INTRODUCTION

Molecules, cells and organisms display information about themselves in the form of glycoconjugates. This information is decoded by a structurally diverse class of proteins called lectins that bind carbohydrates with considerable specificity but moderate affinity [1]. As a consequence, lectins have found widespread application in probing the architecture and dynamics of cell surface carbohydrates during cell division, differentiation, and malignancies as well as in the isolation and characterization of glycoconjugates [2, 3]. The earliest studies on lectin-mediated mitogenesis and their proliferative effects on various cell types have stimulated interest in the properties of lectins while advancing knowledge of immunology [4, 5]. Their ability to stimulate lymphocytes as well as other cells, have made lectins an important diagnostic and experimental tool, which has made substantial contribution to study the various aspects of cell growth and differentiation, taking lymphocytes as the model cell type [6, 7]. Apart from this, it also has opened up a new arena for scientists to study the probable role of lectins in cell growth and development [8, 9].

Dietary lectins which are plant protein components with the ability to bind, selectively, free or conjugated saccharide in a reversible way by two or more binding sites, have been shown to induce lymphocyte proliferation or modulate several immune functions [2, 7]. They are able to induce cell division or proliferation in different kinds of cells mainly lymphocytes from lymph organs or blood [5, 7]; the most well-known among are Phytohemagglutinin (PHA), Pokeweed mitogen (PWM) and concanavalin A (Con A) [4, 6]. Studies with lectins and monoclonal antibodies have established that large variety of cell-surface molecules influence the initiation and regulation of lymphocyte activation and proliferation [6]. However, not all plant lectins are mitogenic; they can be grouped as mitogenic, non-mitogenic or anti-mitogenic [10, 11]. Actually, WGA has been found to be non-mitogenic, anti-mitogenic, and mitogenic for either T cells or B cells depending on the concentration of the lectin or the purity of the examined cells [5]. As lectins are reportedly being identified and isolated from the vast number of dietary sources with the desirable biological activity, it will be interesting to study the mitogenic potential of dietary lectins on lymphocytes, as they cause in vivo modulation of lymphoid tissues [4, 10, 11]. Potato lectin (Solanum tuberosum agglutinin; STA) is a blood group-nonspecific lectin (pan-agglutinin) present in potato tubers, and is specific for chitin (poly N-acetyl-D-glucosamine). STA is dimeric comprising of two identical monomers of ~55 kDa, fifty percent of its mass being carbohydrates [12]. The bulbs of garlic contain two mannose specific lectins (Allium sativum agglutinin ASA I & II) which are the predominant proteins in garlic bulbs. The heterodimeric ASA I consists of two slightly different subunits of 11.5 and 12.5 kDa, whereas the homodimeric ASA II consists of 12 kDa subunits [13].

Garlic extracts are known to maintain cardiovascular homeostasis in mice and rats [14] and modulate an immune response by inducing proliferation of T lymphocytes [15]. Previously it is reported that STA is involved in degranulating the mast cells and basophils thereby resulting in non-allergic food hypersensitivity reactions [16]. However, the mitogenic and immunomodulatory activity of purified potato lectin, have not been reported so far and information on the immunomodulatory potential and interaction with immune cells of garlic lectin is very limited [17]. Present study focus to investigate the modulator effects of the raw and heat processed potato and garlic extracts along with the purified mannose-specific garlic lectins (ASA I and ASA II) and oligo-GlcNAc specific potato lectin (STA) on human and murine lymphocytes in relation to mitogenicity to provide insights about the immunomodulatory activity with relevance to native, heat processed and purified lectins.
along with known mitogen Con A to understand, mitogenic, co-
mitogenic and anti mitogenic nature with its synergistic behavior.

