COMPARATIVE EVALUATION OF ALCOHOLIC AND AQUEOUS EXTRACTS OF *OCIMUM SANCTUM* FOR IMMUNOMODULATORY ACTIVITY

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ABSTRACT

**Background:** Traditionally the aqueous and alcoholic extracts as well as seed oil of *Ocimum sanctum* is reported to modulate the immune response. So the present study was undertaken to compare the immunomodulatory activity between alcoholic and aqueous extract of *Ocimum sanctum*.

**Objective:** To comparatively evaluate the immunomodulatory activity of alcoholic and aqueous extracts of *Ocimum sanctum*.

**Methods:** Alcoholic and aqueous extracts of *Ocimum sanctum* were administered orally at doses of 50, 100 and 200 mg/kg/day for 14 days to healthy albino Swiss mice. The assessment of immunomodulatory activity was carried by testing the humoral (haemagglutination antibody titer model) and cellular immunity (delayed type hypersensitivity reaction models).

**Results:** On oral administration of aqueous and alcoholic extracts had stimulatory effect on delayed type hypersensitivity (DTH) and significantly (p<0.01) improved humoral immunity.

**Conclusion:** The study shown stimulatory effect on the humoral and delayed type hypersensitivity but the alcoholic extract was more potent in producing immune stimulation than aqueous extract.

**KEY WORDS:** *Ocimum sanctum*, immunomodulatory activity, HA titer, DTH

INTRODUCTION

The immune system is designed to protect the host from invading pathogens and to eliminate disease. At its functioning best, the immune system is exquisitely responsive to invading pathogens while retaining the capacity to recognize “self” antigens to which it is tolerant. Protection from infection and disease is provided by the innate and the adaptive immune system1.

The innate immune system is the first line of defense against an antigenic insult and includes physical (skin), biochemical (complement, lysozyme and interferon) and cellular components (neutrophils, monocytes, macrophages). When the innate immune response is inadequate to cope with infection, the adaptive response culminates in the production of antibodies, which are the effectors of humoral immunity and the activation of T lymphocytes, which are the effectors of cell-mediated immunity. Humoral immunity or B-cell immunity develops circulating antibodies, which are globulin molecules that are capable of attacking the invading agent. Cell-mediated immunity is achieved by the formation of large number of activated lymphocytes that are specifically design to destroy foreign agent2.

The immune system is known to be involved in the etiology and pathophysiological mechanisms of several diseases3. The function and efficiency of the immune system may be influenced by many exogenous and endogenous factors resulting in either immunosupression or immunostimulation. Several agents have been shown to possess an activity to normalize or modulate pathophysiological processes and are called immunomodulatory agents4.

There is need to evaluate the potential of Indian Ayurvedic remedies as adjuvants to counteract side effects of modern therapy and compare the cost effectiveness of certain therapies vis-a-vise modern therapeutic schedules4. There are several synthetic chemical agents have been discovered to have immuno-modulating property. Cyclophosphamide is an alkylating agent with most efficacious immunosuppressive property. It
destroys proliferating lymphoid cells but also appear to alkylate resting cells. Levamisole is a levo isomer of
tetramisole, originally introduced as an anthelmintic for use in human beings more than 20 years ago, but now
widely recognized and employed for its immunomodulatory activity. The levamisole acts by elevating cGMP
levels in lymphocytes in vitro and enhance their proliferate response to nitrogen or foreign cells. The imidazole
ring seems to be one of the active moieties of levamisole responsible for the functional increase of peripheral T-
cells and macrophages5.

Ocimum sanctum has ability to modulate humoral immune responses by acting at various levels in immune
mechanism such as antibody production; release of mediators of hypersensitivity reactions and tissue responses
to these mediators in the target organs6. Therefore aim of the present study was to evaluate the comparative
efficacy of alcoholic and successive aqueous extract of Ocimum sanctum.

MATERIALS AND METHODS

Chemicals / Drugs
Cyclophosphamide (Sigma Aldrich. Ltd), Di- potassium EDTA (S.D. Fine Chem. Limited) and WBC diluting
fluid (Himedia Laboratories Pvt. Ltd.)

