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RESEARCH ARTICLE

Immunosuppressive effects of *Euphorbia hirta* in experimental animals

Sheikh Fayaz Ahmad · Beenish Khan · Sarang Bani · Anpurna Kaul · Phalisteen Sultan · Sheikh Abid Ali · N. K. Satti · Saleh A. Bakheet · Sabry M. Attia · Khairy M. A. Zoheir · Adel R. A. Abd-Allah

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Abstract *Euphorbia hirta* L. (Euphorbiaceae) (*E. hirta*) is a tree locally used as a traditional medicine in Africa and Australia to treat numerous diseases such as hypertension, respiratory ailments, tumors, wounds, antipyretic, anti-inflammatory activities, etc. Therefore, we undertook to investigate their immunomodulatory effect on T lymphocytes (CD3+, CD4+ and CD8+ receptors) and Th1 cytokines (IL-2, TNF-α, IFN-γ) in a dose-dependent manner. *E. hirta* ethanol extract at 25, 50, 100 and 200 mg/kg doses was given orally for 7 days from the day of immunization. *E. hirta* maximum inhibition at 100 and 200 mg/kg p.o. was found to significantly block the production of the cell-mediated immune response, (CD3+, CD4+ and CD8+ receptors) and (IL-2, TNF-α, IFN-γ) and also prolongs graft rejection. *E. hirta* also showed a decrease of delayed hypersensitivity (DTH) response and dose-related decrease in the primary antibody response, respectively. Based on the data, it can be suggested that *E. hirta* is a potent and non-toxic immunosuppressor, which can be further explored for the development of potent immunosuppressor.

Keywords Cytokines · T-lymphocyte · Cyclosporine · Levamisole · Flowcytometry

S. F. Ahmad (✉) · S. A. Bakheet · S. M. Attia · K. M. A. Zoheir · A. R. A. Abd-Allah
Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, PO Box 11451, Riyadh, Saudi Arabia
e-mail: s_fayazahmad@yahoo.com; fashaikh@ksu.edu.sa

B. Khan · S. Bani · A. Kaul · P. Sultan · S. A. Ali · N. K. Satti
Indian Institute of Integrative Medicine formerly known as Regional Research Laboratory (CSIR), Jammu, Jammu and Kashmir, India

Introduction

Activation of immune system involves coordinated interaction of various signaling molecules with several immune cells and facilitation of the cross-talk between these immune cells to evoke a desired immune response. In this respect, lymphocytes and antigen presenting cells are important parts of the immune system which switch over disease-specific cytokines and chemokines secretion during the pathological conditions (Khan et al. 2009). IFN-gamma and IL-2 secreted by Th1 cells can block the proliferation of Th2 cells, and high concentrations of IL-4 or IL-10 can block the generation of Th1 cells from naive T cells (Kidd 2003). Cyclosporine, tacrolimus, mycophenolic mofetil—the prodrug for mycophenolic acid—and rapamycin are immunosuppressive drugs in use for organ transplantation and to treat some autoimmune diseases. However, these drugs are critical dose related, they exhibit a high degree of interindividual and intraindividual pharmacokinetic and pharmacodynamic variability, which increases the possibility of therapeutic failure if these agents are used at uniform doses in all patients (Wong 2003). There are several medicinal plants that are considered to possess immunomodulatory properties (Mathur et al. 2010). Recently, the understanding of research on immunomodulators has come up as a new field of immunopharmacology (Archana et al. 2011). As a consequence, there continues to be a high demand and challenge to the medical system for new immunosuppressants without any or less side effects (Suna et al. 2005). Medicinal plants serve as therapeutic alternatives, safer choices (Gautam et al. 2007) and a larger number of these plants and their isolated constituents have shown beneficial therapeutic effects including antioxidant, anti-inflammatory, anticancer, antimicrobial, and immunomodulatory effects (Salem 2005).

