To Evaluate the Immunomodulatory Activity of Hydroalcoholic and Methanolic Extract of *Quisqualis indica* Linn in Rats

**Keywords:** Immunomodulation, *Quisqualis Indica*, Myelosuppression and immunity

**ABSTRACT**

The goal of the present study is to evaluate the immunomodulatory activity of hydroalcoholic and methanolic extract of *Quisqualis Indica Linn* in wistar rats. *Quisqualis indica* (also known as Rangoon Creeper, Madhumalti, or Laal-chamiel) hydroalcoholic and methanolic extracts were given orally at dosage of 100 mg/kg and 200 mg/kg. Levamisol 50 mg/kg used as standard. The carbon clearance test, Cyclophosphamide-induced myelosuppression, Total Leukocyte Count (TLC), and Differential Leukocyte Count (DLC) were used to determine immunomodulatory activity on specific and non-specific immunity (DLC). The carbon clearance test was used to determine the effect of *Quisqualis indica* (QI) flower extract on phagocytic activity. In carbon clearance test, QI flower extract treated all groups, exhibited significantly high phagocytic index. The phagocytic index of (100 mg/kg) and QI flower extract (200 mg/kg) showed significant (p<0.05) increased in phagocytic index when compared to control group. This indicates stimulation of the reticuloendothelial system. Cyclophosphamide at the dose of 30 mg/kg, caused a significant reduction in total WBC count in rat as compared to control group. The rise in the total WBC count lowered by Cyclophosphamide was observed at 100 mg/kg and 200 mg/kg of *Quisqualis indica* flower extract. As a result, the current study found some pharmacological evidence to back up the folklore argument that *Quisqualis indica* L. has immunomodulatory properties.
INTRODUCTION:

Immunomodulators:

Immunomodulators are biological or synthetic substances that can activate, inhibit, or modulate any part of the immune system, including both the innate and adaptive arms of the immune response. A number of clinical conditions, like cancer\textsuperscript{1, 2} surgery\textsuperscript{3} or administration of drugs are known to affect the different components of the immune system, thereby making the host susceptible to infections. Also, stress, be it physical or psychological, causes immunosuppression.\textsuperscript{4} As opposed to the need for immunostimulants, there also are cases of immune-hypersensitivity reactions, such as asthma, autoimmunity, graft rejection, arthritis, allergy and inflammatory disorders, in which an immuno-suppressor is indicated.\textsuperscript{5} Most of such agents in clinical use are cytotoxic drugs such as azathioprine\textsuperscript{6}, cyclophosphamide\textsuperscript{7}, prostaglandins\textsuperscript{8}, cyclosporine A\textsuperscript{9}, thicarbomate\textsuperscript{10}, levamisol\textsuperscript{11}, niridazole\textsuperscript{12} and pencillamine\textsuperscript{13}. The main disadvantage of these drugs is their cytotoxicity and associated side effects.\textsuperscript{13}

Classification of immunomodulatory:

Immunomodulators may be classified as immunoadjuvant, immunostimulants and immunosuppressant.

Immunoadjuvant:

These agents are used to improve vaccine effectiveness and thus may be classified as particular immune stimulants\textsuperscript{14}. A good example is Freund's adjuvant.\textsuperscript{15} The immunoadjuvant hold the promise of being the true modulators of immune response. It has proposed to exploit them for selecting between cellular and humoral, Th1 (helper T1 cells) and Th2, (helper T2 cells) immunoprotective and immunodestructive, and reagenic (IgE) versus immunoglobin G (IgG) type of immune responses, which poses to be a real challenge to vaccinedesigners.\textsuperscript{16}

Immunostimulants:

Since they were designed to improve the body's resistance to infection, these agents are generally non-specific. They have the ability to function both by innate and adaptive immune responses. Immunostimulants are intended to function as prophylactic and promoter agents in
healthy people, acting as immunopotentiators by improving the basic level of immune response, and as immunotherapeutic agents in people with impaired immune responses.\textsuperscript{17}

**Immunosuppressant:**

These are a group of structurally and functionally diverse medications that are often used in combination regimens to treat different forms of organ transplant rejection and autoimmune diseases.\textsuperscript{18}