Experimental Animals
Albino Swiss mice of either sex (18-25 gms) were obtained from the colonies maintain at Central Animal
Facility, Natural Remedies Pvt. LTD. Bangalore and housed three animals per cage with paddy husk as bedding.
Animals were housed at temperature of 25±2°C and relative humidity of 30-60%. A 12:12 h light and dark cycle
was followed. The animals were allocated to different treatment groups (each 6) and each animal in a group was
recognized by mark of picric acid on the fur. Animals had free access to pelleted feed and purified water ad
libitum.

Experimental designs
Animals were divided into nine groups each having eight mice and treated accordingly,
- Group-I: Vehicle control received Tween-20 (0.5%W/V, 10 ml/kg)
- Group-II: Animals treated with Cyclophosphamide (60 mg/kg, i.p.)
- Group-III: Animals treated with Levamisole (50 mg/kg, p.o.)
- Group-IV: Animals treated with aqueous extract of Ocimum sanctum (50 mg/kg, p.o.)
- Group-V: Animals treated with aqueous extract of Ocimum sanctum (100 mg/kg, p.o.)
- Group-VI: Animals treated with aqueous extract of Ocimum sanctum (200 mg/kg, p.o.)
- Group-VII: Animals treated with alcoholic extract of Ocimum sanctum (50 mg/kg, p.o.)
- Group-VIII: Animals treated with alcoholic extract of Ocimum sanctum (100 mg/kg, p.o.)
- Group-IX: Animals treated with alcoholic extract of Ocimum sanctum (200 mg/kg, p.o.)

Preparation of Alsever’s solution

Composition:
Dextrose: 2.05 gm
Sodium citrate : 0.8 gm
Sodium chloride: 0.4 gm
Citric acid: 0.05 gm
All the ingredients were weighed and dissolve in 100ml of distilled water. Alsever’s solution was used in the
proportion of 1:2 (Sheep blood: Alsever’s solution) for washing sheep blood.

Antigen suspension (SRBC suspension)
Sheep blood was collected in alsever’s solution and washed twice with buffer saline (PBS). The number of
SRBC was then adjusted to a concentration of 1X10⁸ cells after the RBC count. RBC count was carried out
using Neubers chamber and RBC pipette. This RBC suspension was used for immunization and challenge.

Hemagglutination antibody titer⁷,⁸
On 0th day all the animals were immunized by injecting 1X10⁸ sheep red blood cells (SRBC’s) intraperitoneal
(Zero day). The test extracts were administered to all the animals from day 0 to day 7 as shown above. Three
doses of Cyclophosphamide (50 mg /kg body wt.) were administered orally to the group II on 4th, 5th and 6th
day. Blood samples were collected from individual animals of all the groups by retro orbital bleeding on day 7
and serum was separated. Antibody levels were determined by the haemagglutination technique, this is
performed by using 96 wells (12x8) V bottomed titre plate. The wells were marked from 1 to 12. In the first and
last well 25 µl of serum collected from treated animals is added and inactivated at 56⁰ C for 30 minutes.
Afterwards 25 µl of PBS was added to all the wells except well number 12 and mixed well. Then 25 µl of
sample from first well was taken and added to 2nd well, again 25 µl from second well was taken and added it to third well and continued the same procedure up to well number 10. After this 25 µl of sample from well number 10 was discard. Finally 25 µl of 1% SRBC was added to all the wells and kept at room temperature for two hours. Each well was examined for haemagglutination. The reciprocal of highest dilution just before the button formation was considered as a titer value.

**Delayed type hypersensitivity**

On 7th day the thickness of the right hind foot pad was measured using plethysmometer by using mercury displacement method. The animals were then challenged by injecting 1X10^8 SRBC’s in right hind foot pad. Foot pad thickness was measured again 24 hours after the challenge, by mercury displacement method using Plethysmometer, wherein paw was marked with ink at the level of lateral malleolus and immersed in the mercury up to this mark. The difference in paw thickness was taken as a measure of delayed hypersensitivity and the mean value obtained for treatment groups were compared with that of control group.

