Immunomodulatory effect of *Moringa oleifera* Lam. extract on cyclophosphamide induced toxicity in mice

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Immunomodulatory effect of ethanolic extract (50%) of *M. oleifera* leaves (MOE) has been studied in normal and immunosuppressed mice models. Different doses of MOE i.e. 125, 250 and 500 mg/kg body weight of mice were administered orally for 15 days. Cyclophosphamide at a dose of 30 mg / kg body weight was administered orally for the next 3 days. On day 16 and 19, hematological parameters like white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin level (Hb), percent neutrophils and organ weight were recorded. Effect of MOE on phagocytic activity of mice macrophages was determined by carbon clearance test. MOE showed significant dose dependent increase in WBC, percent neutrophils, weight of thymus and spleen along with phagocytic index in normal and immunosuppressed mice. The results indicate that MOE significantly reduced cyclophosphamide induced immunosuppression by stimulating both cellular and humoral immunity.

**Keywords:** Amelioration, Cyclophosphamide, Immunomodulation, Immunosuppression, *Moringa oleifera*, Phagocytic index

Immunodeficiency disorder impairs the immune system's ability to defend the body against foreign or abnormal cells which is a major drawback in conventional therapy (radiotherapy and chemotherapy) of cancer¹. Modulation of immune responses, by various plant materials, for alleviation of diseases has been an interesting approach since ancient time and also the basic concept of ’rasayana’ in Ayurveda².

**Immunomodulatory activity of Ocimum sanctum³, Viscum album⁴, Piper longum Linn.⁵, Picrorhiza kurroa⁶, Salicornia herbacea⁷, Opilia celtidifolia⁸, Panax ginseng⁹ and Asparagus recemosus¹⁰ have been reported earlier.**

*Moringa oleifera,* commonly known as ‘drumstick’ or ‘horseradish’ tree (Family: Moringaceae), is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree, used for several medicinal and industrial purposes¹¹,¹², has been reported to exhibit chemomodulatory¹³ and antioxidant¹⁴ activities. Moreover, it also regulates thyroid status and cholesterol levels¹⁵.

However, the immunomodulatory activity of *M. oleifera* has not been reported so far. The present investigation has been carried out to evaluate the immunomodulatory activity of ethanolic extract of *M. oleifera* leaves.

**Preparation of extract—**Leaves from authenticated *M. oleifera* Lam, plant were obtained from botanical garden of National Botanical Research Institute, Lucknow and voucher specimen (NBR 182376) was deposited in the departmental herbarium for future reference. Air dried powdered leaves of *M. oleifera* (1000 g) was extracted with 50% ethanol (3×10 L) and concentrated under reduced pressure to yield 7.5% w/w (MOE).

**Animals**—Swiss albino mice (males 25-30 g) were procured from CDRI, Lucknow and were provided with standard dry pellet diet (Amrut, India) and water ad libitum. All studies were performed in accordance with the guidelines on regulation of scientific experiments on animals as approved by the Institutional Animal Ethics Committee (registration no. 222/2000/CPCSEA).

**Drugs**—Drug used was cyclophosphamide (CTX) (Khandelwal Laboratories Ltd, Mumbai). Drug was dissolved in normal saline (0.9% NaCl) and administered orally to mice at the dose of 30 mg/kg body weight.

**Effect of chronic administration of MOE on normal mice**—Swiss albino mice (6 nos; Group I) were treated orally with three doses (125, 250, and 500 mg/kg body weight) of MOE for 15 days. Animals receiving 1% aqueous carboxymethyl cellulose
(CMC) in the same volume were kept as control. Blood was collected from retro – orbital plexuses and parameters such as WBC count, RBC count, Hb level were recorded on day 16 by using autoanalyser (CA620 Medonic, Boule, Sweden). The differential leukocyte count was performed by fixing the blood smears and staining with Leishman’s stain and percent neutrophils in each sample was determined 16. Weight of vital organs such as thymus, spleen, liver and kidney were also recorded on the same day after sacrificing the animals.

Effect of MOE on immunosuppressed mice induced by CTX—Swiss albino mice (6 nos; Group II) were treated orally with three doses (125, 250, and 500 mg/kg body weight) of MOE for 15 days. CTX at a dose of 30 mg / kg body weight was administered orally to the same mice for the next 3 days. Animals receiving 1% aqueous CMC in the same volume were kept as control. Parameters described above were recorded by the same method 16 on day 19. In addition to above parameters, levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) 17 and serum alkaline phosphatase (SALP) 18 were also recorded.

