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**RESEARCH ARTICLE**

# Immunostimolatory activities of *Vigna mungo* L. extract in male Sprague–Dawley rats

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**Abstract**

*Vigna mungo* L. (*Fabaceae*) is a popular food legume used in the traditional Indian system of medicine for the treatment of a variety of disease conditions. The objective of the study was to evaluate any immunostimulatory activities of the extract of *V. mungo* seeds in an animal model. The induction of any immunostimulatory effects were evaluated using measures of sheep red blood cells (SRBC)-induced humoral antibody titer, SRBC-induced delayed-type hypersensitivity (DTH), neutrophil adhesion, and *in vivo* phagocytosis (via the carbon clearance method) after host treatment with the extract. The results here indicated that primary and secondary antibody titers in the rats were significantly increased by treatment with the *V. mungo* extract as compared with those noted among rats in a control group. Increases in DTH response, the percentage (%) neutrophil adhesion, and *in situ* phagocytosis were also observed after treatment with the extract. We summarize that the apparent immunostimulatory effect of the *V. mungo* seed extract might be attributed to an augmentation of humoral and cell-mediated responses, phagocytosis, and hematopoiesis in the treated rats. The findings in this study suggest that *V. mungo* seed extract possesses profound immunostimulatory activities. Whether such outcomes are also evidenced by consumption of the intact seeds themselves, as is most likely to be the case with humans, remains to be determined. Nonetheless, the present study provides evidence that could help explain how the traditional use of *V. mungo* has been successful in the treatment of various disorders in humans.

**Keywords:** *Delayed-type hypersensitivity; immunostimulatory; neutrophil adhesion; phagocytosis*



## Introduction

The immune system is the major defense mechanism used by the body against foreign pathogens. Abnormalities of the immune system result in a variety of pathophysiological states—autoimmune disorders, cancers, transplant rejection, hypersensitivity reactions, altered tolerance, and immunodeficiency diseases (Abbas and Lichtman, 2001). There are only few drugs available in the modern system of medicine to treat immunological disorders; however, many of these have serious limitations (i.e., potential toxicities, etc.). Hence, increasingly, researchers have focused their attention on screening plants for any potential immunomodulatory activities.

Herbal drugs are known to possess immunomodulatory properties and generally act by stimulating both specific and non-specific immunity (Wagner and Proksh, 1985). Many plants described under 'Rasayana' in the traditional Indian system of medicine are immunomodulatory. Some of these plants generally stimulate both humoral and cell-mediated immunity, while others only selectively activate certain cellular components of either system (Atal et al., 1986). As of 2000, about 34 plants had been identified in the Indian Ayurvedic system of medicine as having documentable immunomodulatory activities (see Agarwal and Singh, 1999).

*Vigna mungo* L. (*Fabaceae*) is a popular food component of South Asia. *V. mungo* is a rich source of protein, carbohydrates, oil, iron, potassium, and vitamin $B_1$ (Swaminathan and Jain, 1975). It also contains phenolics (vitexin and isovitexin) (Peng et al., 2008), polyphenolics (phytic acid and tannic acid), trypsin inhibitors, and aromatic constituents like hexanol, benzyl alcohol, γ-butyrolacetone, methyl-2-proponal, and pentanol (Lee and Shibamoto, 2000). Apart from its role in the diet, this plant has also been used in the traditional Indian system of medicine for the treatment of lipid disorders, liver and stomach disorders, fever, and general weakness, as well as being used as a blood purifier (Warrier et al., 1996). The boiled seeds of the plant in particular have been used as a traditional treatment by immunocompromised patients.

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To date, V. mungo L. has not been adequately characterized in detail for its immunomodulatory (i.e., immunostimulatory) properties. Thus, the present investigation was undertaken to evaluate any immunostimulatory activities of *V. mungo* seed extract (VMSE) in an experimental animal model. It is hoped that through these series of studies, the groundwork will be laid for subsequent extensive studies of the modes of action by—and a clearer determination of the active constituents within—these seed preparations.