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Keeping in mind the above-mentioned pharmacological benefits of medicinal plants and requirement of novel immunosuppressor, we have made an effort to identify and explore the immunosuppressive properties of *E. hirta*. The genus family *Euphorbia* (Euphorbiaceae or spurge family) is one of the largest families of plant world, with about 300 genera and 7,500 species in non-tropical areas such as the Mediterranean, the Middle East, South Africa, and southern USA (Chellaiah et al. 2006). *E. hirta* exhibits antiamoebic, antibacterial, antimalarial and antioxidant (Tona et al. 2000; Liu et al. 2007, Sharma et al. 2007; Suresh et al. 2008). *E. hirta* is also known to have anti-allergic, antipyretic, anti-inflammatory (Singh et al. 2006; Shih et al. 2010), etc. Recent studies have indicated that *E. hirta* has potent long-term antioxidant properties (Subramanian et al. 2011). The stem sap is used in the treatment of eyelid styes and a leaf poultice is used on swelling and boils (The Wealth of India 2005). *E. hirta* slows down matrix metalloproteinase's (MMPs) and tissue inhibitors of matrix metalloproteinase's (TIMPs) in the rat articular cartilage was investigated (Lee et al. 2008). In the present study, we have investigated whether oral administration of *E. hirta* suppresses the immune function, particularly the humoral, cell-mediated immune responses, T-lymphocytes (CD3+, CD4+ and CD8+) and Th1 cytokines (IL-2, TNF-α, IFN-γ) in a dose-dependent manner.

Materials and methods

Extraction of test material

Test material was ground to coarse powder. 500 g of the powdered material was extracted with 95 % ethyl alcohol (2 l) at room temperature by mechanical stirring for 2 h. The extraction process was repeated three times more under similar conditions. Pooled extract was concentrated under reduced pressure and the gummy residue (44 g) was stored in desiccating conditions till further use.

Markers

The marker was isolated from ethanolic extract by column chromatography. 20 g extract was subjected to Silicagel (100–200 mesh) chromatography. The column was eluted using a gradient of $CHCl_3$–CH_3OH (100:0–0:100) to afford 20 fractions. All the 20 fractions were checked on TLC (run in *n*-butanol:acetic acid:water 4:1:5), spots were visualized by freshly prepared borinate–PEG solution (2-aminoethyldiphenylborinate, 1 % in CH_3OH:polyethylene glycol-4000, 5 % in C_2H_5OH, 1:1 v/v). Out of 20 fractions, fraction-8 (eluted in 15 % CH_3OH in $CHCl_3$) showed one major spot in TLC. The fraction was subjected to repeated column chromatography on silica gel to obtain a compound. The compound was identified as quercitrin (Eldahshan 2011). It was finally purified by crystallization and identified with the help of ^1H, ^{13}C NMR and in comparison with data reported in the literature.

Chemoprofiling

Equipment

The Water HPLC system comprising two Waters 515 HPLC pumps, automatic sampling unit (Waters 717 plus auto sampler), column oven, photodiode array detector (Waters 2996), Merck Rp-18 column (5 µm, 250 × 4.00 mm ID), temperature control module II and Waters Empower software was used for data analysis and data processing.

Experimental conditions

Quercitrin, was quantified in the extract at 30° C, the analysis was performed at a flow rate of 1.0 ml/min using mobile phase consisted of ACN(B):1.5 % AcOH in water (A) [gradient: time in minute (B %): 0 (12), 25 (21), 30 (25), 40 (50), 50 (75), 60 (90), 70 (12)]. The photodiode array detector was set at wavelength of 340 nm for quantification.