Effect of MOE on phagocytic index—Phagocytic index was determined by carbon clearance test. For this purpose, mice (6 nos; Group III) were treated intraperitoneally with MOE at the doses of 125, 250 and 500 mg/kg body weight daily for 5 consecutive days. Animals treated with phosphate buffer saline (PBS, pH 7.4) in equal volume were kept as control whereas those without any treatment were considered as normal. After 48 hr of last treatment colloidal carbon (Indian ink), which was diluted with PBS eight times before use, was injected via the tail vein at the dose of 10 μl/g body weight. Blood was collected from retro – orbital plexuses of the individual animal at the intervals of 0 and 15 min. The blood was suspended in 0.1% sodium carbonate and the absorbance was measured at 660 nm. The phagocytic index, K, was calculated by equation:

\[ K = \frac{\ln OD_1 - \ln OD_2}{t_2 - t_1} \]

Where, OD1 and OD2 depict the optical densities at t1 = 0 time and t2 = after 15 min, respectively.

Statistical evaluation—All the values were expressed as mean ± SD for 6 mice. Statistical analysis was carried out by using PRISM software package (version 3.0). Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Newman-Keuls multiple comparison test. The value of probability less than 5% (P < 0.05) was considered statistically significant.

Effect of MOE on hematological parameters of normal and immunosuppressed mice—There was a significant dose related increase in the WBC count and percent neutrophils in normal mice after treating with MOE whereas no significant changes were observed in other hematological parameters. There was a significant reduction in the WBC count and percent neutrophils of mice treated with CTX, but the restoration of these parameters was observed after the combined treatment with CTX and MOE (Table 1).

Effect of MOE on organ weight of normal and immunosuppressed mice—There was a significant dose related increase in size and weight of the thymus

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Table 1 — Effect of M. oleifera extract at different concentration on RBC (million/mm³), WBC (thousand/mm³), neutrophil (%) and hemoglobin (gm/dl) of normal and immunosuppressed mice

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Control (mg/kg MOE)</th>
<th>125 (mg/kg MOE)</th>
<th>250 (mg/kg MOE)</th>
<th>500 (mg/kg MOE)</th>
<th>CTX 30 (mg/kg)</th>
<th>CTX+ 125 (mg/kg MOE)</th>
<th>CTX+ 250 (mg/kg MOE)</th>
<th>CTX+ 500 (mg/kg MOE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>4.2500 ± 0.2863</td>
<td>4.6233 ± 0.3601</td>
<td>4.5917 ± 0.2816</td>
<td>4.6733 ± 0.3590</td>
<td>4.2683 ± 0.4718</td>
<td>4.3433 ± 0.3034</td>
<td>4.3817 ± 0.3973</td>
<td>4.8667 ± 0.3815</td>
</tr>
<tr>
<td>WBC</td>
<td>9.6467 ± 0.3879</td>
<td>10.4317 ± 0.1220</td>
<td>11.1700 ± 0.1000</td>
<td>12.1200 ± 0.1307</td>
<td>10.2517 ± 0.7317</td>
<td>10.6513 ± 0.4973</td>
<td>10.7183 ± 0.1295</td>
<td>10.8933 ± 0.0659</td>
</tr>
<tr>
<td>% Neutrophil</td>
<td>52.0100 ± 2.9643</td>
<td>56.0200 ± 2.2854</td>
<td>60.2700 ± 2.0027</td>
<td>63.0667 ± 2.7685</td>
<td>58.4667 ± 1.7239</td>
<td>64.1695 ± 1.0100</td>
<td>67.2485 ± 1.2016</td>
<td>70.8180 ± 0.5058</td>
</tr>
<tr>
<td>Hb</td>
<td>10.8633 ± 0.3008</td>
<td>10.8600 ± 0.4693</td>
<td>11.1700 ± 0.5583</td>
<td>11.2800 ± 0.4917</td>
<td>9.7383 ± 0.4029</td>
<td>9.7517 ± 0.2932</td>
<td>9.8150 ± 0.2812</td>
<td>10.8917 ± 0.7002</td>
</tr>
</tbody>
</table>

P: a < 0.002 compared to control group; P: b < 0.000 compared to control group; P: c < 0.000 compared to only CTX- treated group; P: d < 0.025 compared to control group.
and spleen after MOE treatment. Administration of CTX caused a significant reduction in the weight of thymus and spleen, while in the animals treated with CTX along with MOE, these parameters were found to be increased. No significant differences were observed in the weight of the liver and kidney after administration of CTX and/or MOE (Table 2).

Effect of MOE on serum enzyme level of immunosuppressed mice—No significant changes were observed in the level of serum enzymes by administration of CTX alone and CTX along with MOE. The results indicated that MOE only affects the immune system (Table 3).

Phagocytic activity—A significant dose dependent increase in phagocytic index was observed in mice treated with MOE compared to control group. The observed values of phagocytic index were 2.0210 \pm 0.2616, 2.9550 \pm 0.2616 and 4.3700 \pm 0.7817 for the groups treated with MOE at the doses of 125, 250 and 500 mg/kg body weight respectively.