MATERIALS AND METHODS

Materials

This study was undertaken after clearance by the Institutional
Human as well as Animal Ethics Committees; informed consent was
obtained from all human volunteers for obtaining peripheral venous
blood in the age range of 18-60 y. Potato lectin and garlic lectins
were purified in the laboratory as described previously [12, 13].
Spleen and thymus were obtained from BALB/c mice procured from
Central Animal House Facility, Indian Institute of Science, Bangalore,
India. Concanavalin A (Con A), phytohemagglutinin (PHA), Ficol-
hyphaque, RPMI-1640 medium were products of Sigma-Aldrich Co.,
St. Louis, MO, USA. Tissue culture grade sodium bicarbonate, glutamine,
MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl
tetrazolium bromide A. R.] and NBT (Nitroblue tetrazolium A. R.)
were purchased from Hi Media Laboratories Ltd., Mumbai, India.
Fetal calf serum (FCS) was obtained from Sera-lab (Sussex, England).
Tissue culture plates were products of Costar Ltd., Cambridge, MA,
USA. All other chemicals and reagents were of analytical grade.

Raw and heat-processed garlic and potato extracts and their lectins

Raw and heat-processed (boiling for 30 min at 100 °C) potato/garlic
extracts (50% w/v) were prepared using phosphate buffered saline
(PBS). The extract was then centrifuged in the cold at 5200 x g rpm
for 15 min. Raw and heat-processed extracts were analyzed for
protein content by dye-binding assay. Potato lectin (STA) and garlic
lectins (ASA I and ASA II) at pH 9.6 at 4 °C overnight. After the blocking step using
Ficol-hyphaque, RPMI-1640 medium were products of Sigma-Aldrich Co.,
St. Louis, MO, USA. Tissue culture grade sodium bicarbonate, glutamine,
MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl
tetrazolium bromide A. R.] and NBT (Nitroblue tetrazolium A. R.)
were purchased from Hi Media Laboratories Ltd., Mumbai, India.
Fetal calf serum (FCS) was obtained from Sera-lab (Sussex, England).
Tissue culture plates were products of Costar Ltd., Cambridge, MA,
USA. All other chemicals and reagents were of analytical grade.

Preparation of 2% rabbit erythrocytes and haemagglutination
(HA) assay

Five milliliters of fresh rabbit blood was collected and put into 5 mL of
Alsever’s solution. The contents were mixed by swirling in order to
remove the plasma layer, a circle of white translucent coat containing
lymphocytes was washed 4-5 times using PBS at 4 °C, and finally
re suspended in RPMI-1640 medium.

Glycoprotein binding assay

Microtiter wells were coated with 10-20 µg protein of various potato
and garlic extracts or purified potato (STA) and garlic lectins (ASA I
and ASA II) at pH 9.6 at 4 °C overnight. After the blocking step using
3% gelatin in PBS, the microtiter wells were incubated with HRP
(100 µL of 0.1 mg/ml), or avidin-AP conjugate (100 µL of 1:2000
dilution) in PBS containing 1% BSA/0.05% Tween-20 at 37 °C for 2
h. Following the addition of the respective substrate (o-
phenylenediamine/H₂O₂ for HRP or p-nitro phenyl phosphate for
alkaline-phosphatase), the absorbance was measured at 405 nm.

SDS PAGE pattern of processed extract and pure lectins

Protein quantification was carried out following the standard protocol
of Bradford assay, using BSA as the standard. The protein pattern of
the potato & garlic extracts (Raw and heat processed) were analyzed by 12% or 15% SDS-PAGE (reducing), using a
Bio-Rad mini electrophoresis unit. The protein bands were visualized by
staining with Coomassie Brilliant Blue R-250.

Isolation human and murine lymphocytes

Human lymphocytes were isolated from the peripheral blood
by peripheral blood lymphocytes whereas, the murine lymphocytes
were obtained from the spleen and thymus of mice as splenocytes
and thymocytes and are maintained in RPMI cell culture medium.

Preparation of complete RPMI-1640 media for proliferation assay

RPMI-1640 cell culture medium was used for all experiments. For
incomplete medium, powder medium was added to triple distilled
filtered water, and dissolved by gentle stirring. Later, tissue culture
grade sodium bicarbonate was added to strength of 7.5 %, the final
volume was made with water, and the pH adjusted to 7.2.
Incomplete medium was supplemented with 10 % v/v fetal calf serum,
1 % sodium pyruvate, 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin to obtain a complete
medium, which was used for the proliferation assay.

