Immunomodulatory activity of aqueous extract of *Ocimum sanctum* in rat

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**ABSTRACT**

**Background:** Biochemical, haematological and Immunomodulatory effect of *Ocimum sanctum* in rat was studied.

**Objective:** To evaluate the immunomodulatory effect of *Ocimum sanctum* in rat

**Methods:** Aqueous extract of *Ocimum sanctum* were administered oraly at doses of 100, 200 mg/kg/day for 45 days in wistar albino rats. Immunomodulatory effect and biochemical and haematological changes were tested by standard methods.

**Results:** Aqueous extract of the *Ocimum sanctum* showed increasing antibody production in dose dependent manner. It enhance the production of RBC, WBC and haemoglobin. It dose not affect the biochemical parameters.

**Conclusion:** An oral administration of the aqueous extract of *O. sanctum* showed immunomodulatory effect in rat.

**Introduction**

The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000 – 5000 B.C and Chinese used first the natural herbal preparations as medicines. In India, earliest references are available in Rigveda which is said to be written between 3500 – 1600 B.C. (Sirkar, 1989). Now a day large number of drugs in use are derived from plants, like morphine from *Papaver somniferum*, Aswagandha from *Withania somnifera*, Ephedrine from *Ephedra vulgaris*, Atrophine from *Atropa belladonna* etc. Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against much pathology (Borchers \textit{et al.}, 2000). Plant extracts are potentially curative. Some of these extracts can boost the humoral (Rehman \textit{et al.}, 1999) and cell mediated immunity (Upadhyay \textit{et al.}, 1992) against viruses(Calixto \textit{et al.}, 1998), bacteria (Boyanova and Neshev, 1999), fungi (Ali \textit{et al.}, 1999), protozoa (Sharma \textit{et al.}, 1998) and cancer (Wong \textit{et al.}, 1994).

Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials. *Ocimum sanctum* (Tulsi or holy basil) has a very special place in the Hindu culture. Tulsi is a Sanskrit word which means “matchless one”. Several medicinal properties have been attributed to the Tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani systems of medicine (Shankar mondal, 2010). In Ayurveda, Tulsi used as antiasthmatic and antikaphic drugs (Sirkar, 1989). *Ocimum sanctum* L. (Tulsi) is an erect, much branched sub-shrub 30-60 cm tall, with simple opposite green or purple leaves that are strongly scented and hairy stems. Leaves have petiole and are ovate, up to 5 cm long, usually somewhat toothed. Flowers are purplish in elongate racemes in close whorls. Tulsi is native throughout the world tropics and widespread as a cultivated plant and an escaped weed. It is cultivated for religious and medicinal purposes and for its essential oil. *O. sanctum* has several medicinal properties. Different parts of Tulsi plant e.g. leaves, flowers, stem, root, seeds etc. are known to possess therapeutic potentials and have been used as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic and antistress agents. It is also used in treatment of fever, bronchitis, arthritis, convulsions etc (Prakash and Guptha, 2005).

**Materials and Methods**

**Plant extract**

Fresh plants were cleaned, dried at 37 C for 3 days and powered well. From this dried powdered extracts were prepared.

**Experimental designs**

Animals were divided into three groups, each having five rats and treated accordingly, Group I: control Group II: Animals treated with aqueous extract of *O.sanctum* (100 mg/kg)
Group III: Animals treated with aqueous extract of *O. sanctum* (200mg/kg)

**Antigen Preparation**
Crystalline Bovine Serum Albumin fraction V (BSA) was used as non cellular antigen for the present investigation.

**Soluble Bovine Serum Albumin (S - BSA)**
S - BSA was prepared by overlaying the BSA powder in isotonic saline 1.0mg/ml of saline (0.15 N). It was allowed to dissolve without agitation and used as antigen.

**Collection of Sheep red blood cells**
SRBC were collected in Alserver’s solution from animal husbandry without contamination. To avoid allogenic difference the Sheep red blood was used throughout the study.