Damage to the immune system is one of the major side effects of chemotherapy. Cytotoxic drugs such as cyclophosphamide acts on the cells of the immune system at various levels. Suppression of bone marrow activity as well as innate immune reponses is the major drawback of these types of drugs. Several sources of immunomodulatory material are being tried since long time to overcome the toxic effects of cytotoxic drugs. Indian medicinal plants are a rich source of substances which are claimed to induce para-immunity and non-specific immunomodulation of granulocytes, macrophages, natural killer cells and complement functions.

Present investigation was carried out to detect the immunomodulatory activity of *M. oleifera* on animal model. Chronic administration of MOE significantly increased WBC count and percent neutrophils of normal mice in a dose related manner. MOE was also capable to significantly reduce the leucopenia induced by sub lethal dose of cyclophosphamide in mice. These results indicate that the extract could stimulate the haemopoetic system.

Moreover, the administrations of extract also stimulated the increase in size and weight of spleen as well as thymus in both normal and immunosuppressed mice. However, there is no effect on kidney and liver as they do not participate in the immune activity. The results indicated that MOE may restore the production of immune cells, which was decreased by cyclophosphamide. Thus, it is clear that MOE has the ability of amelioration.

Phagocytosis represents an important innate defence mechanism against ingested foreign materials. The blood monocytes, neutrophils and tissue macrophages are specialized phagocytic cells. In carbon clearance test the rate of clearance of carbon from blood by phagocytic cells is governed by tissue macrophages which are specialized phagocytic cells. In this study, an increase in phagocytic index in dose-dependent manner was observed, which may be due to increased production of phagocytic cells stimulated by MOE.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>125 (mg/kg MOE)</th>
<th>250 (mg/kg MOE)</th>
<th>500 (mg/kg MOE)</th>
<th>CTX 30 (mg/kg MOE)</th>
<th>CTX + 125 (mg/kg MOE)</th>
<th>CTX + 250 (mg/kg MOE)</th>
<th>CTX + 500 (mg/kg MOE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>0.1274</td>
<td>0.1401</td>
<td>0.1531</td>
<td>0.1596</td>
<td>0.0088</td>
<td>0.0212</td>
<td>0.2984</td>
<td>0.7787</td>
</tr>
<tr>
<td></td>
<td>\pm 0.0021</td>
<td>\pm 0.0035</td>
<td>\pm 0.0051</td>
<td>\pm 0.0044</td>
<td>\pm 0.0007</td>
<td>\pm 0.0030</td>
<td>\pm 0.0148</td>
<td>\pm 0.0376</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.0102</td>
<td>0.0107</td>
<td>0.0119</td>
<td>0.0129</td>
<td>0.0016</td>
<td>0.0109</td>
<td>0.0736</td>
<td>0.0958</td>
</tr>
<tr>
<td></td>
<td>\pm 0.0001</td>
<td>\pm 0.0002</td>
<td>\pm 0.0004</td>
<td>\pm 0.0006</td>
<td>\pm 0.0006</td>
<td>\pm 0.0028</td>
<td>\pm 0.0060</td>
<td>\pm 0.0073</td>
</tr>
<tr>
<td>Liver</td>
<td>1.5074</td>
<td>1.4752</td>
<td>1.5300</td>
<td>1.5963</td>
<td>1.4742</td>
<td>1.4874</td>
<td>1.4701</td>
<td>1.4846</td>
</tr>
<tr>
<td></td>
<td>\pm 0.064</td>
<td>\pm 0.125</td>
<td>\pm 0.304</td>
<td>\pm 0.226</td>
<td>\pm 0.045</td>
<td>\pm 0.0042</td>
<td>\pm 0.0108</td>
<td>\pm 0.0032</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.3438</td>
<td>0.401</td>
<td>0.3821</td>
<td>0.3936</td>
<td>0.3403</td>
<td>0.3303</td>
<td>0.3268</td>
<td>0.3523</td>
</tr>
<tr>
<td></td>
<td>\pm 0.040</td>
<td>\pm 0.045</td>
<td>\pm 0.044</td>
<td>\pm 0.043</td>
<td>\pm 0.0054</td>
<td>\pm 0.0060</td>
<td>\pm 0.0032</td>
<td>\pm 0.0229</td>
</tr>
</tbody>
</table>

*P: ^a^ < 0.000 compared to control group; *P: ^b^ < 0.000 compared to CTX- treated group*
Moringa leaves contain significant amounts of vitamins A, B and C, calcium ions, iron, potassium, proteins, traces of carotenoids, saponins, phytates and phenolic constituents. These constituents may be responsible for its immunomodulatory activity. The exact mechanism of action of MOE in stimulation of both the cellular and the humoral immunity is not yet clear. It may be due to an enhanced production of growth factors. Results of the present study showed that Moringa oleifera may alleviate the myelosupression and subsequent leucopenia induced by cyclophosphamide in mice.

References