytosis assay.

Statistical Analysis

All the values were expressed as mean $\pm SD$ for six animals. Values for the in vitro assays were expressed as mean standard deviation for implicate independent experiments. Statistical significance was analyzed using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. P values less than 0.05 were considered significant.

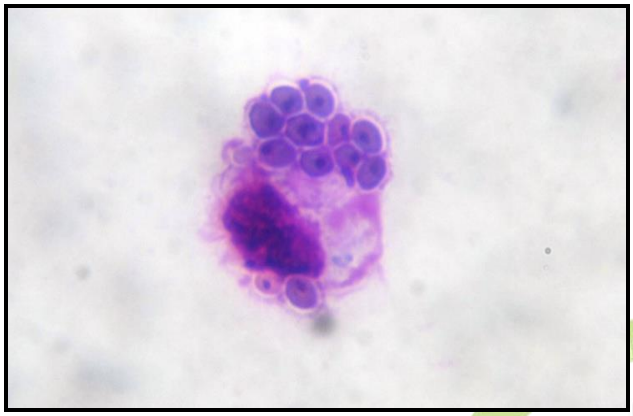

Figure 1: Phagocytosis of yeast cells by murine peritoneal macrophages

RESULTS AND DISCUSSION

Administration of *V. zizanioides* extracts enhances the phagocytic activity of murine peritoneal macrophages. A dose dependent response was observed *in vitro* and *in vivo* treatment.

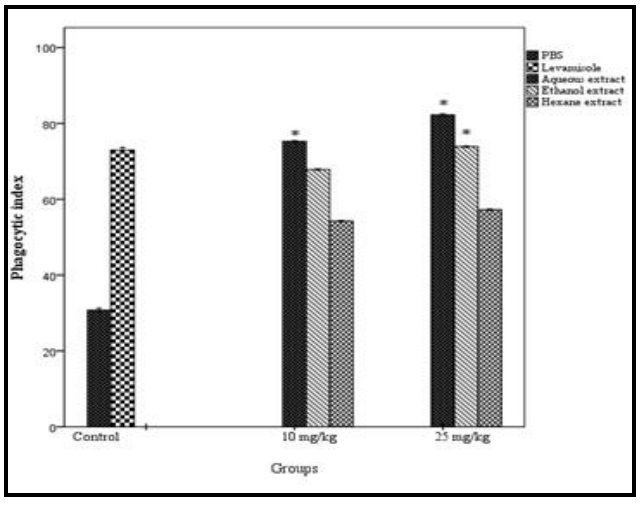

Figure 2: *In vivo* effect of *Vetiveria zizanoids* extract on phagocytic activity of peritoneal macrophages.*p<0.05 *vs* levamisole treated group

Figure 2. Shows *in vivo* effect of *V. zizanioides* extract on phagocytic activity of peritoneal macrophages. Aqueous extract treated group exhibited significant phagocytic activity at dose of 25mg/kg b. w. when compared with the normal control group. Among others two extracts treated group ethanol extract treated group showed significant (p<0.05) phagocytic activity over hexane extract treated group.

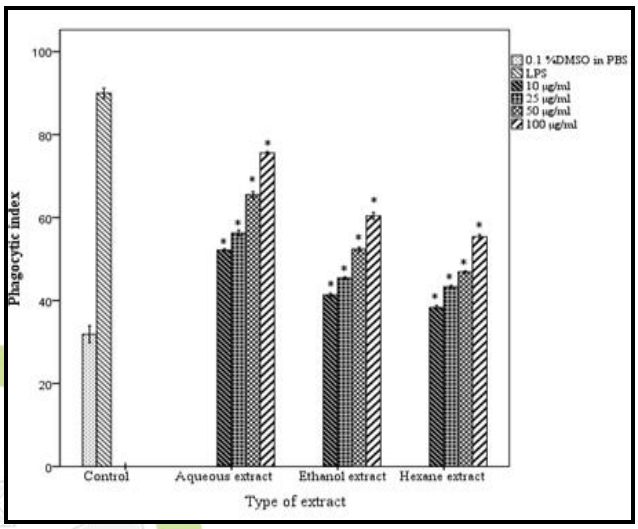

Figure 3: *In vitro* effect of *Vetiveria zizanoids* extracts on phagocytic activity of peritoneal macrophages.*p<0.05 *vs*. untreated macrophage cells

In *in vitro* effect of *V. zizanioides* extracts on phagocytic activity of peritoneal macrophages is showed in fig 3. All extract shows phagocytotic modulation. At 100 µg/ml concentration aqueous extract gave maximum phagocytic index followed by ethanol and hexane

The results of present study showed that *V. zizanioides* has significant effect on non-specific immune response (phagocytosis). Immunomodulatory phytocompounds from herbal agents activates host defense mechanism and can provide an alternative therapy to conventional chemotherapy (Wagnar et al., 1984). Antioxidant activity and antibacterial activity of *V. zizanioides* was reported (Subradevi et al., 2010) Antifungal activity (Derprakah et al, 2011) Hepatoprotective activity (GD Chaudhary et al., 2010) Antitubercular activity (Dharmendra saikia et al., 2012) Mosquito repellent activity (N. Athira et al., 2011) Hyperglycaemic activity

(Sanjaykumar et al., 2012) Antidepresent activity (Glory Josephine I et al., 2012). Earlier reports indicate that whatever zizanoids is a multifarious plant with immense potential.

In the present study, administration of *V. zizanioides* extracts enhances the phagocytosis activity of murine peritoneal macrophages both *in vivo* and *in vitro*. A dose dependent response was observed in *in vitro* and *in viv*o treatment.

Macrophages are mononuclear phagocytes that are widely distributed throughout the body. These cells can contribute to development and homeostasis of innate and adaptive immune response. Macrophages are prodigious secretory cells, and in that role can promote and regulate immune responses and contribute to autoimmune pathologies. Macrophages are highly phagocytic and in this capacity have long been considered to be essential immune effector cells (Zang et. al., 2008). Macrophages play an important role in inflammation. They have three major functions in inflammation; antigen presentation, phagocytosis and immunomodulation through production of various cytokines and growth factors. Macrophages play a critical role in the initiation, maintenance and resolution of inflammation. Because macrophages produce a wide range of biologically active molecules participated in both beneficial and detrimental outcomes in inflammation, therapeutic intervention targeted macrophages and their products may open new avenues for controlling inflammatory diseases (Fujiwara and Kobayashi, 2005).

CONCLUSION

The results of the present study showed that *V. zizanioides* had significant enhancing effect on phagocytic activity of macrophages. Since this effect was observed on oral administration of extract, it can reasonably be assumed that daily consumption of *V. zizanioides* as thirst quenchers might have some desirable effect on host defense mechanism against infection and inflammatory diseases in which macrophages play important role. Further detailed studies will be helpful in elucidating the mechanism of immune-modulation by this plant. Studies on the effect of extracts of *V. zizanioides* on the humoral and cellular immune function are in progress in our laboratory.

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