Isolation of human peripheral blood lymphocytes (PBLS)

Ten milliliters of venous blood drawn from healthy normal subjects
were transferred to HAAnticlot vials (heparin-coated flat-bottom
polystyrene vials from HiMedia Laboratories Ltd., Mumbai, India). The heparinized blood was layered carefulliy on Ficol-hyphaque (density = 1.077 g/ml) contained in a polystyrene tube. The tubes were centrifuged at 250 x g at 25 °C for 20 min. Centrifugation at a lower temperature (4 °C) was avoided since this result in cell
crushing and poor recovery [14]. After centrifugation, below the plasma layer, a circle of white translucent coat containing
lymphocytes was aspirated carefully using a pasteur pipette. The cells were then re suspended in isonicot phosphate-buffered saline (PBS) and mixed by gentle aspiration. The buffy coat containing
lymphocytes was washed 4-5 times using PBS at 4 °C, and finally
were placed in complete RPMI-1640 medium.

Isolation of murine splenocytes and thymocytes

Spleen and thymus were collected under aseptic conditions from
normal BALB/c mice (23-25 g, 12-weeks-old) after sacrifice,
placed in isonicot phosphate buffered saline (PBS). These tissues
were separately minced using a pair of scissors and passed through
a fine steel mesh to obtain a homogenous cell suspension. The cells
were pelleted to remove the tissue debris. After centrifugation (380 x g at 4 °C for 10 min), the pelleted cells were washed three times with PBS (400 x g at 4 °C for 10 min). The pellet obtained after washing with PBS was re suspended in a modified ammonium chloride buffer (150 mM NaCl, 10 mM KHCO₃, pH 7.4 containing 10
mM sodium acetate) and incubated at 4 °C for 5 min to remove the
erythrocytes. After the cells were centrifuged at 400 x g at 4 °C for 20
min, the pellets were further washed in physiological salt solution (PSS) [137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1.6 mM CaCl₂, 10 mM HEPES, 0.05 % gelatin and 6.45 mM NaN₃, pH 7.4], washed three times in the same buffer, and finally re suspended in RPMI-
1640 medium.

Counting of lymphocytes and determination of viability

The isolated lymphocytes from normal human subjects and mice
spleen and thymus were counted using hemocytometer and crystal
violet stain. The cell concentration was adjusted to 2.5 x 10⁵ cells/ml
and used for proliferation assay. Percentage viability of lymphocytes
in the isolated cell suspension was checked by Trypan blue exclusion
method. For the cell viability determination, an aliquot of cell
suspension was taken and mixed with 0.2 % Trypan blue at 1:1 dilution and kept at 25 °C for ~2 min. The cell suspension charged to
hemocytometer was observed under the microscope. Cells, which are
dead or partially damaged, appear as dark blue against a light
blue background, since they take up the dye. The viable cells appear
clear without any stain against the light blue background.

MTT assay for cellular proliferation

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide] assay was carried out using the method described previously [10, 11]. The assay was performed in tissue culture plates (96 wells microtiter plates); the wells were added with complete medium followed by lectins (0.01 to 10 µg/ml) and lymphocytes (100 µL of 2.5 x 10⁵ cells/ml). Plates were incubated in a CO₂
incubator at 37 °C with 5% CO₂ for 72 h. Lymphocytes in the absence of lectins represent control, and blank was carried out with
complete medium only as other incubation for 72 h, 5 µL of 5 mg/ml
MTT solution (MTT was dissolved in 0.1 M Tris-buffered saline,
which was then filtered to remove any insoluble residues) were
added and incubated for further 4 h under the same conditions.
After removing the plates, the samples were aspirated to micro
centrifuge tube, and centrifuged at 750 x g at 4 °C for 15 min.

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Supernatant was removed and the blue formazan crystals were resolubilized in 500 μL of isopropanol with 0.04 N HCl under agitation. After dissolving the crystals, 100 μL of each sample were taken in microtiter plates. Plates were read in a microplate reader at 570 nm.