**Statistical analysis**

All the data’s were analyzed using One-Way ANOVA method followed by Dunnett T3 test for heterogeneous data and One-Way ANOVA followed by Bonferroni test for homogeneous data. All values were reported as mean ± SEM. P≤0.05 was considered to be statistically significant.

**RESULTS**

**Haemagglutination Antibody Titer in Albino Swiss Mice**

The oral administration of cyclophosphamide (50 mg/kg) on the day 4, 5 and 6 of post immunization showed significant decrease in haemagglutination antibody titer (36.00 ±6.60) when compared with vehicle control (104.00 ±11.72). Levamisole did not show any significant increase in haemagglutination antibody titer (96.00 ±12.10) when compared with vehicle control (104.00 ±11.72).

Administration of alcoholic and aqueous extracts of *Ocimum sanctum* produced dose dependent significant (P<0.05) increased in haemagglutination antibody titer (for aqueous extract from 7.70% to 76.93% and for alcoholic extract 0% to 80.77%) compared to vehicle control. The alcoholic extract at 200 mg/kg body weight dose is showing more value of antibody titer compared to aqueous extract (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/ kg)</th>
<th>HA titer Mean ± SEM</th>
<th>% Changes in HA titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Vehicle Control</td>
<td>10 ml/kg</td>
<td>104.00 ± 11.72</td>
<td>-</td>
</tr>
<tr>
<td>II Cyclophosphamide</td>
<td>60</td>
<td>36.00 ± 6.60*</td>
<td>-65.39</td>
</tr>
<tr>
<td>III Levamisole</td>
<td>50</td>
<td>96.00 ± 12.10</td>
<td>-</td>
</tr>
<tr>
<td>IV Aq Extract of OS</td>
<td>50</td>
<td>112.00 ± 10.48</td>
<td>7.70</td>
</tr>
<tr>
<td>V Aq Extract of OS</td>
<td>100</td>
<td>180.00 ± 56.07*</td>
<td>73.08</td>
</tr>
<tr>
<td>VI Aq Extract of OS</td>
<td>200</td>
<td>184.00 ± 54.68*</td>
<td>76.93</td>
</tr>
<tr>
<td>VII Aq Extract of OS</td>
<td>50</td>
<td>96.00 ± 12.10</td>
<td>-</td>
</tr>
<tr>
<td>VIII Aq Extract of OS</td>
<td>100</td>
<td>176.00 ± 48.00*</td>
<td>69.23</td>
</tr>
<tr>
<td>IX Alc Extract of OS</td>
<td>200</td>
<td>188.00 ± 54.24*</td>
<td>80.77</td>
</tr>
</tbody>
</table>

Table 1: Effect of *Ocimum sanctum* extracts on Haemagglutination antibody (HA) titer

Values are expressed as mean ± SEM; n=8, *p<0.05 Control Vs Cyclophosphamide / Levamisole/ Test substance
Delayed type hypersensitivity in albino Swiss mice

Levamisole showed increase in paw edema (0.227 ± 0.010 ml) when compared with vehicle control (0.190 ± 0.011 ml) but response was not significant. Aqueous and alcoholic extracts of Ocimum sanctum showed non-significantly increased in Delayed Type Hypersensitivity in all treated groups when compared with vehicle control group. Aqueous extract at the dose of 100 mg/kg body weight and alcoholic extract at the dose of 50 mg/kg body weight showed the maximum immunostimulatory activity (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Paw volume (ml)</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Vehicle Control</td>
<td>10 ml/kg</td>
<td>0.164 ± 0.005</td>
<td>0.190 ± 0.011</td>
<td></td>
</tr>
<tr>
<td>II Levamisole</td>
<td>50</td>
<td>0.172 ± 0.006</td>
<td>0.227 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>III Aq Extract of OS</td>
<td>50</td>
<td>0.160 ± 0.005</td>
<td>0.224 ± 0.012</td>
<td></td>
</tr>
<tr>
<td>IV Aq Extract of OS</td>
<td>100</td>
<td>0.159 ± 0.008</td>
<td>0.239 ± 0.014</td>
<td></td>
</tr>
<tr>
<td>V Aq Extract of OS</td>
<td>200</td>
<td>0.160 ± 0.008</td>
<td>0.234 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>VI Alc Extract of OS</td>
<td>50</td>
<td>0.158 ± 0.006</td>
<td>0.238 ± 0.009</td>
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<tr>
<td>VII Alc Extract of OS</td>
<td>100</td>
<td>0.154 ± 0.006</td>
<td>0.225 ± 0.010</td>
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</tr>
<tr>
<td>VII Alc Extract of OS</td>
<td>200</td>
<td>0.167 ± 0.009</td>
<td>0.244 ± 0.005</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n=8, Control Vs Cyclophosphamide/Levamisole/Test substance

