Immunomodulatory Effect of Water Soluble Polysaccharides Isolated from *Metroxylon sagu* in Animal Models of Immunosuppression

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**ABSTRACT**

**Aim:** This study was aimed to investigate the immunomodulatory activity of water soluble polysaccharides isolated from *Metroxylon sagu* (PSMS) by dilute acid extraction, ethanol precipitation in rats by using three different *in-vivo* experimental models of immunosuppression. **Methodology:** Three models of immunosuppression include metronidazole (MTZ) induced immunosuppression, pyrogallol induced immunosuppression and Ethanol-induced immunosuppression. Immunological indices like humoral antibody titer values, cellular immune response, percent change in phagocytosis, serum immunoglobulins were estimated. Histopathology of spleen was done in all control and treated groups. The doses of 500 and 250 mg/kg of PSMS were administered orally to evaluate the immunomodulatory activity. **Results:** Though PSMS was demonstrated to have immunostimulatory activity in almost all three models of immunosuppression, PSMS was found to be more efficacious against ethanol-induced immunosuppression when compared with pyrogallol induced immunosuppression and MTZ induced immunosuppression. However, dose-dependent improvement in immunological indices was evident in all three models. **Conclusion:** In summary, water soluble polysaccharides isolated from *M. sagu* stimulate the immunity in the animal models of immunosuppression.

**Keywords:** Immunomodulation, immunosuppression, *Metroxylon sagu*, oxidative stress, polysaccharides

**INTRODUCTION**

Immune system dysfunction is responsible for various diseases such as arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer, and infectious diseases. Chemotherapeutic agents available today have mainly immunosuppressive activity and most of them are cytotoxic and exerts a variety of side-effects. Medicinal plants and their active components as a source of immunomodulatory agents are gaining importance.¹ Many herbs such as *Centella asiatica*, *Azadirachta indica*, *Phyllanthus debelis*, *Asparagus racemosus*, and *Chenopodium ambrosioides* have been shown to alter the immune function and to possess a wide array of immunomodulatory effects.² True sago palm is one of genus *Metroxylon* belongs to family arecaceae or palmae. The plant accumulates a huge amount of starch in its stem, very often more than 100 kg/plant. It contains mainly 80% starch, 16% water, 2% nitrogenous substance, and very little ash. Its utilization includes a wide range of consumption types, containing staple food, noodle-making, confectionery and fuel alcohol.³ Traditionally, stem sap of *M. sagu* is applied to the forehead to ease headaches. Starch derived from the plant trunk is mixed with water and drunk to treat diarrhea and stomach pains. Starch paste is also applied on to burn. The Leaf is used to cover fresh or infected sores until they heal. Liquid starch is given to newborn to treat enlarged spleen.⁴ Since there is no scientific data on the immunomodulatory activity of this plant, this study was undertaken to evaluate the immunomodulatory activity of water soluble polysaccharides isolated from the *M. sagu* in rats.

**MATERIALS AND METHODS**

**Drugs and chemicals**

Metronidazole (MTZ) was gifted by Zydus Cadila Health Care Limited (Hyderabad, India), India. Pyrogallol
was a gifted by Hi Media Laboratories Private Limited (Hyderabad, India), Levamisole was gifted by Leo Bio-Care Private Limited (Hyderabad, India). All the chemicals were purchased from local agents, and these are of analytical grade.

Plant material

The seeds of *M. sagu* belongs to the family Arecaceae were collected at the local areas of Anantapur district (Andhra Pradesh, India) in winter season in the month of November 2010 and were authenticated by Dr. J. Ravindra Reddy and voucher specimen (Raghavendra Institute of Pharmaceutical Education and Research-05/11) was preserved in the departmental herbarium (Pharmacognosy and Ethnopharmacology Division) for future reference.