Sample preparation and quantification

Accurately weighed quantity of the dried extract (21 mg) was dissolved in 2 ml methanol:water (1:1 v/v mixture) HPLC grade. The sample was centrifuged and filtered through Millipore micro filter (0.45 µm) and was used for analysis. 10 µl from it was injected into the HPLC system (Fig. 1). Quercitrin (1.2 mg) was dissolved in 5 ml methanol:water (1:1 v/v mixture) HPLC grade. From the

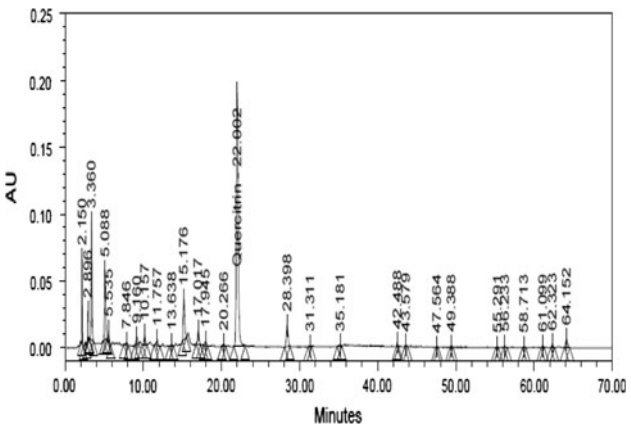

Fig. 1 HPLC chromatogram of ethanol extract of *E. hirta*

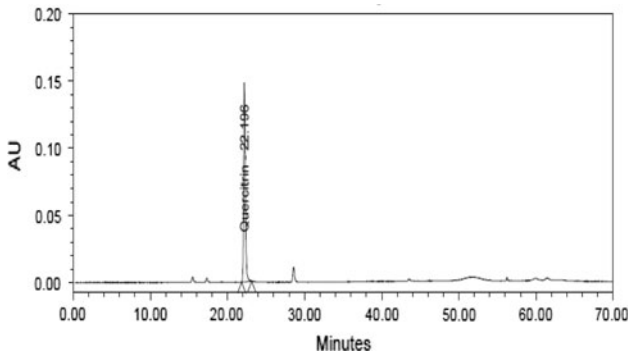

Fig. 2 HPLC chromatogram of standard mixture of quercetin used for identification and quantification. Following are the retention time (Rt) of marker compound

solution 2, 4, 6, 8, 10 µl was injected in the HPLC system for plotting of calibration curve (Fig. 2). Linearity in the concentration range of 480–2,400 µg/ml was observed. The marker compounds in the extract were quantified using the calibration curve. It was found that extract contained 0.55 % quercitrin.

Animals

Female inbred Balb/c mice (20–24 g, 10–12 week old) were obtained from animal house of IIIM, Jammu. Animals were employed in groups of six for the study. All the animals were maintained in transparent polycarbonate filter top cages in animal isolator cabins at 22 ± 2 °C with 12 h light/dark cycle and free access to pellet food (Ashirwad India Ltd) and autoclaved water. All experimental protocols and the number of animals used for the experimental work were duly approved by the Institutional Animals Ethics Committee (IAEC) of Indian Institute of Integrative Medicine, CSIR, Canal Road, Jammu, J&K, India, (CPCSEA registration no. 67/99/CPCSEA) according to the Government of India accepted principles for laboratory animal use and care under No. 10/1998-99.

Antigen

Fresh sheep red blood cells (SRBC) were collected aseptically from the jugular vein of sheep and stored in cold sterile Alsever's solution, was washed three times with pyrogen-free sterile saline (NaCl, 0.9 % w/v) and adjusted to the concentration of 5×10^9 cells/ml for immunization and challenge at the required time schedule.

Effect on general behavior and maximum dose tolerance in mice

The maximum dose tolerance in mice were carried out following (OECD 1996), guidelines No. 423. Graded doses of the test drug were administered orally to group of 8 rats and 10 mice by the method of (Singh et al. 1978). The animals were observed for first 2 h continuously and then at half-hourly interval for next 6 h for changes in reactivity, gait, motor activity, ptosis, respiration rate, writhing, etc. A high-dose toxicity effect resulting into mortality was recorded over 1-week period and the acute oral LD_{50} was calculated.

