Immunomodulatory effect of methanol extract *of Solanum xanthocarpum* fruits

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ABSTRACT

Purpose : To study the immunomodulatory activity of methanol extracts of fruits of *Solanum xanthocarpum* (Solanaceae) on Swiss albino mice.

Methods

Model -Cyclophosphamide induced immunosuppression model

Animal used - Swiss albino mice.

Dose – Cyclophosphamide-30mg/kg body weight, i.p route.

Extract-100mg/kg body weight, oral route.

The extent of protection against immunosuppression caused by Cyclophosphsmide was evaluated after 14 days of drug administration, by estimating hematological parameter and neutrophil adhesion test.

Results

Methanol extracts of fruits of *Solanum xanthocarpum* showed pronounced immunoprotective activity by increasing the depleted levels of total WBC count and RBC, % Hb, and % neutrophils adhesion.

Conclusions

The extract was found to be effective immunomodulatory agents.

Keywords: *Solanum xanthocarpum, methanol extract,* Immunomodulatory activity, Cyclophosphamide, immunopotentiation

INTRODUCTION

In recent years, there has been growing interest in the field of herbal medicines research and search for for promising potential area of investigating of immunomodulatory agents from natural products. The immune system is designed to protect the host from invading pathogen and to eliminate disease. Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases. Herbal drugs are believed to enhance the natural resistance of the body against infection and their immunomodulatory activities have been reported in numerous plants [1&2].

The concept of immunomodulation relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells and lymphocytes and also to the production of various effector molecules generated by activated cells. It is expected that theses nonspecific effects give protection against different pathogens including bacteria, viruses, fungi etc. and constitute an alternative to conventional chemotherapy [3& 4].

Kantkari (*Solanum Xanthocarpum*) is one of the members of the dashmula (ten roots) of the Ayurveda[5]. It is a very spiny diffuse herb up to 1.2 m tall, commonly found throughout India, used in medicine in various forms, such as decoction, electuary, ghrita, etc [6]. A decoction of the root is given with the addition of long pepper and honey, in cough and catarrh, and with rock salt and assafoetida in spasmodic cough [7]. Plant has been investigated for much of responses and as well a pilot study on the clinical efficacy of *Solanum xanthocarpum* as a dried whole plant shown significant improvement in some respiratory diseases like bronchial asthma [8]. The present study aimed at investigating the immunomodulatory potency of the methanol extract of fruit of the plants using cyclophosphamide induced immunosuppression model and neutrophil adhesion test.

Cyclophosphamide acts on both cyclic and intermitotic cells, resulting in general depletion of immune-competent cells. Cyclophosphamide (CP) is an alkylating agent widely used in anti-neoplastic therapy [9]. It is effective against a variety of cancers such as lymphoma, myeloma and chronic lymphocytic leukemia [10]. CP-induced immunosuppression is reported to prompt various types of infection [11&12].

Haematological parameter such as Total WBC, RBC, Haemoglobin and neutrophil constitutes the key components of the immune system. A rise or fall in the concentration of these cells affects the health/immune constitution of the body as they are known to recognize the foreign antigens and mount an immune response

[13]. Hence these parameter is chosen to study the Immunomodulatory activity of the methanol extract of fruits of *Solanum xanthocarpum*.

The present study is aimed at investigating the immunomodulatory potency of the methanol extract of fruits of the *Solanum xanthocarpum* using Cyclophosphamide induced immunosuppression model by evaluating the effect of the extract on various hematological parameters and Neutrophil adhesion test in Swiss albino mice.

MATERIALS AND METHODS

Plant material:

The fresh fruits of *Solanum xanthocarpum* were collected form APRC Lab Chennai and authentication no APRC/78/22/08-09.

Drugs and Chemicals

Cyclophosphamide (Sigma, life science) was used as standard immunosuppressant. All the other reagents and chemicals used in studies were of analytical grade.

Animals:

Eight week-old healthy, laboratory bred, Swiss albino mice of either sex (20-25g) were maintained under standard laboratory conditions such as temperature 22–25^{o}C, 12 hour light/dark cycle and provided with water and pellet food ad libitum. The experiments were conducted in CPCSEA (Committee for the purpose of control and supervision of experiments on animals, Chennai, India) approved animal house after obtaining the prior approval from the Institutional Animal Ethics Committee. Letter no AACP/IAEC/P-33/2006.

Preparation of aqueous and methanol extract

Solanum xanthocarpum fruits were shade dried and reduced to coarse powder # 22. Powdered plant materials were defatted with petroleum ether (60-80ºC) and the marc was refluxed with methanol for 8 hrs. Extract was filtered and concentrated by evaporation under reduced pressure using rotary vacuum evaporator, dried and kept in an air tight container. The percentage yield was noted.