The immune system is one of our body's most complicated biological processes. The immune system's primary function is to differentiate between self and non-self. An infected organism, a transplanted organ, or an endogenous cell that is mistaken for a foreign cell could all be considered non-self.\textsuperscript{19}

![Image: Immunomodulators in the lap of nature](image_url)
Types of immune responses:

The immune responses of the human body against any non-self are of two types:

- Innate immune response (or natural or non-specific)
  - Humoral immunity
  - Cellular immunity

- Adaptive immune response (or acquired or specific)
  - Humoral immunity
  - Cellular immunity

MATERIALS AND METHODS:

PLANT PROFILE:

**QUISQUALIS INDICA**

*Quisqualis indica* Linn. (Combretaceae) is a good climber with a ligneous vine that can grow up to 8 meters tall. Rangoon creeper is its common name. It's native to Africa and the Indo-Malaysian region, and it's grown all over India.

<table>
<thead>
<tr>
<th>Scientific classification</th>
<th>Local Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom- Plantae</td>
<td>English (RangoonCreeper),</td>
</tr>
<tr>
<td>Division- Magnoliophyta</td>
<td>Hindi (Madhumalti),</td>
</tr>
<tr>
<td>Class- Magnoliopsida</td>
<td>Bengali (Modhumalati),</td>
</tr>
<tr>
<td>Order- Myrtales</td>
<td>Telgu (RadhaManoharam),</td>
</tr>
<tr>
<td>Family- Combretaceae</td>
<td>Filipino (Niyog-niyogan),</td>
</tr>
<tr>
<td>Genus- Quisqualis</td>
<td>Spanish (Quiscual),</td>
</tr>
<tr>
<td>Species- <em>Q. indica</em></td>
<td>China (Shih-chun-tzu),</td>
</tr>
</tbody>
</table>
It is a vining, evergreen plant with vigorous growth that requires sturdy support and can get out of hand on its preferred growing site; it does not need thick, anchoring roots. It is widely distributed throughout the world, especially in China, the Philippines, Bangladesh, Myanmar, and Malaysia, and is now widely grown as an ornamental plant in most gardens in India. Throughout the Philippines, it can be found in 1) thickets and secondary forests. 2) Its flowers are ornamentally planted. 3) It's also found in India and Malaya. 4) It has been introduced to the majority of tropical countries.

Pharmacognostic Studies:

Macroscopy- Morphological studies were done by using simple microscope. The shape, apex, base, margin, taste and odor of leaves and flowers were determined.

Phytoconstituents:

Every plant contains several phytoconstituents in its different parts showing various pharmacological activities and / toxicities, likewise *Quisqualis indica* Linn. also showing many pharmacological activities due to the presence of medicinally active compounds. *Quisqualis indica* Linn contains phytoconstituents such as Trigonelline (alkaloid), L-proline (α-amino acid), L-asparagine (α-amino acid), quisqualic acid (agonist for both AMPA receptors), rutin (flavonoid) and two forms of the cysteine synthase, isoenzyme A and isoenzyme B (enzyme). Rutin and pelargonidin-3-glucoside have also been isolated from flowers.

Extraction Procedure:

About 180 gm of dry powder was taken in a closed bottle and it was defatted with Petroleum ether. The defatting was continued for 9-10 days with occasional shaking. The Petroleum ether extract was filtered. The marc left after Petroleum ether defatting was taken out and dried under shade to get a dry mass, then extracted with Methanol and water (hydroalcoholic) by using cold maceration extraction. The extraction was continued for 9-10 days with occasional shaking. The hydroalcoholic extract was filtered, concentrated under reduced pressure to a semisolid mass and was made free from solvent. The final obtained extract was weighed; percentage yield was calculated and stored in a cool place.
TABLE NO. 1: LIST OF CHEMICAL AND DRUGS