## Materials and methods

### *Plant collection and identification*

The seeds of *V. mungo* were purchased from a local market in Anand, Gujarat, India. The seeds were then botanically identified by Dr. G. C. Jadeja, Professor and Head of the Department of Agricultural Botany, B. A. College of Agriculture, Anand, Gujarat, India. The specimens of the sample used in these studies were stored in the museum of the department (Specimen number: 0702).

### *Extraction*

The dried powdered (mesh no. 40) seeds of *V. mungo* were defatted with petroleum ether and then extracted with 50% (v/v) alcohol by maceration. The solvents were evaporated at 60°C to yield a pasty mass referred to as a VMSE. These protocols yielded the final product that comprised 22.12% VMSE (by weight) of the dry powder. Numerous tests on the product used here (and in other studies at the L. M. College of Pharmacy) have indicated that there was no endotoxin contamination in the samples (i.e., Limulus amebocyte lysate results all below level of detection [maximum sensitivity of assay was 0.005 endotoxin units /mL]).

### *Chemicals and reagents*

All the chemicals used were of analytical grade. Dexamethasone (DMS) was received as gift samples from Zydus Research Centre, Ahmadabad, Gujarat, India. The reagents and solvents were of analytical grade and purchased from S. D. Fine Chemicals Limited, Mumbai, Maharashtra, India.

### *Pharmacological evaluation*

#### Animals

Male albino rats (Sprague–Dawley strain) weighing 150–200 g were obtained from the central facility and acclimatized for 6 days. Throughout the studies, all rats were housed individually under specific pathogen-free conditions in polypropylene cages at ambient temperature (25 ± 1°C), relative humidity (55% ± 5%), and under a 12/12 h light–dark cycle. Animals had free access to standard commercial pellet diet (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum* throughout the study period. This study was approved by the institutional animal ethics committee in accordance with the guidelines of Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA, 2003). For each experiment outlined below, rats were randomized into various groups (unless elsewise indicated, $n = 6$/group) that then received VMSE, vehicle, or DMS as a reference immunomodulant drug.

#### Treatment regimens

The VMSE was suspended in distilled water using 1% (w/v) gum acacia. DMS was suspended at a concentration of 0.8 μg/mL in distilled water using 1% (w/v) carboxymethylcellulose (CMC). In the studies herein, unless elsewise indicated, treatment rats received VMSE at 500 mg/kg body weight (BW) in 1 mL doses daily by gavage. The control group rats received vehicle, i.e., a single 2 mL bolus bearing 1 mL each of the 1% (w/v) gum acacia and 1% (w/v) CMC solutions, in parallel daily. The rats in the reference drug group received DMS at the dose of 0.25 mg/kg BW (in a 1 mL volume) daily by gavage.

#### Antigen preparation

Fresh blood was collected from sheep sacrificed in a local slaughterhouse, and placed in Alsever's solution. During the experiment, an adequate amount of stock solution of sheep red blood cells (SRBC) stored in Alsever's solution was taken and allowed to stand at room temperature. It was washed three times with normal saline. The settled SRBC were then suspended in normal saline. The SRBC in this suspension were adjusted to a concentration of $5 \times 10^9$ SRBC/mL for immunization and challenge (Bafna and Mishra, 2005).

#### Acute toxicity studies

Acute oral toxicity refers to those adverse effects occurring usually within 24 h after oral administration of a single dose, or multiple doses, of a test substance. Here, rats were treated with different doses of VMSE up to 1000 mg VMSE/kg BW, by gavage and then observed for any mortality or other pathologies for a period of up to 72 h.

### *Immunomodulatory activities*

#### SRBC-induced antibody (HA) titer

The method described by Atal et al. (1986) was utilized to examine the rats provided VMSE once daily by gavage, starting 7 days prior to sensitization and continuing up to the second time of challenge (i.e., Day –7 up to and through Day + 14; for a total of 21 days). Control and DMS-treated rats received vehicle or the drug, respectively, in parallel each day.