Immunomodulatory Activity

The methanol extract of the plant was subjected to evaluation of Immunomodulatory Activity using Cyclophosphamide induced immunosuppression model and neutrophil adhesion test.

Preparation of sample

The methanol extract of the plant were suspended in 0.5% Carboxy methyl cellulose solution in distilled water. The extract was administered orally at a dose of 100 mg/kg b/w.

Preparation of Cyclophosphamide

The Cyclophosphamide was suspended in 0.5% Carboxy methyl cellulose solution in distilled water. The solution was administered intraperitoneally at a dose of 30 mg/kg b/w.

Cyclophosphamide induced immunosuppression

The animals were divided into the 4 groups containing 6 animals in each group. Group1 (Control group) received Carboxy Methyl Cellulose (CMC) for 14 days and group 2 (Challenge group) received CMC for 10 days, on 11th, 12th and 13th day Cyclophosphamide intraperitonially at a dose of 30mg/kg b/w. Groups 3 (Test group) received methanol extract of the drug at a dose of 100mg/kg body weight orally for 14 days. On days 11, 12 and 13th day Cyclophosphamide solution was given intraperitonially at a dose of 30mg/kg b/w one hr after the administration of the extract.

Hematological Test

At the end of the treatment, mice were light anaesthetized by using di-ethyl ether. The blood was collected from the retro-orbital plexus using heparinised capillary tubes and Hematological tests were carried out.

The WBC count was done by Turke's method [14], RBC by Hayem's method [15], and haemoglobin by Sahli's method [16]. The results are shown in Fig 1-4.

Neutrophil adhesion test [17]

Total leukocyte counts (TLC) and differential leukocyte counts (DLC) were analyzed by fixing blood smears and staining with Field stain I and II- Leishman's stain. After initial counts, blood samples were incubated with 80mg/ ml of nylon fibers for 15 min at 37ºC. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample.

Percent neutrophil adhesion was calculated as shown below

$$\text{Neutrophil adhesion (\%)} = \frac{NI\ u - NI\ t}{NI\ u} \times 100$$

Where

NI u = Neutrophil index of untreated blood sample.

NI t = Neutrophil index of treated blood sample.

Statistical Analysis

The data were expressed as the mean ± standard deviation of the means (S.D) and statistical analysis was carried out employing student's t-test and one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

Results

Effectiveness against drug-induced immunosuppression

Administration of Cyclophosphamide (30 mg/kg, i.p) produced a significant decrease in the Total Leukocyte Count from 6.2±0.081 to 3.08±0.214, RBC count from 4.91±0.116 to 2.9±0.152, and % hemoglobin from 16.60±0.081 to 10.32±0.153 (P<0.01). This was found to be consistent with earlier studies which state that Cyclophosphamide induces immune dysfunction through reactive intermediate-induced damage to the cells of the immune system [18].

Evaluation of effect of methanol extract of fruits of *Solanum xanthocarpum* on Cyclophosphamide induced immunosuppression indicated good protection by increasing all the hematological parameters. WBC count, RBC count, and % hemoglobin values observed were better than untreated control groups (Fig 1-3).

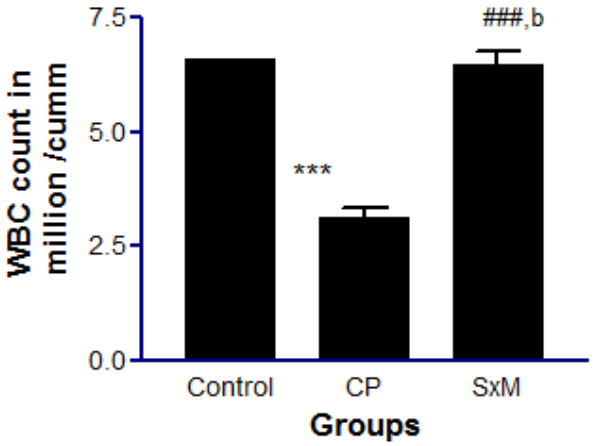

Figure 1. Effect of methanol extract on *Solanum xanthocarpum* fruit on WBC count.
All values are mean±SEM, n=6.
***P<0.001 when compared with control group and, ###P<0.001, when compared with Cyclophosphamide treated group (Students t test).
bP<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