**Nitric oxide (NO) assay**

NO is a gaseous free-radical molecule which is catalytically generated by cellular nitric oxide synthase (NOS) upon conversion of L-arginine to L-citrulline. The amount of NO produced in the medium (incubated with human PBLs and lectins) was determined by assaying its stable end product, NO₃⁻ (nitrate) by the method described previously [18]. Briefly, equal volumes (100 μL) of sample and Griess reagent (1% sulfanilamide, 0.1% N-t-naphthyl ethylenediamine in 5% H₃PO₄) were mixed in a 96-well microtiter plate at room temperature. The absorbance was then measured at 540 nm in a microplate reader. A range of sodium nitrate dilutions served to generate a standard curve for each assay.

**Nitro blue tetrazolium (NBT) assay for reactive oxygen species**

NBT assay was performed according to the procedure explained previously [19]. Briefly, the rat peritoneal exudate cells containing macrophages were isolated using Tyrode buffer (137 mMNaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 11.9 mM NaHCO₃, 0.4 mM NaH₂PO₄, and 5.6 mM glucose, pH 7.4) containing 0.1% BSA. The cells obtained were washed twice with Tyrode buffer by centrifugation at 2000 x g for 20 min. The peritoneal cells (1 x 10⁵ cells/ml) were taken in a volume of 100 μL, 400 μL of Hank’s balanced salt solution (HBSS) containing lectins (0.1, 1, 10 μg/ml) was added with 0.4% BSA. The samples were pre-incubated at 37°C for 10 min, and then 2 mM NBT (40 μL/each tube) was added and further incubated at 37°C for 20 min. Reaction was arrested by the addition of 2.5 mL ice cold HBSS. After the tubes were centrifuged at 400 x g for 10 min, the supernatant was discarded and the blue formazan crystals were dissolved by adding 2 volume of dioxiane. After centrifugation, absorbance of the supernatant was read at 540 nm.

**Statistical analysis**

Data presented in this study were expressed as mean±Standard Error (SEM) of three experiments (n=3). One-way analysis of variance (ANOVA) test followed by multiple Tukey’s comparison test was applied. A p-value of <0.05 was considered to be statistically significant.

**RESULTS**

**SDS-PAGE analysis, hemagglutination and glycoprotein binding assay**

The SDS-PAGE gel profile shown in Fig.1 depicts the protein pattern of raw/heat processed extracts and purified lectins of potato and garlic. The specific activity of potato and garlic lectins as measured by hemagglutination assay was summarized in table 1. The specific HA activity of potato lectin (STA) was 3891 units/mg whereas that of garlic lectins ASA I and II were 1779 and 604 units/mg respectively.

**Table 1: Hemagglutination and glycoprotein binding properties of raw and heat processed extract and lectins of potato and garlic**

<table>
<thead>
<tr>
<th>Samples</th>
<th>HA activity (Units/mg protein)</th>
<th>Glycoprotein bind in #</th>
<th>HRP (A405) (0.1 mg/ml)</th>
<th>Avidin-AP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Potato</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE</td>
<td>58.20</td>
<td>0.345*</td>
<td>0.311*</td>
<td></td>
</tr>
<tr>
<td>HPPE</td>
<td>29.05</td>
<td>0.214</td>
<td>0.245</td>
<td></td>
</tr>
<tr>
<td>HPPE supernatant</td>
<td>No agglutination</td>
<td>0.023</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>STA</td>
<td>3891.0</td>
<td>0.419**</td>
<td>0.411**</td>
<td></td>
</tr>
<tr>
<td><strong>Garlic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RGE</td>
<td>21.5</td>
<td>0.057</td>
<td>0.146</td>
<td></td>
</tr>
<tr>
<td>HPGE</td>
<td>14.3</td>
<td>0.036</td>
<td>0.072</td>
<td></td>
</tr>
<tr>
<td>HPGE supernatant</td>
<td>No agglutination</td>
<td>0.014</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>ASA I</td>
<td>1779</td>
<td>0.395**</td>
<td>0.489*</td>
<td></td>
</tr>
<tr>
<td>ASA II</td>
<td>604</td>
<td>0.263*</td>
<td>0.344*</td>
<td></td>
</tr>
</tbody>
</table>

#20 μg protein coated on microtiter wells; HRP concentration: 0.1 mg/ml; avidin-AP dilution-1:1500. Values for control protein (BSA): 0.009 (HRP-BA); 0.015 [Avidin-AP].