To specifically assess effects on antibody formation, groups of six rats per treatment were each immunized with 20 μL of SRBC suspension ($5 \times 10^9$ SRBC/mL) injected subcutaneously into their right hind footpad. This day of immunization was referred to as Day 0. Seven days later (Day + 7), each rat was challenged intradermally by injection of 20 μL of SRBC suspension ($5 \times 10^9$ SRBC/mL) into the left hind footpad. Blood samples were then collected from each animal by retro-orbital puncture (under light ether anesthesia) on Day + 7 (post-challenge) for assessing primary antibody titer and again on Day + 14 (post-challenge) for secondary antibody titer. Antibody levels were determined by the method of Shinde et al. (1999). Specifically, after allowing the collected

blood to clot, serum was isolated and 25 µL was placed into one well of a 96-well microtiter plate. Serial two-fold dilutions of the serum were then made using 25 µL of normal saline each time of transfer across the plate. Thereafter, to the 25 µL of diluted serum in each well was added 25 µL of a 1% (v/v) SRBC suspension in normal saline. The plate was maintained at room temperature for 1 h and then the well contents examined for hemagglutination, i.e., until control wells showed unequivocally negative patterns. The value of the highest serum dilution showing hemagglutination was defined as the antibody titer for the given rat.

**SRBC-induced delayed-type hypersensitivity response**

The method of Lagrange et al. (1974) was used to analyze effects on delayed-type hypersensitivity (DTH) responses in the treated rats. Daily treatment with the VMSE (500 mg/kg BW, by gavage) began 14 days prior to the challenge, i.e., starting on the same day as immunization with SRBC. Control and DMS-treated rats received vehicle or the drug, respectively, in parallel each day.

On Day 0, all rats were each immunized with 20 µL SRBC solution ($5 \times 10^9$ SRBC/mL) injected subcutaneously into their right hind footpad. After 14 days of gavage treatments, the thickness of each rat's left footpad was measured just prior to an SRBC challenge using a Schnelltaster caliper (H.C. Kroplin Hessen, Schluchtern, Germany) that could measure to a minimum unit of 0.01 mm. The rats were then challenged by injecting 20 µL SRBC solution ($5 \times 10^9$ SRBC/mL) intradermally into their left hind footpad (designated time 0). Foot thickness was then re-measured after 24 h. The difference between the thickness of the left footpad just before and 24 h after challenge (in mm) was taken as a measure of DTH (Doherty, 1981).

***Neutrophil adhesion test***

The method described by Wilkonson (1978) was used for evaluating the effect of VMSE on neutrophil adhesion. After 14 days of gavage treatments, blood samples were collected from rats in each group by retro-orbital puncture (under light ether anesthesia) into heparinized vials and subjected to total as well as differential leukocyte counts. After performance of the initial counts, each blood sample was incubated with 80 mg/mL of nylon fibers at 37°C for 15 min. The incubated samples were then reanalyzed for total and differential leukocyte counts. The product of the total leukocyte count and the percentage (%) neutrophil (known as neutrophil index) was determined for each rat in their respective group (Fulzele et al., 2002). The percentage (%) neutrophil adhesion associated with each test rat was then calculated using the formula '% neutrophil adhesion' = $100 \times (NI_u - NI_t)/NI_u$, where $NI_u$ is the neutrophil index of the blood samples before nylon fiber treatment and $NI_t$ the index after the nylon fiber treatment.

***Carbon clearance test***

The method of Biozzi et al. (1953) was used to analyze phagocytic activity among the white blood cells (WBC) in the rats. For each treatment regimen, a total of six rats were utilized. Daily treatment with the VMSE (500 mg/kg BW, by gavage) occurred for 5 days prior to the assessment of *in situ* phagocytic activity. Control and DMS-treated rats received vehicle or the drug, respectively, in parallel each day.