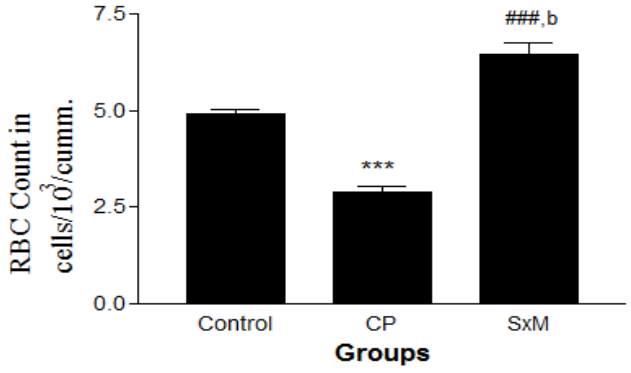

Figure 2. Effect of methanol extract on *Solanum xanthocarpum* fruit on RBC count.
All values are mean±SEM, n=6.
***P<0.001 when compared with control group and , ###P<0.001, when compared with Cyclophosphamide treated group (Students t test).
bP<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

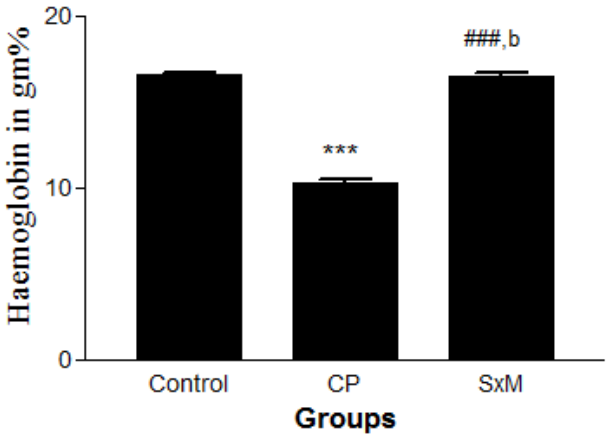

Figure 3. Effect of methanol extract on *Solanum xanthocarpum* fruit on Haemoglobin estimation.
All values are mean±SEM, n=6.
***P<0.001 when compared with control group and , $^{###}$P<0.001, when compared with Cyclophosphamide treated group (Students t test).
^{b}P<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

Neutrophil adhesion test

This test is an indicative of the marginalization of phagocytic cells in the blood vessels, i.e. an indication of immunostimulation. The % neutrophil adhesion in control group animals was, 25.76±1.585, in CP treated group was 14.44±1.08, in methanol treated group it was 26.07±1.043 (Fig 4). The results of neutrophil adhesion test indicating that there was significant (P<0.001) increase in neutrophil adhesion after administration of methanol extract.

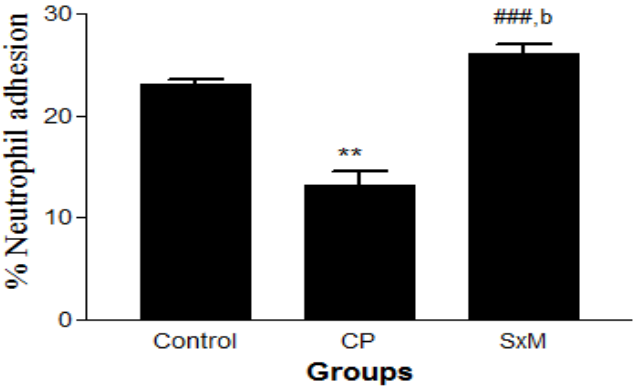

Figure 4. Effect of methanol extract of fruits of *Solanum xanthocarpum* on neutrophil adhesion test on Cyclophosphamide treated mice.
All values are mean±SEM, n=6.
**P<0.01 when compared with control group, $^{###}$P<0.001 when compared with Cyclophosphamide treated group.(Students t' test).
^{b}P<0.01 when compared with Cyclophosphamide treated group (one way Anova).

DISCUSSION

Solanum xanthocarpum is known for several medicinal uses and has been investigated for different pharmacological properties[6-9]. Use of herbs for improving the overall resistance of body against common infections and pathogens has been a guiding principle of Ayurveda. *Solanum xanthocarpum* has been used and reported in many such formulations. However, there is no systematic study of its immunomodulatory activity.

Hence in the present study the immunomodulatory activity of methanol extract of fruit of this plant was investigated. Cyclophosphamide induced immune-suppressive mice model was used because the dyanamic and complex nature of the immune system in which a drug elicits its effect can be detected more reliably after immune challenge. The study affirms that methanol extract of the fruits of *Solanum xanthocarpum* is effective immunomodulatory agent. The effectiveness of extract-treated animals in overcoming the side effects of cyclophosphamide induced immunosuppression provides evidence for balancing and adaptogenic effectiveness of extract. The extract potentiated the non-specific immune response. This may attributed to different phytoconstituents. Increase in percent neutrophil is attributed to marginalization of phagocytic cells i.e. improved defensive response under normal circumstances. Thus with the result of this preliminary study it can be conclude that the plant holds promise for being used as an immunostimulating agent.

CONCLUSION

The Methanol extract of fruits of *Solanum xanthocarpum* have protected the animal against Cyclophosphamide induced immunosuppression indicating its profound immunostimulatory activity.

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