**Immunization**
After 3days of exposure to the toxicant, rats were immunized with optimum dose of 0.5 ml of antigen. The antigen was injected through the intraperitoneal route using 3ml tuberculin syringe. Secondary immunization was also done with the same dose of antigen through the same route on the 15th day after primary immunization. Antigen administration and serial bleeding of animals were always done between 2 – 4pm to avoid circadian rhythmic variations on the immune response.

**Blood Collection from Test Animal**
Blood samples were collected from a tail vein by snipping the tip of the tail. The tip of the tail was cleaned with spirit and snipped with clean scissors. The blood was collected in EDTA rinsed vials for hematological studies and antigen-antibody titration.

**Normal Serum and Antiserum Collection**
The blood was collected from the control and test animals by snipping the caudal vein rinsed with 1% EDTA and kept at room temperature for 20 min. The serum was separated by spinning down the clot at 3000 rpm for 15-20 min and then collected in sterilized storage vials. It was kept at 57ºC in a water bath for 30mts to inactivate complement and stored at 20ºC until use.

**Antibody Titration**

**Passive Haemoagglutination Assay**
Chromic chloride method
This assay was used to determine anti-BSA antibodies in the serum. Two fold dilutions of the antiserum (50 µl per well) were made with saline in ‘U’ bottom microtitre plate 50µl of 2% BSA coupled SRBC in saline was added to each well. For effective mixing the microtitre plate was hand shaken and incubated for an overnight at 37 ºC. The highest dilution of the serum samples showed detectable macroscopic agglutination was recorded and expressed as Log 2 antibody titre of the serum.

**Coupling of BSA to SRBC**
The chromic chloride method for immunological purposes was followed by Goding (1976). In this present study, CrCl3 used as a coupling agent for the coupling of BSA to SRBC. Fresh sheep erythrocytes were washed thrice by using phosphate buffered saline and stored at 4ºC. One volume of the chromic chloride solution was added to an equal volume of the protein antigen in 0.15M saline and then added to one volume of packed red cells immediately. Then it was mixed well and kept at room temperature for 4 min. The coupled red cells are then washed three times in 10-20 volumes of 0.11 M NaCl and resuspended in 0.15 M NaCl with 2% BSA.

**Haematological analysis**
The fresh whole blood samples were used for the estimation of leucocyte, erythrocyte counts and haemoglobin, RBC, WBC.

**Biochemical tests**
Total plasma protein, albumin, globulin, alkaline phosphatase, SGOT, SGPT were analysed by Semi auto analyzer (Chem 400).

**Results**
Administration of aqueous extract of ( 100 mg/kg) and 200 (mg/kg) *Ocimum sanctum* produced dose dependent significant increased in antibody titre compared to control. The results were given in the table (1).

**Haematological changes**
WBC, RBC count in *O. sanctum* treated groups was significantly higher compared with the control group during the experimental period Fig.2. and 3. haemoglobin content also increased. The results were given in the Figure 4.
The results showed that the increasing level of total protein in low and high dose *O. sanctum* treated animals. When compared to control, albumin level was not significantly changed for both low and high dose. SGOT was slightly increased for low dose. But when compared to control, significant changes were not observed in high dose. SGPT was decreased during the study period for both low and high dose. ALP was increased for both low and high dose during the experimental period. The results were given in the table. 1.

**Discussion**

*O. sanctum* is found throughout the semitropical and tropical parts of India. This is used as medicinal plant in Ayurveda and Siddha systems of medicine. It has anti-inflammatory, analgesic and immunostimulatory properties. In this present study immunomodulatory effect of *O. sanctum* was studied in wistar albino rat.

The immune system is a complex system, to protect the host from invading and to eliminate diseases. Immunomodulators are being used as an adjuvant in conditions of immunodeficiency in cancer and other immunodeficiency syndrome (Mathew and Kuttan, 1999). In this present study, *O. sanctum* showed increasing antibody production. It may be the release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs by *O. sanctum* (Godhwani, et al., 1988). The same result was proved by the methanol extract and aqueous suspension of *Ocimum sanctum* leaves by Mediratta et al., (1988). Both humoral and cell mediated immune response of *Ocimum sanctum* seed oil was proved by Mitra et al., (1999). He concluded that it may be due to the activity on GABA energetic pathways. Alcoholic and aqueous extracts of *Ocimum sanctum* increase the in haemagglutination titer in mice (Vaghasiya et al., 2010).