RPE: raw potato extract; HPPE: heat processed potato extract. STA: Solanum tuberosum agglutinin (potato lectin); ASA: Allium sativum agglutinins (garlic bulb lectins). Data represents results of three independent experiments. Values are mean±SEM, n = 3, one way ANOVA followed by Tukey’s multiple comparison test. Significant values are *p<0.05; **p<0.01.

Heat processed potato and garlic extracts have retained nearly 50% of HA activity when compared to their raw counterparts (table 1). The results of glycoprotein binding assay revealed that potato (STA) and garlic lectins (ASA I and II) showed a strong binding to HRP and avidin. Among the garlic lectins ASA I showed significantly stronger binding to the glycoprotein. Raw potato extracts (RPE) showed a good binding to glycoprotein than raw garlic extracts (RGE). It is obvious from the results that heat processing did not completely reduce the glycoprotein binding efficiency in both potato and garlic extracts indicating its heat stability.

**Mitogenic stimulation of human PBLs by garlic and potato lectins**

The dietary lectins from potato tuber (STA) and garlic bulb (ASA I and ASA II) were purified to homogeneity as described previously [12, 13]. The purified lectins were tested for their ability to proliferate human PBLs. Initially, all the three lectins along with

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positive reference mitogen, Con A, were checked in the concentration range of 0.01 to 10 µg/ml concentration. The results are shown in fig. 2. Both garlic lectins ASA I and II show stimulatory effects with human PBLs where as STA shows inhibitory effects at higher concentrations (1 and 10 µg/ml) and no effect at lower concentrations (0.01 to 0.1 µg/ml). ASA I behaves similar to Con A in its potential to cause proliferation of human PBLs (fig 2A). However, ASA II shows only a mild mitogenic effect as compared to control.

The results are shown in fig. 3A. ASA I show a similar effect as that of the known mitogen Con A, and an increased response is seen in the presence of Con A (significant at P ≤ 0.001). ASA II shows approximately half of the response seen for ASA I and Con A, but in the presence of Con A, ASA II shows only a slight increase in cell proliferation (compared to Con A alone) which is not significant at P ≤ 0.05. Potato lectin (STA) does not show any stimulatory effect; in the presence of Con A STA reduces the response induced by Con A by 30-35%, which indicates the inhibitory effect of potato lectin towards human PBLs.

The proliferative effect of garlic lectins, potato lectin and garlic/potato extracts on human PBLs are shown in terms of proliferation index in fig. 3B. The index for control (untreated cells) taken as 1.0, and for others are represented as fold increase or decrease over the control. Proliferation index is calculated by dividing the absorbance of test by absorbance of control. Raw garlic extract (RGE) has mitogenic, whereas heat-processed garlic extract (HPGE) has half the mitogenic effect shown by RGE. Potato extracts (RPE and HPPE) do not show any mitogenic activity. ASA I and Con A shows 3.5 fold increase, whereas ASA II shows only 1.5 fold, indicating that both ASA I and ASA II are mitogenic. On the other hand, STA showed a proliferation index of 0.65 and appears to be anti-mitogenic.

Mitogenic and co-mitogenic effect of extracts and lectins on human PBLs

Both garlic lectins, ASA I and ASA II, were found to be mitogenic and co-mitogenic for human PBLs, whereas STA was neither mitogenic by itself nor co-mitogenic when added with a known mitogen.
Modulatory effects of garlic lectins on murine splenocytes and thymocytes

Murine splenocytes were isolated from the spleen obtained from adult BALB/c mice. The splenocytes were tested for proliferation by garlic lectins ASA I and ASA II at 0.1-10 µg/ml concentration. Con A and PHA which are known T-cell mitogens were used as reference positive mitogens, and the cells in the absence of any lectin served as control. The result of splenocytes stimulation is shown in fig. 4A. There is a significant difference (at p ≤ 0.001) in the proliferation of splenocytes by ASA I at 1 and 10 µg/ml and this effect is comparable to those of the reference mitogens. ASA II shows only a slight increase in stimulatory effect and is not significant at p ≤ 0.001 as compared to Con A, but in comparison to control the effect is significant at p ≤ 0.05.