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drugs/Chemical</th>
<th>Manufacturer/Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Indomethacin</td>
<td>Cayman chemical company.</td>
</tr>
<tr>
<td>2.</td>
<td>Fruends adjuvant</td>
<td>Sigma-Aldrich chemicals Ltd.</td>
</tr>
<tr>
<td>3.</td>
<td>Carboxymethylcellulose</td>
<td>Merck specialties Pvt. Ltd</td>
</tr>
<tr>
<td>4.</td>
<td>Formaldehyde</td>
<td>CDH private Ltd.</td>
</tr>
<tr>
<td>5.</td>
<td>Diclofenac sodium</td>
<td>Day’ Pharmaceutical Ltd.</td>
</tr>
<tr>
<td>6.</td>
<td>Levamisol</td>
<td>Sigma Aldrich Ltd.</td>
</tr>
<tr>
<td>7.</td>
<td>Sodium bi carbonate</td>
<td>Merck specialties Pvt. Ltd</td>
</tr>
<tr>
<td>8.</td>
<td>Indian ink</td>
<td>Camel Ltd.</td>
</tr>
<tr>
<td>9.</td>
<td>Cyclophosphamide</td>
<td>Biochem pharmaceutical Ltd.</td>
</tr>
<tr>
<td>10.</td>
<td>Glacial acetic acid</td>
<td>Merck specialties Pvt. Ltd</td>
</tr>
<tr>
<td>11.</td>
<td>Leishman’s stain</td>
<td>HD fine Ltd.</td>
</tr>
<tr>
<td>12.</td>
<td>Methanol</td>
<td>Merck specialties Pvt. Ltd</td>
</tr>
<tr>
<td>13.</td>
<td>Petroleum ether</td>
<td>Merck specialties Pvt. Ltd</td>
</tr>
<tr>
<td>14.</td>
<td>Di ethyl ether</td>
<td>Merck specialties Pvt. Ltd</td>
</tr>
</tbody>
</table>

EXPERIMENTAL METHODS:

Acute oral toxicity:

The dose limit was selected on the basis of previously performed oral acute toxicity studies in albino mice in accordance with the OECD guidelines. Acute toxicity studies on *Quisqualis indica* aerial parts extract were performed in mice containing 6 animals in each group, the graded doses of the methanolic and hydroalcoholic extracts of *Quisqualis indica* aerial parts extract doses selected for the study were 100 mg/kg, 200 mg/kg, 400 mg/kg, 800 mg/kg, 1600 mg/kg, 2000 mg/kg were administered orally and the animals were observed for 2 weeks following administration, change in body weight gain, food consumption, any kind of behaviorally changes and mortality were noted. It was found that the methanolic extract has produced significant toxicity at the dose of 2000 mg/kg as 2 animal of this group was died. Thus, the extract was highly tolerable up to 1500 mg/kg for methanolic extract and 2000 mg/kg for hydroalcoholic extract.
IMMUNOMODULATORY ACTIVITY:

Carbon Clearance test:22

Procedure:

To evaluate the Phagocytic activity of the reticuloendothelial system in-vivo, the Animals were divided in 4 groups each having 4 animals.

Group-1 Vehicle (Distilled Water),

Group-2, (Plant extract low dose) 100 mg/ kg, Treated,

Group-3, (Plant extract high dose) 200 mg/kg p.o Treated,

Group-4 Standard (levamisol) 50 mg/kg,

Oral Treated a carbon clearance test was performed after completion of the drug pretreatment. On day 14, the treated rats received an intravenous injection (tail vein) of carbon suspension (1:50 dilution of Indian ink, Camel) in a dose of 0.5 mL/100 g body weight. Blood was withdrawn from the retro-orbital venous plexus at 2 min and 15 min after injection of the carbon suspension. 0.05 mL of blood will be lysed with 4 mL of 0.1% Na2CO3 and the optical density was measured spectrophotometrically at 650 nm wavelength.

Parameters observed:

The Phagocytic index K will be calculated using the following equation: Log (OD0) - log (ODt)/t.

Where OD0 is the OD at 0 min and ODt is the OD at t min.