DISCUSSION

The immune system is a complex system, involving an interwoven network of biochemical mechanisms. The modulation of immune response by various agents in order to alleviate the disease has been of interest since many years and the concept of Indian Rasayana in Ayurveda has similarity with it9.

Immunomodulators are being used as an adjuvant in conditions of immunodeficiency in cancer and to a limited extent in acquired immunodeficiency syndrome. Many of the presently available immunomodulators such as levamisole, glucans, telerones, L-fucose as well as Corynebacterium parvum bacterium, are not free from side-effects which include fever, neutropenia, leucopenia and, at times, allergic reactions. Hence screening for new immunomodulators is an urgent need10.

Immunomodulatory agents of plant origin enhance the immune responsiveness of the organism against a pathogen by activating the immune system. However these agents should be subjected to systemic studies to substantiate the therapeutic claims made with regard to their clinical utility11. Ocimum sanctum has ability to modulate humoral immune responses by acting at various levels in immune mechanism such as antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs9. Methanol extract and aqueous suspension of Ocimum sanctum leaves have ability to stimulate humoral response12. Ocimum sanctum seed oil also has been proved to modulate both humoral and cell mediated responsiveness in both non-stressed and stressed experimental animals and mechanism behind these immunomodulatory properties could be due to their activity on GABAergic pathways13.

The present study was carried out to compare the immunomodulatory activity between alcoholic and aqueous extracts of Ocimum sanctum at various dose levels. Antibody molecules, a product of B-lymphocytes and plasma cells, are central to humoral immune responses; IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxins etc5. Cyclophosphamide at a dose
of 50 mg/kg body weight, for three days p.o., showed a significant inhibition of antibody responses. Alcoholic and aqueous extracts of *Ocimum sanctum* showed significant (p< 0.05) increased in haemagglutination titer. In case of aqueous extract the increase in haemagglutination titer is lower compared to alcoholic extract showing that alcoholic extract (200 mg/kg) may be superior compared to aqueous extract. But our study was only of 7 days duration and the extract may not be so much potent to elicit humoral immune response in such shorter duration, hence further study is required to be conducted to confirm the effectiveness of extracts on long term treatment.

During cell mediated immunity responses, sensitized T-lymphocytes, when challenged by the antigen, are converted to lymphoblasts and secrete lymphokines, attracting more scavenger cells to the site of reaction. The infiltrating cells are thus immobilized to promote defensive (inflammatory) reaction. In our study, foot volume was enhanced after *Ocimum sanctum* treatment suggesting cell mediated immune enhancement. It was found that alcoholic and aqueous extracts of *Ocimum sanctum* potentates the DTH reaction induced by both SRBC. Increase in DTH reaction in mice in response to thymus-dependent antigen by the stimulatory effect of alcoholic and aqueous extracts might indicate the involvement of T lymphocytes and accessory cell types, which are required for the DTH reaction.

The present investigation suggests that alcoholic and aqueous extracts of *Ocimum sanctum* stimulate the cell-mediated immunity significantly and also stimulated the humoral immunity. Further studies are to be conducted to evaluate the effect on humoral and non-specific immunity on chronic treatment.

CONCLUSION

From the experimental study it can be concluded that the aqueous extract of *Ocimum sanctum* at higher dose (200mg/kg body weight) showed increase in delayed type hypersensitivity response to SRBC’s but the alcoholic extract is more potent than aqueous extract in producing delayed type hypersensitivity response. Both extracts have marginal stimulatory effect on humoral immunity.

REFERENCES