Humoral antibody response

Mice were immunized by injecting 20 µl of 5×10^9 SRBC/ml intraperitoneally (i.p.) on day 0, and the blood samples were collected on day +7 (before challenge) for primary antibody. Haemagglutination antibody titres were determined following the microtitration technique described by (Nelson and Mildenhall 1967), BSA-saline alone served as a control.

Skin allograft rejection

The modified method of Billingham and Medawar (1951) was followed to study the skin allograft rejection time in mice. Graded doses of test material were administered to the animals for 7 days and graft rejection time (GRT) was recorded by daily observation of epithelial skin layer survival. Control group was given vehicle only, and another group received cyclosporine as standard at 5 mg/kg body weight daily for 7 days.

Induction and evaluation of delayed type hypersensitivity reaction

The method of Doherty (1981) was followed. *E. hirta* was administered 2 h after SRBC injection and once daily on consecutive days. Six days later, the thickness of the left hind food was measured with a spheromicrometer (pitch, 0.01 mm) and was considered as a control. The mice were then challenged by injecting the same amount of SRBC intradermally into the left hind footpad. The foot thickness was measured again after 24 h.

Lymphocyte immunophenotyping

Immunophenotyping focuses on lymphocyte populations involved in acquired immunity. A specific molecule present on the cell surface defines characteristics of lymphocytes such as state of activation or functional capabilities. Immunization of Balb/c mice was carried out by injecting 20 µl of 5×10^9 SRBC/ml (i.p.) *E. hirta* ethanol extract was carried out for 5 days. Same amount of SRBC was then injected into the mice for the challenge on day 6 and blood was collected after 24 h of challenge in

heparinised tubes from retro-orbital plexus for estimation of CD3+, CD4+ and CD8+ surface activation markers. Murine monoclonal antibodies conjugated to a fluorochrome and directed against co-receptors CD3+, CD4+ and CD8+ were used in a multiparametric flowcytometric assay to quantify the lymphocyte subsets associated with the cell-mediated immune response. These antibodies were added directly to 100 µl of whole blood, which was then lysed using whole blood lysing reagent (BD Biosciences). Following the final centrifugation, samples were resuspended in phosphate buffer saline (pH 7.4) and analyzed directly on the flowcytometer (LSR, BD Biosciences) using Cell Quest Pro Software (BD Biosciences).

Intracellular cytokine estimation

Whole blood (100 µl) was pipetted directly into a 12 × 75 mm fluorescence-activated cell sorting tube containing 20 µl of monoclonal antibodies for the T-helper surface antigen CD4+ and CD8+ (BD Biosciences) and incubated at room temperature in the dark for 10 min. Then, 1 % paraformaldehyde (0.5 ml) was added for 10 min to stabilize the monoclonal antibody–surface antigen complex. RBCs were lysed using 2 ml of 1× lysing solution (BD Biosciences) for 10 min. After centrifugation at 300*g* for 5 min, the supernatant was aspirated and 1× permeabilizing solution (500 µl, BD Biosciences) was added into the pellet and incubated for 10 min at room temperature in the dark. After washing with 3 ml buffer (1 % bovine serum albumin, 0.1 % NaN3, 1× PBS), cytokine-specific antibodies (20 µl, IL-2 TNF-α and IFN-γ BD Biosciences) were added to the cells and incubated for 30 min at room temperature in the dark. After one final wash, cells were resuspended in 1 % paraformaldehyde (500 µl) and stored at 4 °C until flowcytometry analysis. Cells were acquired using a (LSR, BD Biosciences) flowcytometer and data were analyzed using Cell Quest software. A minimum of 10,000 cells was counted from each sample.

Statistical analysis

Data represents mean ± SEM of eight animals. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons).

Result

Effect on general behavior and maximum dose tolerance in mice

Mice and rats treated with *E. hirta* at a maximum oral dose of 2,500 mg/kg did not show any difference in gross general behavior compared with the control group of animals that were administered only the vehicle. No mortality was observed over an observation period of 7 days.