Extraction of polysaccharides

*M. sagu* seeds were collected and pulverized into a coarse powder and used for extraction of polysaccharides. About 160 g of the seeds of *M. sagu* was allowed to stand in 1 L of 0.1 N HCl for overnight at room temperature. The extract was filtered through an atypical woman’s nylon sock. Later, the filtrate was neutralized with 1 N NaOH and polysaccharides were precipitated with three volumes of ethanol. After centrifugation for 30 min, the precipitate was re-dissolved in distilled water. The pH of the suspension was adjusted to 2.0 with 1 N HCl, and CaCl₂ was added to the final concentration of 2 M. The resulting precipitate was removed by centrifugation and the supernatant was treated with three volumes of ethanol. The ethanol precipitation was repeated twice and the precipitate was re-dissolved in distilled water, dialyzed at 4°C against water for 48 h, and then freeze-dried.

Experimental animals

Wistar rats of 180-200 g were used to carry out the immunomodulatory activity. The animals had free access to standard commercial diet and water *ad libitum* and were housed in cages under standard laboratory conditions, i.e., 12:12 h light/dark cycle at 25 ± 2°C. The experimental protocol was approved by the Institutional Animal Ethics Committee (Protocol number is PIPER/IAEC/05/2011 and all experiments were carried out in compliance with CPCSEA guidelines. (878/ac/05/CPCSEA/003/2011).

Acute toxicity study of the polysaccharides isolated from *Metroxylon sagu* (PSMS)

The PSMS was subjected to acute toxicity studies to determine the dose for the *in-vivo* studies. Wistar mice of either sex were selected randomly and divided into six groups (*n* = 6). The animals were fasted overnight and the PSMS at a dose of 200, 400, 800, 1000, 2000, and 4000 mg/kg body weight, were given orally to the mice. The animals were observed carefully for any sign of morbidity, mortality, and behavioral changes immediately after being dosed at 4 h and at 24 h intervals and twice daily for the subsequent 7 days.

Assessment of immunomodulatory activity

For the assessment of immunomodulatory activity, we selected three models like MTZ induced immunosuppression, Pyrogallol induced immunosuppression and ethanol induced immunosuppression. Animals were treated with polysaccharides isolated from *M. sagu* (PSMS) at a dose of 250 and 500 mg/kg. Experimental design for immunological studies was shown in Table 1.

Immunological studies

Hemagglutinating antibody (HA) titer

Blood was withdrawn from the jugular vein of a sheep red blood cells (SRBCs) were preserved in Elsevier solution. It was then suspended in phosphate buffered saline for further use. All rats were anti-genically challenged twice with SRBC (0.025 × 10⁹ cells/100 g, i.p.).

Blood samples were collected from the retro-orbital plexus and rat serum was used for determination of hemagglutination titer. The blood samples were centrifuged to collect serum and equal volume of individual serum samples of each group was pooled. Sera were serially diluted (in doubling dilutions) in phosphate buffered saline (PBS) and placed in the wells of a U-shape 96-Microtiter plates. Aliquots (25 μl) of two-fold diluted sera in PBS were challenged with 25 μl of 1% v/v SRBCs suspension and mixed. After mixing, the plates were incubated at 37°C for 1 h and examined for hemagglutination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody title.

Delayed-type hypersensitivity (DTH) response

The rats were challenged by injection of 0.5 × 10⁹ cells SRBCs in right hind footpad. The increase in the paw volume induced by injection of SRBC (0.025 × 10⁹ cells) in the sub plantar region of right hind paw was assessed after 48 h of this challenge. The mean percent increase in paw volume was considered as DTH reaction and considered as an index of cell-mediated immunity. The volume of left hind paw injected similarly with phosphate buffered saline, served as control.
Pulla, et al.: Protective effects of water soluble polysaccharides isolated from Metroxylon sagu