Cyclophosphamide-induced myelosuppression:23

Procedure:

Animals were divided into four groups of four animals each. Group I (Normal control group) and Group II (Cyclophosphamide-treated group) received the vehicle (distill water) for period of 13 days. Group III, IV were given Plant extract 100mg/kg and 200mg/kg respectively, p.o., daily for 13 days. The animals of groups II-, IV were injected with Cyclophosphamide (30
mg/kg, i.p.) on the 11th, 12th, and 13th day, 1 h after the administration of the respective drug treatments. Blood samples were collected from retro orbital plexus on the 14th day of the experiment. Determination of total and differential white blood cells was carried out.

**Parameters monitored:**

**Total White Cell (Leukocyte) Count:**

A sample of whole blood was mixed with a weak acid solution that lyses non nucleated red blood cells. Following adequate mixing, the specimen is introduced into a counting chamber where the white blood cells (leukocytes) in a diluted volume are counted. White-count diluting fluid. Either of the following diluting fluids may be used: 2% acetic acid. Add 2 ml glacial acetic acid to a 100 ml volumetric flask. Dilute to the mark with distilled water.

1. Draw well-mixed capillary or venous blood exactly to the 0.5 mark in a white blood cell diluting pipet. This blood column must be free of air bubbles.

2. Wipe the excess blood from the outside of the pipet to avoid transfer of cells to the diluting fluid. Take care not to touch the tip of the pipette with the gauze.

3. Immediately draw diluting fluid to the "11" mark while rotating the pipet between the thumb and forefinger to mix the specimen and diluent. Hold the pipet upright to prevent air bubbles in the bulb.

4. Mix the contents of the pipet for 3-5 minutes to ensure even distribution of cells. Expel unmixed and relatively cell-free fluid from the capillary portion of the pipette (usually 4 drops).

5. Place the forefinger over the top (short end) of the pipet, hold the pipet at a 450 angle, and touch the pipette tip to the junction of the cover glass and the counting chamber.

6. Allow the mixture to flow under the cover glass until the chamber is completely charged. Similarly, fill the opposite chamber of the hemacytometer. Count the white cells in the four 1 sq mm corner areas and calculated WBC count by following formula.

The formula is as follows:

\[
\text{WBCs per cu mm} = \text{avg. no of chambers} \times \text{WBCs counted} \times \text{dilution} / \text{volume}
\]
Differential Leukocyte Counts

A drop of blood was added on the centre line of the glass slide about 1 cm from one end and blood smear was prepared. Then smear was stained with diluted Leishman's stain for 30 min and washed with distilled water and dried at room temperature. For counting of DLC, the slide was examined under microscope at 100x using Cedarwood oil. Finally, total number of Neutrophils, Lymphocytes, eosinophils and Monocytes in the 100 cells were counted and results were expressed in percentage.

Statistical analysis:

The mean ± SEM values were calculated for each group. Significant difference between groups was determined using analysis of variance (ANOVA) followed by Dunnett’s t-test. P<0.05 was considered as significant.

RESULT AND DISCUSSION:

Immunomodulatory activity:

1. Carbon clearance test of hydroalcoholic and methanolic extract of *Quisqualis indica*:

Increase in phagocytic activity was observed in the present study when treated groups were compared with control (Table 2). The rate of carbon clearance which was determined in terms of phagocytic index was 0.0135±0.00155 and 0.0150±0.001 found in low dose and high dose hydroalcoholic extract treated group 3 and group 4 respectively and 0.0127±0.00256, 0.014±0.00195 found in low dose and high dose methanolic extract treated group respectively.