Humoral immune response

Euphorbia hirta (25–200 mg/kg p.o.) produced a dose-related decrease in the primary antibody synthesis. Maximum effect was observed at 100 mg/kg (4.7 ± 0.16 % decrease) after which the suppressive effect influence and was 4.5 ± 0.14 % at 200 mg/kg oral dose. Cyclosporine used as a standard drug showed 3.6 ± 0.13 % decrease in antibody synthesis at the dose of 5 mg/kg oral dose (Table 1).

Skin allograft rejection

Oral administration of *E. hirta* at 25, 50, 100 and 200 mg/kg delayed the skin allograft rejection time in mice (days) by 17.5 ± 1.17, 18.0 ± 1.11, 20.16 ± 1.08 and 22.12 ± 1.05, respectively. Cyclosporine at 5 mg/kg increased the rejection time by 23.2 ± 1.01 % (Table 2).

Delayed type hypersensitivity (DTH) response

Maximum effect was observed of *E. hirta* ethanol extract at 50, 100 and 200 mg/kg that showed the DTH response

Table 1 Effect of *E. hirta* ethanol extract or cyclosporine on humoral immune response (antibody titer) in mouse model (mean ± SE)

Groups	Dose (mg/kg)	Antibody titer (%)
Sensitized control	0	6.5 ± 0.22
E. hirta extract	25	5.6 ± 0.21*
E. hirta extract	50	5.01 ± 0.19**
E. hirta extract	100	4.7 ± 0.16***
E. hirta extract	200	4.5 ± 0.14***
Cyclosporine	5	3.6 ± 0.13***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

Table 2 Effect of *E. hirta* ethanol extract or cyclosporine on homologous graft rejection in mouse model (mean ± SE)

Groups	Dose (mg/kg)	Mortality	Rejection time (days)
Sensitized control	0	Nil	13.0 ± 1.25
E. hirta extract	25	Nil	17.5 ± 1.17*
E. hirta extract	50	Nil	18.0 ± 1.11**
E. hirta extract	100	Nil	20.16 ± 1.08***
E. hirta extract	200	Nil	22.12 ± 1.05***
Cyclosporine	5	Nil	23.2 ± 1.01***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

Table 3 Effect of *E. hirta* ethanol extract or cyclosporine on delayed type hypersensitivity (DTH) response in mouse model (mean ± SE)

Groups	Dose (mg/kg)	Foot pad thickness (mm)
Sensitized control	0	0.72 ± 0.08
E. hirta extract	25	0.66 ± 0.02
E. hirta extract	50	0.60 ± 0.07**
E. hirta extract	100	0.42 ± 0.04***
E. hirta extract	200	0.40 ± 0.02***
Cyclosporine	5	0.36 ± 0.02***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

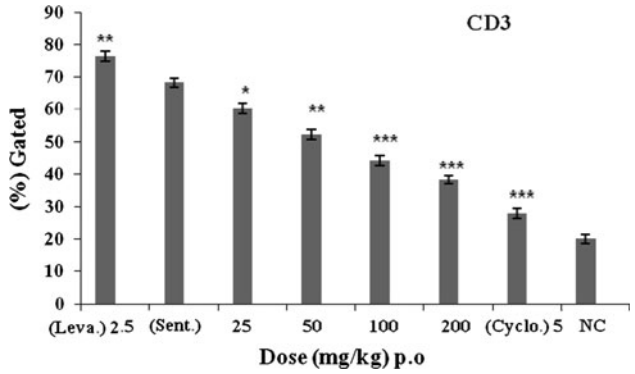

Fig. 3 Effect of *E. hirta* ethanol extract on CD3+ receptors in whole blood. Cyclosporine 5 mg/kg and levamisole 2.5 mg/kg were used as standard control. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