Murine thymocytes were isolated from the thymus obtained from adult BALB/c mice. Thymocytes were stimulated by garlic lectins ASA I and ASA II at 0.1-10 µg/ml concentration (fig. 4B). The stimulatory response of garlic lectins for murine thymocytes seems to be more as compared to human PBLs and murine splenocytes. Similar trend of activation by garlic lectins is seen as in the case of murine splenocytes. The effect, of ASA I is comparable to that of Con A and PHA. However, ASA II shows a slightly higher degree of activation for thymocytes compared to splenocytes (fig. 4C).

Effect of garlic and potato lectins on nitric oxide (NO) production from human PBLs

The induction of nitric oxide synthesis from human PBLs by extracts and lectins was studied. A calibration curve was prepared using sodium nitrate in the concentration range of 0-10 µM showing a linear correlation with R² of 0.9995. Garlic extracts and purified garlic lectins do not show a significant increase in the NO production from human PBLs (fig. 5A). However, HPGE and ASA II shows a slightly lower production as compared to RGE and ASA I. Reference lectins (Con A and PHA) do not show a remarkable NO production. Nitric oxide production by garlic lectins ranges from 1.5 to 2 µM as measured from the calibration curve. Nitric oxide induction by garlic lectins is not very significant. A similar trend has been observed even with potato extracts and potato lectin. Nitric oxide production is marginal and the result is not significant (fig. 5A). Potato extracts and potato lectin induce NO in the range of 0.5 to 1 µM.

Superoxide generation from rat PECs by garlic lectins and potato lectin

All the three purified lectins (ASA I, ASA II and STA) and RGE were checked for their ability to induce reactive oxygen species from isolated rat PECs containing macrophages. The lectins were used in the concentration range of 0.1 to 10 µg/ml for stimulation. The results are shown in fig. 5B. All the three lectins do not induce superoxide production, and only the raw garlic extracts (RGE) at 10 µg/ml induces superoxide production (~3.5 fold compared to the control).

DISCUSSION

Mitogenic activities of plant lectins are consequences of their carbohydrate binding ability as evidences indicate that cell-surface glycoconjugates are involved in mitogenic process [4, 7]. Various studies have established that a surprisingly large variety of cell-surface molecules influence the initiation and regulation of lymphocyte activation and proliferation [5, 20]. Unlike typical antigens, which perhaps stimulate 0.01-0.1% of the lymphocyte population, mitogenic lectins can stimulate up to 20% [21]. The in vitro mitogenicity of lectins is typically measured as their ability to induce proliferation of lymphocytes from lymph organs or blood; the best described and most used are PHA, PWM and Con A [6, 11, 22]. However, not all plant lectins are mitogenic. Some lectins like WGA and datura lectin were reported to be anti-mitogenic in nature [23]. Lectins are biological recognition molecules and can induce varied responses on interaction with cell surface glycoproteins [1, 3]. Many plant lectins have been known to induce different signal on binding with cells of immune system [7]. The potato lectin (STA) and Garlic lectins (ASA I and II) are capable of interacting with the RBC cell surface glycans and agglutinate the cells and can bind model glycoproteins HRP and avidin as evident by hemagglutination and glycoprotein binding assays. Both heat processed potato (HPGE) and garlic extracts (HPGE) retained the lectin activity with hemagglutination and glycoprotein binding efficiency indicating the thermal stability of lectins. Most plant dietary lectins are known to be heat stable and digestive resistant [24] which make them potential to bind cells in the gastrointestinal epithelium and cross the mucosal barrier and can enter into systemic circulation to encounter different cellular system to induce various biological responses [25, 26]. Garlic lectin was reported to be stable in the gut and can induce specific immune response [24].