A colloidal carbon ink suspension was injected via the tail vein into each rat 48 h after the final treatment. From each rat, blood samples (25 µL) were then withdrawn from the retro-orbital plexus under mild ether anesthesia, immediately after the injection and then 5, 10, and 15 min thereafter. Each blood sample was lysed with 2 mL of 0.1% acetic acid and the absorbance of the resulting solution evaluated at 675 nm (Damre et al., 2003). A graph of absorbance versus time post-injection was prepared for each animal and the *in situ* phagocytic index (PI) calculated using 'PI' = $K_{sample}/K_{standard}$, wherein $K_{sample}$ represents the slope of the absorbance versus time curve of blood samples from rats in the extract-treated or DMS-treated group and $K_{standard}$ represents the slope of the absorbance versus time curve of blood samples for the rats in the control group.

***Hematological profile***

After 8 days of the repeated gavage treatment, blood was collected from each rat via their retro-orbital plexus under light ether anesthesia. Various parameters such as total WBC, differential WBC, red blood cells (RBC), and platelet counts, as well as hemoglobin (Hb) levels, were then evaluated using a Sysmax XS800i automated hematology analyzer (TOA Medical Electronic Co., Tokyo, Japan).

***Statistical analysis***

Statistical analysis was carried out using one way ANOVA followed by Tukey's test, using the SigmaState 2.03 software and computer with Intel Pentium dual core processor. A value of $P < 0.05$ was considered a statistically significant difference between analyzed groups.

## Results

***Acute toxicity study***

A single oral dose of VMSE was found to be safe up to the dose of 1 g/kg BW as there was no mortality or other obviated pathologies detected for up to 72 h post-treatment.

***Immunomodulatory activity***

Daily VMSE treatments, at 500 mg/kg BW by gavage for 8 days, significantly ($P < 0.05$) increased total WBC, lymphocyte, and neutrophil counts relative to control rat values (Table 1). In contrast, each of these parameters was significantly and expectedly decreased by DMS treatments. While platelet levels were not altered by either VMSE or DMS treatments, both RBC counts and Hb levels were significantly decreased by both DMS and VMSE.

The studies to assess any effects of the treatments on the adhesion activity of neutrophils revealed that the % neutrophil adhesion levels were significantly decreased after 14 days of gavage treatments with DMS when compared with values associated with cells from the control group rats, i.e., ≈ 14%

versus 23%, respectively (Table 2). In contrast, the repeated VMSE treatments resulted in significantly ($P < 0.05$) increased % neutrophil adhesion values, i.e., ≈ 38% versus 23% for the controls.

In the SRBC-sensitized rats, primary, and secondary antibody titers (as $\log_2$ values) were significantly elevated by VMSE treatments when compared with corresponding values measured in the blood of rats in the control group (Table 3). Specifically, the titers were nearly tripled and doubled, respectively, as a result of the VMSE treatments. In contrast, daily immunosuppressive DMS treatment caused significant decreases in both primary and secondary titers in the rats.

The DTH response, measured in terms of hindpaw thickness, was increased to 14 days of treatment with VMSE by ≈ 98% above the level of change in pad thickness noted in the hind paw of rats in the control group (Table 4). As expected, the repeated DMS treatments resulted in significantly decreased DTH responses, i.e., by ≈ 42% relative to values in the controls. The degree of *in situ* phagocytic functionality, as measured by the rate of clearance of injected carbon after five consecutive days of VMSE treatments, was significantly increased relative to activity levels noted in the control rats (Table 4). Once again, contrary to this finding, parallel DMS treatments significantly decreased the PI in rats.