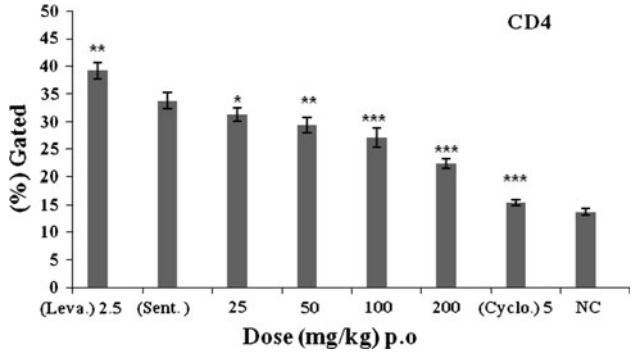

Fig. 4 Effect of *E. hirta* ethanol extract on CD4+ receptors in whole blood. Cyclosporine 5 mg/kg and levamisole 2.5 mg/kg were used as standard control. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

0.60 ± 0.07, 0.42 ± 0.04 and 0.40 ± 0.02 against the sensitized control showing 0.72 ± 0.08. Cyclosporine at 5 mg/kg p.o. produced 23.2 ± 1.01 % decrease, respectively (Table 3).

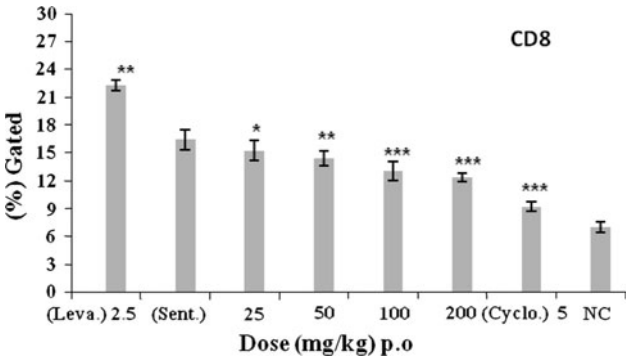

Fig. 5 Effect of *E. hirta* ethanol extract on CD8+ receptors in whole blood. Cyclosporine 5 mg/kg and levamisole 2.5 mg/kg were used as standard control. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

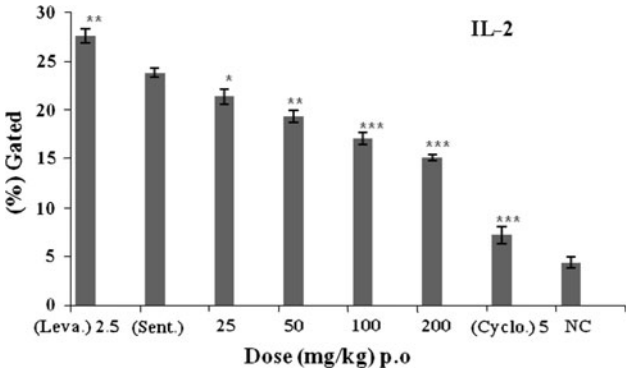

Fig. 6 Effect of *E. hirta* ethanol extract on intracellular IL-2 secreting CD4+ T-cell in whole blood. Cyclosporine 5 mg/kg and levamisole 2.5 mg/kg were used as standard control group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

Lymphocyte immunophenotyping

Euphorbia hirta showed effect of 38.3 % of CD3+, 22.36 % of CD4+ and 12.36 % of CD8+ surface activation markers at 200 mg/kg (p.o.) dose, respectively. The sensitized control values were 68.3 % of CD3+, 33.7 % of CD4+ and 16.4 % CD8+ T cells. This shows a significant decrease in CD3+, CD4+ and CD8+ T cells against sensitized group (Figs. 3, 4, 5). Cyclosporine 5 mg/kg and levamisole 2.5 mg/kg were used as standard drugs.