Carbon clearance test

In all the three models, Wistar rats were treated with the drug or vehicle as per treatment schedule. After 3 h of the last dose of the drug, animals were injected 0.1 ml of carbon ink (camel fountain pen ink) suspension (1.6% v/v in 1% gelatin, in saline) through the tail vein. Blood samples were withdrawn (in 0.15% w/v disodium ethylenediaminetetraacetic acid [EDTA] solution) from a retro-orbital vein at intervals of 0 and 15 min after injection. A 50 μl blood sample was mixed with 4 ml of 0.1% sodium carbonate (Na₂CO₃) solution and the absorbance of this solution was determined at 660 nm, taking 0.1% Na₂CO₃ solution as a blank. The percentage increase in phagocytic index was calculated.¹⁰

Serum immunoglobulins (Igs)

Serum Igs like immunoglobulin G (IgG) was estimated¹¹ using the kit (Quantia) on the last day of the treatment, i.e., 15th day in case of MTZ induced immunosuppression, 22nd day in case of pyrogallol induced immunosuppression and 28th day in the case of ethanol-induced immunosuppression, respectively.

In-vivo anti-oxidant parameters

In-vivo antioxidant parameters such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (RSH), and lipid peroxidase (LPO) were estimated on the last day of the treatment, i.e., 14th day in case of MTZ induced immunosuppression, 22nd day in case of pyrogallol induced immunosuppression and 28th day in the case of ethanol-induced immunosuppression, respectively.

Preparation of erythrocyte lysate

The blood samples were withdrawn into EDTA containing eppendorf tubes on the last day of the treatment from the retro-orbital venous plexus of rats. Then, these eppendorf tubes were subjected to centrifugation at 8000 RPM for 15 min. The supernatant was discarded, and erythrocyte lysate was prepared from the sediment.

SOD

It was estimated in the erythrocyte lysate prepared from the 5% RBC suspension. To 50 μl of the lysate, 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA, and 2 mM of pyrogallol were added. An increase in absorbance was recorded at 420 nm for 3 min by spectrophotometer. The activity of SOD is expressed as units/mg protein.¹²

CAT

A volume of 50 μl of the lysate was added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and 1 ml of 30 mM H₂O₂. CAT activity was measured at 240 nm for 1 min using a spectrophotometer. The molar extinction coefficient of H₂O₂, 43.6 M/cm was used to determine the CAT activity. One unit of activity is equal to 1 mmol of H₂O₂ degraded per min and is expressed as units/mg of protein.¹³

RSH

To 1 ml of sample, 1 ml of 10% trichloroacetic acid (TCA) was added. The precipitated fraction was centrifuged, and 2 ml of samples of clear supernatant solution were mixed with 2 ml aq. 0.67% TBA solution. This mixture was heated on a boiling water bath for 10 min. It was cooled in ice for 5 min and absorbance was measured spectrophotometrically at 535 nm. The values were expressed as nanomoles of malondialdehyde formed per milligram of protein values are normalized to protein content of tissues.¹⁵

LPO

About 2 ml of sample was mixed with 2 ml of 20% TCA and kept on ice for 15 min. The precipitate was separated by centrifugation and 2 ml of samples of clear supernatant solution were mixed with 2 ml aq. 0.67% TBA solution. This mixture was heated on a boiling water bath for 10 min. It was cooled in ice for 5 min and absorbance was measured spectrophotometrically at 535 nm. The values were expressed as nanomoles of malondialdehyde formed per milligram of protein values are normalized to protein content of tissues.¹⁵

**Table 1: Experimental design for the immunological studies**

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Treatment and test doses used</th>
<th>Timings of antigen challenge for humoral response</th>
<th>Estimation day for humoral response</th>
<th>Timings of antigen challenge for cellular response</th>
<th>Estimation day for cellular response</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTZ induced immunosuppression model</td>
<td>PSMS 250 mg/kg and 500 mg/kg</td>
<td>On 8th day</td>
<td>On 13th day</td>
<td>On 13th day</td>
<td>On 15th day</td>
</tr>
<tr>
<td>Pyrogallol induced immunosuppression model</td>
<td>PSMS 250 mg/kg and 500 mg/kg</td>
<td>On 7th and 13th day</td>
<td>On 13th and 20th day</td>
<td>On 20th day</td>
<td>On 22th day</td>
</tr>
<tr>
<td>Ethanol induced immunosuppression model</td>
<td>PSMS 250 mg/kg and 500 mg/kg</td>
<td>On 14th and 20th day</td>
<td>On 20th and 27th day</td>
<td>On 27th day</td>
<td>On 29th day</td>
</tr>
</tbody>
</table>