## Discussion

When animal hosts are non-intravenously sensitized with SRBC, this 'antigen' initially becomes diffused within the extravascular space and, ultimately, via the lymphatic system, enters regional lymph nodes. Macrophages in the lymphoid tissues or lining the sinuses are then able to phagocytize the antigen, process it for presentation (in the context of surface major histocompatability Class II molecules), and become antigen-presenting cells (APC) to many cells, including lymphocytes. Another APC is the B-lymphocyte—like macrophages, B-lymphocytes are not very effective at presenting this or other antigen to naïve T-lymphocytes. They are, however, effective in presenting antigen to memory lymphocytes, especially when antigen level is low (as their surface immunoglobulins [Ig] bind antigen with high affinity; Mayer, 2009). Once the antigen has been fragmented and processed, helper $T_H2$ T-lymphocytes

**Table 1.** Effects of treatments (8 days) on hematological parameters.

| Treatment group[a] | Total WBC[b] (cells/μL) × $10^3$ | Neutrophils (cells/μL) × $10^3$ | Lymphocytes (cells/μL) × $10^3$ | RBC[b] (cells/μL) × $10^6$ | Platelets (cells/μL) × $10^6$ | Hemoglobin[b] (Hb g/dL) |
|---|---|---|---|---|---|---|
| Control | 6.14 ± 0.84 | 0.46 ± 0.15 | 4.90 ± 0.96 | 9.16 ± 0.21 | 813.33 ± 53.48 | 16.13 ± 0.30 |
| DMS | 0.97 ± 0.04* | 0.04 ± 0.04* | 0.76 ± 0.07* | 7.27 ± 0.17* | 940.00 ± 47.84 | 13.17 ± 0.31* |
| VMSE | 9.15 ± 1.04* | 0.88 ± 0.12* | 7.99 ± 0.93* | 6.90 ± 0.50* | 792.33 ± 16.56 | 12.47 ± 0.76* |

[a]VMSE, *Vigna mungo* seed extract; DMS, dexamethasone.
[b]WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin.
All values are presented as mean ± SEM.
*Value significantly ($P < 0.05$) different compared with the control group.

**Table 2.** Effect of treatments (14 days) on the adhesion activity of neutrophils recovered from exposed rats.

| | [b]TLC ($10^3$/$mm^3$) (X) | | % Neutrophil (Y) | | Neutrophil index (X × Y) × $10^3$ | | % Neutrophil adhesion |
|---|---|---|---|---|---|---|---|
| Treatment group[a] | [b]UnB | [b]FTB | UnB | FTB | UnB | FTB | |
| Control | 6.38 ± 0.57 | 6.10 ± 0.60 | 14.34 ± 2.55 | 11.41 ± 1.85 | 88.18 ± 10.82 | 67.35 ± 7.86 | 23.33 ± 1.02 |
| DMS | 1.42 ± 0.34* | 1.43 ± 0.31* | 3.51 ± 0.09* | 3.10 ± 0.08* | 5.46 ± 1.32* | 4.62 ± 1.09* | 14.00 ± 3.07* |
| VMSE | 8.52 ± 1.03 | 8.41 ± 0.82* | 6.17 ± 0.72* | 4.65 ± 0.97* | 57.91 ± 12.03 | 36.14 ± 7.84* | 37.98 ± 2.33* |

[a]VMSE, *Vigna mungo* seed extract; DMS, dexamethasone.
[b]TLC, total leukocytes count; UnB, untreated blood; FTB, nylon fiber-treated blood.
All values are presented as mean ± SEM.
*Value significantly different ($P < 0.05$) compared with the control group.

**Table 3.** Effects of treatments on antibody formation by SRBC-sensitized rats.

| Treatment[a] | Primary titer[b] | Secondary titer[b] |
|---|---|---|
| Control | 3.52 ± 0.76 | 5.00 ± 0.76 |
| DMS | 0.15 ± 0.02* | 0.19 ± 0.05* |
| VMSE | 9.37 ± 1.39* | 10.00 ± 1.53* |

[a]VMSE, *Vigna mungo* seed extract; DMS, dexamethasone—treatments began in period starting 7 days prior to sensitization and continuing up to time of challenge (i.e., Day –7 up to and through Day + 7).
[b]All values are presented as $\log_2$ values (mean ± SEM); $n = 6$ per treatment group.
*Value significantly ($P < 0.05$) different compared with the control group.
SRBC, sheep red blood cells.