In this study, garlic lectins ASA I and II induced proliferation of both human and murine lymphocyte population and the degree of proliferation by ASA I is comparable to those of the reference lectins, Con A and PHA. ASA II shows less stimulatory effect on murine splenocytes and human PBLs. Among RGE and HPGE, only RGE shows a significant stimulatory effect against both human and murine lymphocytes. The non-protein components may co-stimulate and cause synergetic effect with the lectins in the extract. The varying stimulatory response between ASA I and ASA II might be because of their difference in the carbohydrate binding ability [13, 151].
Potato extracts as well as potato lectin do not show the mitogenic stimulation of human and murine lymphocytes. STA was found to be non-mitogenic at lower concentration, but at higher concentration induces an inhibitory response for lymphocytes. Though the initial step in mitogenic stimulation is binding of the lectin to the cell surface carbohydrate moieties, this alone is not sufficient, since certain lectins are non-mitogenic even though they bind well to human lymphocytes [28]. Thus, it is believed that mitogenic lectins interact with unique membrane components that may act as 'stimulatory receptors' and that non-mitogenic lectins may not bind to these membrane components, or alternatively bind to 'inhibitory receptors' [5].

Although some lectins are polyclonal activators both in vivo and in vitro, others may display a broad range of activities toward human lymphocytes. Indeed, the same lectin may be mitogenic, co-mitogenic, or anti-mitogenic, depending on the experimental conditions [5, 29]. Several lectins (WGA, LEA, DSA) were surprisingly found to be anti-mitogenic [30]; in other words they act to antagonize the stimulatory activity of mitogens with which they are co-cultured [31]. The response shown by STA strongly agrees with earlier findings, where potato lectin (LEA), wheat germ agglutinin (WGA) and Datura lectin (DSA) have been found to be non-mitogenic and inhibit lymphocyte proliferation [22, 23, 29, 31]. The Solanaceae lectins have basically similar saccharide specificities, and it is likely that they all act as anti-mitogens by binding to the same receptor [29]. The non-mitogenic nature of these lectins could be explained by their ability of blocking the receptor with an essential role in T-cell activation mechanism of mitogenic stimulation by lectins still need to be understood with respect to the finer details.

Potato lectin and garlic lectin were tested for their ability to induce ROS and nitric oxide production which are signal molecules and serves as a secondary messenger for various biochemical signaling mechanisms [30]. All the three purified lectins, raw and heat processed extract did not induce NO production from human PBLs. These lectins also do not possess the ability for the generation of reactive oxygen species, whereas RAW induced ROS production indicating other non-protein components present in raw garlic have ROS stimulatory activity. Production of ROS is an indication of pro-inflammatory oxidative stress that results in the generation of certain cytokines from leukocytes critical for an inflammatory process involved in pathogenesis [34]. The activation of inducible nitric oxide synthase (iNOS) triggers the production of NO in activated cells. iNOS is stimulated in a pro-inflammatory or inflammatory condition and produces temporary NO [35]. No induction of ROS and NO by lectins indicate these does not stimulate cells to produce any pro-inflammatory cytokines and hence does not cause are related to any pathogenesis.

CONCLUSION

The observations in this study reveals the chitinobiose specific potato lectin (STA) and mannospecific garlic lectins (ASAI and II) induce agglutination of human erythrocytes by binding to cell surface glycans. Potato lectin and extracts were non-mitogenic and anti-mitogenic on human and murine lymphocytes whereas garlic lectins and extracts were found to have potent mitogenic stimulation on human and murine lymphocytes. ASA I in particular had significant stimulatory potential and comparable with standard mitogens confirming to be synergistic and co-mitogenic in nature. Moreover, none of the purified lectin or extract induces the superoxide and nitric oxide in stimulated cells indicating these does not induce inflammation or pathogenesis to cells. Garlic lectin ASA I can be a potential stimulator to boost immune response in immunodeficiency condition. These dietary lectins can be a reagent for activating lymphocytes in diagnostic work and to study immunomodulatory functions. The lectin-cell interactions and stimulation and inhibition of cells encourage developing lectin based cell based biological response tools in health and disease.

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ABBREVIATION


CONFICT OF INTERESTS

The authors declare that there are no known conflicts of interest associated with this publication.

REFERENCES


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