PSMS: Polysaccharides isolated from Metroxylon sagu, MTZ: Metronidazole
Histopathological examination

All groups of rats except PSMS 250 mg/kg lower dose were sacrificed by cervical dislocation on the 15th day in case of MTZ induced immunosuppression, 22nd day in case of pyrogallol induced immunosuppression and 28th day in the case of ethanol-induced immunosuppression, respectively. Spleen of each rat was then collected, fixed in 10% formalin and sectioned. However, histopathological studies were not carried out for a lower dose (PSMS 250 mg/kg). Hence, histopathological changes were presented (Figures 1-7) in the spleen were observed under light microscope.

Statistical analysis

The results were expressed as mean ± standard error of the mean. The differences were compared using one-way analysis of variance followed by Tukey’s test.

RESULTS

Acute oral toxicity

It was observed that water soluble PSMS was not lethal even at the dose of 4000 mg/kg, body weight following oral administration in mice.

Effect of PSMS on immunological parameters

Animals treated with MTZ, pyrogallol and ethanol alone showed significant ($P < 0.05$) decrease in the immunological parameters such as a humoral immune response, cellular immune response, carbon clearance test, and serum Igs, respectively.

Animals treated with PSMS showed significant ($P < 0.05$) and dose-dependent increase in the immunological parameters such as humoral immune response, cellular immune response, carbon clearance test, and serum Igs in all three models (Tables 2-4).

Figure 1: Normal spleen showing both white pulp and red pulp.

Figure 2: Metronidazole treated animals showing congested red pulp and atrophy of white pulp.

Figure 3: Metronidazole + polysaccharides isolated from Metroxylon sagu treated animals showing normal looking red pulp and white.

Figure 4: Pyrogallol treated animals showing congestion of red pulp.
Effect of PSMS on in-vivo oxidative and antioxidant parameters

Animals treated with MTZ, pyrogallol and ethanol alone showed significant ($P < 0.05$) decrease in the SOD, CAT, RSH levels and significant ($P < 0.05$) increase in the lipid peroxidation levels. Animals treated with PSMS showed significant ($P < 0.05$) and dose-dependent increase in the SOD, CAT, RSH levels and a significant decrease in the lipid peroxidation levels in all three models (Tables 5-7).

Effect of PSMS on histopathology of spleen tissue

Light microscopic examinations of spleen showed remarkable differences between MTZ, pyrogallol and ethanol-treated animals and PSMS treated animals. Atrophy in the spleen white pulp and congestion of red pulp was observed in control animals but not in PSMS treated animals (Figures 1-7).

DISCUSSION

Despite the overt use of MTZ as an antibacterial and anti-parasitic in humans, there is little information about its potential influence on the immune system cellularity and function. MTZ has been shown to induce suppression in the bone marrow, a primary lymphoid organ, and to affect male fertility. Furthermore, it has been observed that MTZ induces DNA single-strand breaks in the lymphocytes of patients on standard doses of the drug; therefore, toxicity in the peripheral lymphoid organs is suspected.6

Pyrogallol is toxic to the biological system, and its toxicity is attributed to its ability to generate free radicals. The literature has documented several evidences of the vulnerability of the immune system to the free radical-induced oxidative stress, which indicate that the cellular and humoral components of the immune system are particularly sensitive to increased levels of reactive oxygen species, which may cause premature immunosenescence.8