The mean phagocytic index of standard i.e. levamisol (group 2) was found to be 0.0147±0.00188. The levamisol (50 mg/kg p.o) also show any significant effect on the phagocytic index in the carbon clearance assay. The mean phagocytic index of control (group 1) was found to be 0.0122 ± 0.00188 which clearly indicates that the amount of residual foreign particles in extract treated rats blood was significantly less ( p<0.01).
TABLE NO. 2: CARBON CLEARANCE TEST OF HYDROALCOHOLIC AND METHANOLIC EXTRACT OF *QUISQUALIS INDICA*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 minutes</th>
<th>15 minutes</th>
<th>Phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5625±0.02096</td>
<td>0.3875±0.03198</td>
<td>0.0122±0.00188</td>
</tr>
<tr>
<td>Standard (Levamisol 150mg/kg)</td>
<td>0.7401±0.01080**</td>
<td>0.4702±0.02041**</td>
<td>0.0147±0.00188**</td>
</tr>
<tr>
<td>Low dose hydroalcoholic (100mg/kg)</td>
<td>0.5402±0.02273***</td>
<td>0.3550±0.01322**</td>
<td>0.0135±0.00155*</td>
</tr>
<tr>
<td>High dose hydroalcoholic (200mg/kg)</td>
<td>0.5850±0.00645**</td>
<td>0.3701±0.01683*</td>
<td>0.0150±0.001**</td>
</tr>
<tr>
<td>Low dose methanolic (100mg/kg)</td>
<td>0.5110±0.03488***</td>
<td>0.3425±0.01547**</td>
<td>0.0127±0.00256*</td>
</tr>
<tr>
<td>High dose methanolic (200mg/kg)</td>
<td>0.5125±0.04028***</td>
<td>0.3275±0.01652***</td>
<td>0.014±0.00195*</td>
</tr>
</tbody>
</table>

The values are Means ±S.E.M. (n=4), * P < 0.05, ** P < 0.01, ***P<0.001 as compared with control group (one-way ANOVA followed by Dunnett’s test).

Figure No. 2: Column statistic of various extracts of *Quisqualis indica* in carbon Clearance test
2. Effect of Hydroalcoholic and Methanolic extracts of *Quisqualis indica* in Cyclophosphamide induced myelosuppression model:

**Total Leucocyte Counts:**

A significant ($P < 0.05$) reduction in total white blood cell count was observed in rats treated with Cyclophosphamide alone (group II) as compared to control group (group I). Hydroalcoholic and methanolic extract; low dose 100 and high dose 200 mg/kg, p.o with Cyclophosphamide increased the levels of total WBC count as compared to Cyclophosphamide treated group. The rise in the total WBC count lowered by Cyclophosphamide was observed at 100 mg/kg and 200 mg/kg of HEQI. The total WBC count was restored back to normal (Table 3).

**Differential leukocyte counts:**

There was a significant ($P < 0.05$) decrease in Neutrophils (N), lymphocytes (L), Basophils (B) and eosinophils (E) in animals and insignificantly decrease in monocytes(M) treated with Cyclophosphamide (group II) as compared to control group (group I). HEQI with Cyclophosphamide at 100 mg/kg dose significantly ($P<0.05$) increased the Neutrophils (N), lymphocytes (L) and eosinophils (E) as compared to group I, but failed to significantly reduce the monocytes count as compared to group II in both HEQI with Cyclophosphamide at 100 and 200 mg/kg dose. HEQI with Cyclophosphamide 100 mg/kg also insignificantly increase of Neutrophils (N), lymphocytes (L) and eosinophils (E) (Table 3).
TABLE NO. 3: EFFECT OF HYDROALCOHOLIC AND METHANOLIC EXTRACTS OF *QUISQUALIS INDICA* IN CYCLOPHOSPHAMIDE INDUCED MYELOSUPPRESSION MODEL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TLC(cells/m (^3))</th>
<th>Platelets(lak hs/mm (^3))</th>
<th>Neutrophils %</th>
<th>Lymphocyte %</th>
<th>Monocyte %</th>
<th>Eosinophils %</th>
<th>Basophils %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.71±0.654</td>
<td>6.20±0.43</td>
<td>56.5±3.5</td>
<td>32.25±3.0</td>
<td>4.42±0.82</td>
<td>4.59±0.32</td>
<td>0.08±0.04</td>
</tr>
<tr>
<td>Standard(Cyclophosphamide 30mg/kg)</td>
<td>3.718±0.157</td>
<td>1.12±0.049</td>
<td>21.5±4.73</td>
<td>19±1.47</td>
<td>0.11±0.01</td>
<td>0.10±0.06</td>
<td>0.03±0.029</td>
</tr>
<tr>
<td>Low dose hydroalcoholic (100mg/kg)</td>
<td>8.825±0.092</td>
<td>3.72±0.33**</td>
<td>51.75±1.54*</td>
<td>30±2.41**</td>
<td>3.825±0.4</td>
<td>4±0.09***</td>
<td>0.01±0.007</td>
</tr>
<tr>
<td>High dose hydroalcoholic (200mg/kg)</td>
<td>8.842±0.333</td>
<td>4.72±0.21**</td>
<td>56.5±2.101**</td>
<td>32.25±2.7**</td>
<td>4.15±0.64</td>
<td>4.37±0.131**</td>
<td>0.017±0.00</td>
</tr>
<tr>
<td>Low dose methanolic(100mg/kg)</td>
<td>8.07±0.064*</td>
<td>3.86±0.12**</td>
<td>49.75±0.85**</td>
<td>28.5±1.84**</td>
<td>3.32±0.44</td>
<td>3.65±0.253*</td>
<td>0.007±0.00</td>
</tr>
<tr>
<td>High dose methanolic(100mg/kg)</td>
<td>8.45±0.265**</td>
<td>3.59±0.31**</td>
<td>52.25±1.10**</td>
<td>27±2.73*</td>
<td>3.3±0.35**</td>
<td>3.32±0.20**</td>
<td>0.015±0.01</td>
</tr>
</tbody>
</table>