Immunomodulatory effect of *Ocimum sanctum* and *Valairasa chendhuram* was studied by Anuradha and Murugesan (2001) against copper acetate induced toxicity on fish *Oreochromis mossambicus*. They found that *O.sanctum* and *Valairasa chendhuram* were efficient in enhancing the immune response and setting back the haematological parameters. The common carp (*Cyprinus carpio*) treated with herbal immunostimulant (*O. basilicum, Cinnamomum zeylanicum, Juglans regia, Mentha piperita*) enhanced bactericidal activity, serum lysozyme, respiratory burst activity, WBC, RBC, haemoglobin, total serum protein, albumin, globulin (Abasali and Mohammed, 2010).

Immunoplus (containing *O. sanctum*), a poly herbal formulation mixed diet enhanced the growth of *Labeo rohita* and also it enhanced the total protein and globulin (Kumari et al., 2007).

The herbal immuno modulator containing *O. sanctum, Phyllanthus emblica, Withania somnifera* and Shilajit is very helpful in boosting the immune system and fighting against *Caecal coccidiosis* (Pangasa, 2005). Lymphocyte proliferation of *O.basilicum, P. Americana, P.virginica* and *Rosa spp.* were studied by Gomez-Flores et al., (2008). He concluded that methanol and aqueous extract of *O. basilicum* showed 80 and 83% of lymphocyte proliferation, respectively. In this present study, lymphocyte count was gradually increased. It is may be due to the presence flavonoids and terpenoids (Grayer et al., 1996, Lemberkovics et al., 1998). Mediratta et al., (2002) reported that *O. basilicum* modulate both humoral and cell-mediated immune responses.

The present study suggests that the aqueous extract of *O. sanctum* stimulate the antibody production in rat. It enhance the production of WBC, RBC and Haemoglobin.

**References**


Figure 1. Effects of Ocimum sanctum on humoral immune response to S-BSA exposed for 30 days
Figure 2. Effect of *Ocimum sanctum* on erythrocyte count of Wistar albino rats

![Figure 2](image)

Figure 3. Effect of *Ocimum sanctum* on leukocyte count of Wistar albino rats

![Figure 3](image)
Figure 4. Effect of *Ocimum sanctum* haemoglobin count of Wistar albino rats

![Haemoglobin count graph showing exposure time (Days) vs. Haemoglobin (g%) for Control, Low dose, and High dose groups.]

Table 1. Effect of *O. sanctum* in biochemical parameters of wistar albino rat

<table>
<thead>
<tr>
<th>Biochemical Parameter</th>
<th>Exposure (Days)</th>
<th>Control</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
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<tbody>
<tr>
<td>Protein (g/dl)</td>
<td>0</td>
<td>6.34 ± 0.45</td>
<td>6.3 ± 0.52</td>
<td>6.58 ± 0.50</td>
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<td></td>
<td>15</td>
<td>6.06 ± 0.19</td>
<td>6.36 ± 0.39</td>
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<td>30</td>
<td>6.2 ± 0.61</td>
<td>6.4 ± 0.56</td>
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<td>Albumin (g/dl)</td>
<td>0</td>
<td>4.16 ± 0.35</td>
<td>4.12 ± 0.43</td>
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<td>15</td>
<td>4.16 ± 0.21</td>
<td>4.1 ± 0.32</td>
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<td>4.26 ± 0.15</td>
<td>4.12 ± 0.19</td>
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<td>Globulin (g/dl)</td>
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<td>2.18 ± 0.28</td>
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<td>30</td>
<td>1.94 ± 0.56</td>
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<td>2.64 ± 0.27</td>
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<td>SGOT (U/L)</td>
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<td>55.5 ± 7.00</td>
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<td>57.34 ± 5.02</td>
<td>64.14 ± 10.13</td>
<td>58.32 ± 8.45</td>
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<td>60.06 ± 5.81</td>
<td>70.78 ± 9.60</td>
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<td>24.2 ± 4.05</td>
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<tr>
<td>ALP (U/L)</td>
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