Effect of *E. hirta* ethanol extract on IL-2 and TNF-α secreting CD4+ T cells

Oral administration of *E. hirta* at specified doses showed a significant dose-dependent down-regulation of Th1 cytokines as compared to sensitized control group. *E. hirta* at 25–200 mg/kg showed a significant down-regulation of IL-2 production where maximum down regulatory effect was

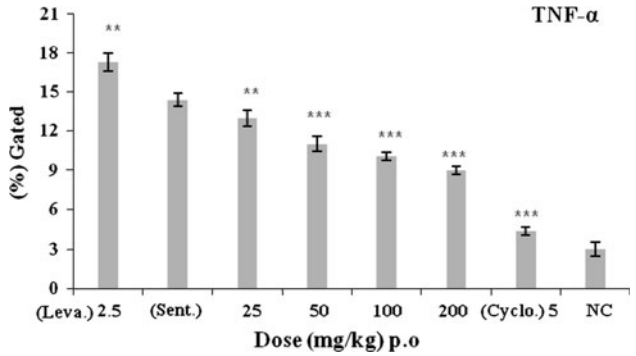

Fig. 7 Effect of *E. hirta* ethanol extract on intracellular TNF-α secreting CD4+ T-cell in whole blood. Cyclosporine 5 mg/kg and levamisole 2.5 mg/kg were used as standard control. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

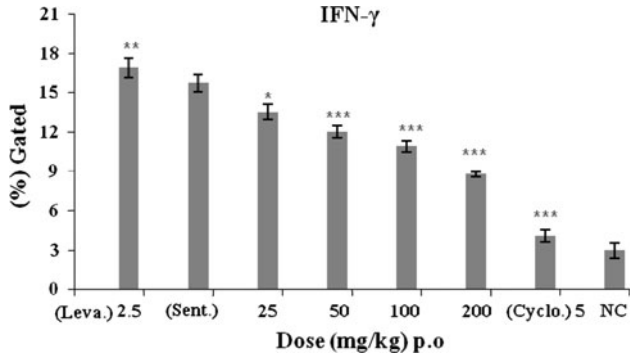

Fig. 8 Effect of *E. hirta* ethanol extract on intracellular IFN-γ secreting CD8+ T-cells in whole blood. Cyclosporine 5 mg/kg and levamisole 2.5 mg/kg were used as standard control. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ compared to sensitized control (analysis of variance, ANOVA followed by Tukey–Kramer for multiple comparisons)

observed at 100 (17.5 %) and 200 mg/kg (15.1 %) dose (Fig. 6), respectively. Similar trend was observed with TNF-α maximum inhibitory effect was seen at 100 (10.04 %) and 200 mg/kg (8.98 %) dose (Fig. 7). Cyclosporine 5 mg/kg and levamisole 2.5 mg/kg were used as standard drugs.

Effect of *E. hirta* ethanol extract on IFN-γ secreting CD8+ T cells estimation

IFN-γ secreting CD8+ T cell estimation was assayed and the effect of *E. hirta* was tested on the release of selected Th1 cytokine IFN-γ. The results are shown in (Fig. 8), indicating that *E. hirta* inhibited IFN-γ production in a dose-related manner. A dose as high as 100 and 200 mg/kg was found to be the most effective for down-regulation of IFN-γ where its effect was (10.91, 8.82 %) of IFN-γ production. The sensitized control values were 15.74 % (Fig. 8), respectively.

Discussion

Plant-based, traditional medicine systems continues to play an essential role in health care, with about 80 % of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi et al. 2007). There are several medicinal plants that are considered to possess immunomodulatory properties (Mathur et al. 2011). This research work focused on the identification of clinically useful and safe products from *E. hirta* ethanol extract that could modulate immune responses. There are now numerous examples in experimental models where modulation of the Th1 balance by administration of recombinant cytokines or cytokine antagonists alters the outcome of the diseases (Stephens et al. 2002). However, their clinical efficacy has been limited and has associated complications (Oberholzer et al. 2000; Wieland et al. 2005). The trends indicate that there is an utmost need for orally active non-peptide compounds that can modulate Th1 balance (Whelan et al. 2003). Levamisole is the only known oral clinically used immunostimulant, which restores suppressed immune function of B and T cells, monocytes and macrophages.