**Table 4.** Effects of treatments on DTH response and the phagocytic index.

| Treatment[a] | DTH response[b] | Phagocytic index[c] |
|---|---|---|
| Control | 0.85 ± 0.02 | 1.00 |
| DMS | 0.36 ± 0.01* (– 42.35%) | 0.24 ± 0.01* |
| VMSE | 1.68 ± 0.02* (+ 98.23%) | 6.41 ± 0.96* |

[a]VMSE, *Vigna mungo* seed extract; DMS, dexamethasone; DTH, delayed type hypersensitivity.
[b]Value in parentheses indicate decrease or increase in DTH response relative to control rat value.
[c]Control value set to 1.00 for comparative purposes.
All values are presented as mean ± SEM; $n = 6$ per treatment group.
*Value significantly ($P < 0.05$) different compared with the control group.

can then interact to assist/stimulate the B-lymphocytes to produce antibody against the SRBC. In general, during a first (primary) response to exposure to the SRBC/antigen, IgM is secreted initially, followed by a switch to (increasingly) IgG (Dale and Forman, 1989; Goldsby et al., 2003). On re-exposure to the antigen, a secondary response is elicited that is characterized by a rapid onset and highly amplified level of antibody production (nearly all as IgG).

In the present study, anti-SRBC antibody titers—during both primary and secondary responses—were seen to be significantly elevated in VMSE-treated rats. At first blush, the titers (as $\log_2$ values) might seem somewhat low in comparison to the vast majority of values reported in the literature. However, the control titers here were, in fact, in line with those of several other studies that utilized rat models (i.e., control host values of 4.4–5.3; Puri et al., 1994; Reeta et al., 2002). A potential reason for the 'low'-appearing titers could be the fact that the initial SRBC administration was through the host footpad rather than via the traditional intraperitoneal route. As already demonstrated in the classic paper by Stein-Streilein and Hart (1980), injection of the same antibody-forming cell (AFC)-inducing amount of SRBC into the footpad resulted in far less AFC in the lymph nodes (i.e., hilar) and spleens of the hosts than occurred in those animals provided the initial SRBC dosage by intraperitoneal (or even intravenous) injection.

Nevertheless, the augmentation of the humoral response by VMSE that is noted here (among all the rats that received the SRBC by the same route throughout—and thus carry any same 'inherent' error outlined above) could indicate that there was an enhanced responsiveness of macrophages/B-lymphocytes subsets in these hosts. The precise mechanism(s) underlying any induced augmentation in one/both cell types remains unclear. Other studies performed here sought to help us formulate initial potential mechanisms of this effect from this natural product.

In general, the rate of *in situ* carbon particle clearance is frequently used as a measure of reticuloendothelial system (RES) competency (Mishra, 2005). Specifically, a faster removal of particles is correlated with an enhanced phagocytic activity of RES cellular components (Abbas and Litchman, 2001). In the study here, prophylactic VMSE treatment enhanced the rate (more than six-fold increase) of carbon clearance seen among control group rats. Normally, a more rapid clearance of exogenous particulates from the blood by macrophages would arise from opsonization of the material with antibodies/complement C3b. However, in this study, the effects from the VMSE were notable within 15 min of particle injection; thus, VMSE-mediated changes in opsonization (overall or rate) is not likely to have been a major factor in our observations. It appears more likely, based on the hematological profile data (see Table 1), there was simply a greater presence of potential phagocytic cells in these treated hosts to ingest the particles over the 15-min period. It must be noted that it is not yet possible to preclude that these cells also have an enhanced phagocytic activity/capacity. The increase in neutrophil function (i.e., adhesion activity) strongly suggests this function in VMSE-treated rats' phagocytes was altered (i.e., immunoenhanced).