The administration of ethanol, over a period of four weeks, not only impaired the immune responses, but also produced oxidative stress, in rats. Since the immunotoxic effects of ethanol may be due to oxidative stress. The literature has documented free radical generation during the metabolism of ethanol. The level of the markers of oxidative stress, observed in ethanol-treated rats substantiates the possibility of extensive generation of free radicals.16
Table 2: Effect of PSMS on immune response in MTZ induced immunosuppression in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>HA titer values</th>
<th>Cellular immune response (percentage change in paw volume)</th>
<th>Percent change in phagocytosis</th>
<th>Serum immunoglobulins (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9.8±0.8</td>
<td>34±1.1</td>
<td>88±2.4</td>
<td>437±11.2</td>
</tr>
<tr>
<td>Disease control</td>
<td>2.3±0.06**</td>
<td>16±4.6</td>
<td>48±1.1**</td>
<td>276±9.5*</td>
</tr>
<tr>
<td>Levimasole (standard)</td>
<td>9.2±3.8***</td>
<td>31±2.3*</td>
<td>76±0.8***</td>
<td>398±16.41*</td>
</tr>
<tr>
<td>PSMS (250 mg/kg)</td>
<td>6.1±7.***</td>
<td>22.5±2.5**</td>
<td>60.4±3.1***</td>
<td>342±8.7**</td>
</tr>
<tr>
<td>PSMS (500 mg/kg)</td>
<td>9.3±4.4***</td>
<td>38±3.5**</td>
<td>77±0.8***</td>
<td>412±7.2**</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM, n=6 in each group, **P<0.01 when compared to normal, ***P<0.001 when compared to control, *P<0.05 when compared to control. PSMS: Polysaccharides isolated from Metroxylon sagu, SEM: Standard error of the mean, HA: Humoral antibody, MTZ: Metronidazole

Table 3: Effect of PSMS on immune response in pyrogallol induced immunosuppression in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>HA titer values</th>
<th>Cellular immune response (percentage change in paw volume)</th>
<th>Percent change in phagocytosis</th>
<th>Serum immunoglobulins (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10.5±0.5</td>
<td>10.7±1.4</td>
<td>34±1.1</td>
<td>88±2.4</td>
</tr>
<tr>
<td>Disease control</td>
<td>1.1±0.06**</td>
<td>5.0±0.5**</td>
<td>9±0.1**</td>
<td>53±0.6**</td>
</tr>
<tr>
<td>Levimasole (standard)</td>
<td>9.6±0.8***</td>
<td>9.9±0.7***</td>
<td>25±3.3*</td>
<td>86±0.3***</td>
</tr>
<tr>
<td>PSMS (250 mg/kg)</td>
<td>4.3±0.6***</td>
<td>7.8±0.9***</td>
<td>61.4±1.7***</td>
<td>335±10**</td>
</tr>
<tr>
<td>PSMS (500 mg/kg)</td>
<td>9.9±0.7***</td>
<td>30±1.1**</td>
<td>82±1.7***</td>
<td>400±16**</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM, n=6 in each group, **P<0.01 when compared to normal, ***P<0.001 when compared with control, *P<0.05 when compared to control. PSMS: Polysaccharides isolated from Metroxylon sagu, SEM: Standard error of the mean, HA: Humoral antibody

Table 4: Effect of PSMS on immune response in ethanol induced immunosuppression in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>HA titer values</th>
<th>Cellular immune response (percentage change in paw volume)</th>
<th>Percent change in phagocytosis</th>
<th>Serum immunoglobulins (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11.0±0.8</td>
<td>11.5±0.4</td>
<td>34±1.1</td>
<td>88±2.4</td>
</tr>
<tr>
<td>Disease control</td>
<td>2.9±0.03*</td>
<td>8±1.3*</td>
<td>8±0.6*</td>
<td>57±0.6*</td>
</tr>
<tr>
<td>Levimasole (standard)</td>
<td>10.0±0.6*</td>
<td>10.2±3.4*</td>
<td>30±1.4*</td>
<td>87±1.4*</td>
</tr>
<tr>
<td>PSMS (250 mg/kg)</td>
<td>6.6±1.8*</td>
<td>9.8±0.5*</td>
<td>21.6±3.4*</td>
<td>75±3.4*</td>
</tr>
<tr>
<td>PSMS (500 mg/kg)</td>
<td>10.9±2.0*</td>
<td>11.7±1.4*</td>
<td>93±1.5*</td>
<td>455±0.5*</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM, n=6 in each group, *P<0.01 when compared to normal, ***P<0.001 when compared to control. PSMS: Polysaccharides isolated from Metroxylon sagu, SEM: Standard error of the mean, HA: Humoral antibody