The antibody titre and DTH responses were determined for the assessment of the effect of *E. hirta* on humoral and cell-mediated immune responses. Humoral immunity involves the production of antibody molecules in response to an antigen and DTH reaction is triggered by antigen-specific T cells. DTH reaction is also an important in vivo manifestation of cell-mediated immune response and is characterized by the expansion of antigen-specific Th1 type CD4+ T cells during the initial phase, and an inflammation response by Th1 cytokines released from CD4+ T cells during the effectors phase. The present study evaluated the effect of the ethanol extract on humoral response; its influence was tested on sheep erythrocyte specific haemagglutination antibody titre in mice. It was found to significantly suppress the production of circulating antibodies (Table 1). Whether its suppressive effect on the antibody responses was a direct result of its action on the B cells or an indirect effect via suppression of helper T cell functions is not known. Supporting the hypothesis of T lymphocytes inhibition is the increase in the homologous skin graft rejection time in mice treated with *E. hirta* ethanol extract showing almost 69.2 % inhibition in the rejection (Table 2). In our studies, we found that *E. hirta* showed highly significant and dose-dependent inhibition of SRBC-induced antibody titre and DTH response at 100 and 200 mg/kg, p.o. (Table 3). Th1 response is considered central to regulation of antigen-specific classical cell-mediated functions such as delayed type hypersensitivity (DTH) response and B cell activation (Bourgeols and Corinne 2003). The results suggest that in normal animals,

levamisole in comparison to *E. hirta* ethanol extract resulted in significantly suppressive humoral and cellular immune responses. Interestingly, in sensitized animals, *E. hirta* extract exhibited dose-dependent suppression of cellular and humoral immune response comparable to cyclosporine (5 mg/kg) indicating unique suppressive profile (Tables 1, 3). The basic mechanism involved in graft rejection time is the suppression of T lymphocytes.

Result suggests that *E. hirta* ethanol extract treatment showed significant decrease in CD3+, CD4+ and CD8+ T-lymphocytes count as compared to sensitized control and levamisole (standard) group (Figs. 3, 4, 5). Hence, comparative study of levamisole and *E. hirta* ethanol extract was planned where effect on Th1 cytokines was studied (in vivo) in immunized Balb/c mice. Since IL-2 is a particularly indicates T-cell activation, *E. hirta* ethanol extract showed significantly lower levels of IL-2 at 100 and 200 mg/kg compared with sensitized control group (Fig. 6). IL-2 plays a central role in the therapeutic manipulation of the immune system. Even more striking was the significantly decreased TNF-α (Fig. 7) and IFN-γ (Fig. 8) at 100 and 200 mg/kg. The results suggested that oral administration of *E. hirta* ethanol extract decreased Th1 response IL-2, TNF-α and IFN-γ cytokines levels against sensitized control. TNF-α is made by many other cells as well as macrophages, which are major source, especially after priming by IFN-γ and inhibition of TNF-α signals is inhibited by the anti-inflammatory effect of *E. hirta* extract. An important reduction of IFN-gamma in *E. hirta* treated groups compared to control indicates inhibition of important cytokine that is implicated in Th1 development by mediating IL-12Rβ2 chain expression (Szabo et al. 1997).

Conclusions

The studies on humoral immune response and cell-mediated immune response on Th1 cytokines and cell surface markers by flowcytometry analysis clearly indicates the immunosuppressive effects of *E. hirta* ethanol extract. It appears very promising in the treatment of autoimmune diseases. The findings demonstrate it to have a potent immunosuppressive potential, which is suggestive of its possible therapeutic usefulness. Its apparent safety over long-term administration is encouraging enough to warrant further studies to explore its possible role in modern clinical practice.

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Conflict of interest The authors declare that there is no conflict of interest.

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