In addition to the above-noted outcomes, the DTH response—the magnitude of which can be directly correlated with competence of a host's cell-mediated immune function (Roitt and Delves, 1998)—was increased in rats that received VMSE. Apart from the key role of memory (sensitized) T-lymphocytes in this reaction, the role of local macrophages (initially) and then recruited monocytes/other phagocytes are critical as well. From the data here, no specific conclusions about the functionality of memory T-lymphocytes can be formulated; however, the increases in anti-SRBC titers in the VMSE-treated rats are suggestive of enhanced activation of T-lymphocytes (as a response to SRBC is considered T-lymphocyte-dependent). Those types of outcomes would be in keeping with the findings of effects on lymphocytes (and accessory cells required for DTH expression) noted by Mitra et al. (1999) in *in situ* studies of immunomodulatory effects of IM-133 (a formulation of the aqueous extracts of *Tinospora cordifolia* stems, *Withania somnifera* roots, *Phyllanthus emblica* fruits, and *Ocimum sanctum* leaves) in mice.

Apart from the potential effects on the lymphocytes, the increased phagocytic activities of local/recruited phagocytes would also be a major factor for the substantive increases in DTH noted in VMSE-treated rats. Clearly, if the local macrophages (here, dendritic cells) also display increases in their amount/rate of SRBC antigen presentation, this would likely also increase the degree of T-helper lymphocyte stimulation and subsequent release of cytokines. Several of these cytokines affect adhesion molecule expression on the local endothelium (and so enhance monocyte/phagocyte recruitment from blood) or activate the now-recruited monocytes and/or phagocytes and other resident macrophages at the challenge site. Our ongoing studies to analyze blood levels of several of the key cytokines involved in these processes, i.e., tumor necrosis factor-$\alpha$ and interferon-$\gamma$, should help us clarify whether VMSE caused augmentation in the release of these products (both basal and in response to SRBC/other antigens). Changes along these lines in the VMSE-treated rats would be similar to effects reported by Fulzele et al. (2002) in their studies of the immunostimulatory activity of *Asthmangal ghrita* (another herbal drug used in Ayurveda) in rats.

The majority of the cells involved in the immune system are produced from common hematopoietic stem cells found in the bone marrow. This site also provides a microenvironment for antigen-dependent differentiation of B-lymphocytes (Raphael and Kuttan, 2003). Since VMSE treatments were seen here to give rise to increased circulating antibody titers (specifically against the SRBC), it would be expected then that there should have also been increases induced in levels of one or more of the cell types involved in the humoral response to this antigen. In the present study, the evaluations of the peripheral blood of VMSE-treated rats confirmed there was an enhancement in total WBC levels, and among lymphocytes (differentiation of T- vs. B-types was not performed here) in particular. These outcomes suggested strongly that one potential effect of VMSE was an impact on hematopoietic

processes and on the bone marrow in particular. Ongoing studies will perform examinations of bone marrow cellularity to confirm if this site is indeed affected by the VMSE treatments.

The results of the current studies have shown that VMSE can impact upon individual functions *within* specific cell types in the immune system, i.e., particle clearance by phagocytes and neutrophil adhesion, as well as upon complex processes *among* various cell types in the immune system, i.e., antibody formation after antigen recognition, DTH responses. Whether such immunostimulatory outcomes also occur via consumption of the intact seeds themselves, as is most likely to be the case with humans, remains to be determined. Still, the present study provides evidence that could help explain how the traditional use of *V. mungo* has been successful in the treatment of various disorders in humans. To further improve the acceptability of this natural product for use by a wider population, the modes of action and the active constituents clearly need to be further investigated and characterized. To date, unlike for several natural products—including many routinely used in the Ayurvedic system of medicine, there are no reports in the literature that have sufficiently characterized the constituents of the *V. mungo* seed or any of its various preparations. Thus, a concerted effort between our laboratory and that of natural product chemists/analytical chemists will also need to be a part of these future investigations as well.

## Declaration of interest

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