Table 5: Effect of PSMS on oxidants and anti-oxidant enzymes in MTZ induced immunosuppression in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Superoxide dismutase (units/mg protein)</th>
<th>Catalase (units/mg protein)</th>
<th>Glutathione μmol DTNB (conjugated/g Hb)</th>
<th>LPO (nmMDA/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>34±0.6</td>
<td>286±6.0</td>
<td>5.7±0.1</td>
<td>96±0.6</td>
</tr>
<tr>
<td>Disease control</td>
<td>16±1.4*</td>
<td>160±2.6*</td>
<td>3.4±0.05**</td>
<td>179±1.7**</td>
</tr>
<tr>
<td>Levimasole (standard)</td>
<td>33±0.5**</td>
<td>213±3.3**</td>
<td>4.2±0.1*</td>
<td>118±1.5**</td>
</tr>
<tr>
<td>PSMS (250 mg/kg)</td>
<td>26±1.9**</td>
<td>182±4.5**</td>
<td>4.1±0.1*</td>
<td>149±4.4***</td>
</tr>
<tr>
<td>PSMS (500 mg/kg)</td>
<td>37±2.5**</td>
<td>213±3.3**</td>
<td>4.3±0.2*</td>
<td>122±1.1***</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM, n=6 in each group, *P<0.01 when compared to normal, ***P<0.001 when compared to control. PSMS: Polysaccharides isolated from Metroxylon sagu, SEM: Standard error of the mean, LPO: Lipid peroxidation, DTNB: 5,5'-dithiobis (2-nitrobenzoic acid), Hb: Hemoglobin, MDA: Malondialdehyde, MTZ: Metronidazole

Table 6: Effect of PSMS on oxidants and anti-oxidant enzymes in pyrogallol induced immunosuppression in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Superoxide dismutase (units/mg protein)</th>
<th>Catalase (units/mg protein)</th>
<th>Glutathione μmol DTNB (Conjugated/g Hb)</th>
<th>LPO (nmMDA/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>34±0.6</td>
<td>286±6.0</td>
<td>5.7±0.1</td>
<td>96±0.6</td>
</tr>
<tr>
<td>Disease control</td>
<td>21±1.2*</td>
<td>195±2.8*</td>
<td>4.4±0.1*</td>
<td>163±2.5**</td>
</tr>
<tr>
<td>Levimasole (standard)</td>
<td>39±1.0***</td>
<td>240±2.0***</td>
<td>5.2±0.1*</td>
<td>97±1.4***</td>
</tr>
<tr>
<td>PSMS (250 mg/kg)</td>
<td>30±2.5***</td>
<td>225±3.2***</td>
<td>4.8±0.08*</td>
<td>136±4.6***</td>
</tr>
<tr>
<td>PSMS (500 mg/kg)</td>
<td>40±1.1***</td>
<td>246±4.6***</td>
<td>5.5±0.1**</td>
<td>10±1.0***</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM, n=6 in each group, *P<0.01 when compared to normal, ***P<0.001 when compared to control. PSMS: Polysaccharides isolated from Metroxylon sagu, SEM: Standard error of the mean, LPO: Lipid peroxidation, DTNB: 5,5'-dithiobis (2-nitrobenzoic acid), Hb: Hemoglobin, MDA: Malondialdehyde
Table 7: Effect of PSMS on oxidants and anti-oxidant enzymes in ethanol induced immunosuppression in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Superoxide dismutase (units/mg protein)</th>
<th>Catalase (units/mg protein)</th>
<th>Glutathione μmol DTNB (Conjugated/g Hb)</th>
<th>LPO (nmMDA/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>34±0.6</td>
<td>286±6.0</td>
<td>5.7±0.1</td>
<td>96±0.6</td>
</tr>
<tr>
<td>Disease control</td>
<td>25±1.0†</td>
<td>198±1.1†</td>
<td>4.7±0.1†</td>
<td>154±1.1†</td>
</tr>
<tr>
<td>Levimazole (standard)</td>
<td>40±0.3**</td>
<td>261±1.3**</td>
<td>5.2±0.1†</td>
<td>93±1.5**</td>
</tr>
<tr>
<td>PSMS (250 mg/kg)</td>
<td>29±2.3**</td>
<td>254±5.6**</td>
<td>4.9±0.09**</td>
<td>123±3.2**</td>
</tr>
<tr>
<td>PSMS (500 mg/kg)</td>
<td>43±1.4**</td>
<td>279±4.7**</td>
<td>5.7±0.1**</td>
<td>87±2.9**</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM, n=6 in each group. *P<0.01 when compared to normal, **P<0.001 when compared to normal, †P<0.001 when compared to control, ‡P<0.05 when compared to control. PSMS: Polysaccharides isolated from Metroxylon sagu, SEM: Standard error of the mean, LPO: Lipid peroxidation, DTNB: 5,5′-dithiobis (2-nitrobenzoic acid), Hb: Hemoglobin, MDA: Malondialdehyde.

Phagocytosis is the process in which phagocytes, ingests and removes microorganisms, malignant cells, inorganic particles, and cellular debris.17 The carbon clearance test was done to evaluate the effect of drugs on the reticuloendothelial system (RES). The RES is a diffuse system consisting of phagocytic cells. Cells of the RES play a vital role in the clearance of particles from the bloodstream.

When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation.18 Water soluble PSMS isolated from M. sagu 500 mg/kg showed a remarkable augmentation in the phagocytic index by exhibiting increase in clearance rate of carbon by the cells of the RES, it is speculated that it might be due to increase in the activity of the RES by prior treatment of animals with water soluble PSMS isolated from M. sagu.

IgG and IgM antibodies are involved in the complement activation, opsonization, neutralization of toxins, etc.17 The successive oral treatment of water soluble PSMS isolated from M. sagu showed a significant response in antibody production against SRBC compared to the control group. The enhancement of antibody responsiveness to SRBC in mice, in this study, indicated the enhanced responsiveness of macrophages and B lymphocyte subsets involved in the antibody synthesis. Therefore, augmentation of the humoral immune response to SRBCs by water soluble PSMS isolated from M. sagu, as evidenced by increase in the antibody titer in rats indicated the enhanced responsiveness of T and B lymphocyte subsets, involved in the antibody synthesis.19 The high values of HA titer obtained in the case of water soluble PSMS isolated from M. sagu indicated that the immunostimulation was achieved through humoral immunity.

Cell-mediated immunity involves effector mechanisms carried out by T lymphocytes and their products (lymphokines).17 In immune inflammatory DTH reaction, macrophages and Th1 cells plays a major role. This reaction requires a specific antigenic substance, which will release cytokines by activation with T-lymphocytes.20 Here, SRBC was used as the antigenic substance, which elicits the hypersensitivity reaction in mice. Therefore, increase in DTH reaction in rats in response to T cell-dependent antigen revealed the stimulatory effect of water soluble PSMS isolated from M. sagu on T cells in all the three models of immunosuppression.

CONCLUSION

The results of the study indicated that water soluble PSMS has remarkable and dose-dependent immunostimulatory activity against three models. The order of immunostimulant activity of PSMS against three models is ethanol-induced immunosuppression model > pyrogallol induced immunosuppression model >MTZ induced immunosuppression model.

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