The values are Means ±S.E.M. (n=4), * P < 0.05, ** P < 0.01, ***P<0.001.as compared with control group (one-way ANOVA followed by Dunnett’s test)

Figure No. 3: Column statistic of various extracts of *Quisqualis indica* in Cyclophosphamid induced myelosuppression (TLC)
Figure No. 4: Column statistic of various extracts of *Quisqualis indica* in Cyclophosphamide induced myelosuppression (DLC)

Figure No. 5: Column statistic of various extracts of *Quisqualis indica* in Cyclophosphamide induced myelosuppression (Platelets)
DISCUSSION:

The findings show that *Quisqualis indica* (QI) flower extract is a powerful immunostimulant that stimulates both specific and non-specific immune mechanisms. The rate of removal of carbon particles from the bloodstream is used to measure the phagocytic activity of the reticuloendothelial system. *Quisqualis indica* extract tended to improve phagocytic function by demonstrating a carbon clearance rate by the reticuloendothelial system's cells. The effect of *Quisqualis indica* flower extract on the phagocytic activity by the carbon clearance test in a carbon clearance survey, all groups treated with QI flower extract had a significantly higher phagocytic index. When compared to the control group, the phagocytic index of (100 mg/kg) and QI flower extract (200 mg/kg) showed a substantial (p<0.05) increase in phagocytic index. This means that the reticuloendothelial system is being stimulated. When compared to the control group, cyclophosphamide at a dose of 30 mg/kg resulted in a substantial reduction in total WBC count in rats. At 100 mg/kg and 200 mg/kg of *Quisqualis indica* flower extract an increase in total WBC count was observed after Cyclophosphamide treatment. The total WBC count was significant restoration of white blood cell count. There was a significant decrease, Neutrophils lymphocytes, eosinophils and monocytes in animals treated with Cyclophosphamide (group II) as compared to control group (groupI) because Cyclophosphamide showed that in rats lymphocytes decrease due to immunotoxic effect as well as decreases in the activity of lymphoid cells especially the CD4+ lymphocyte.

CONCLUSION:

The pharmacological screening included evaluation of immunomodulatory activity of hydroalcoholic and methanolic extract of *Quisqualis indica* at the dose of 100 mg/kg, 200mg/kg in rats with carbon clearance test and cyclophosphamide-induced myelosuppression. Administration of *Quisqualis Indica* was found to increase phagocytic activity by stimulation of macrophages, total WBC and differential leukocytes count.

The phytochemicals analysis revealed the presence of polyphenols and flavonoids. The polyphenols have potent anti-inflammatory activity by inhibiting prostaglandin synthesis. So anti inflammatory activity of Hydroalcoholic and methanolic extract of *Quisqualis indica* can be attributed to bradykinin and PG synthesis inhibition property of polyphenols.

The study reveals that hydroalcoholic and methanolic extract of *Quisqualis Indica* (HEQI) has Immunostimulants activity which strongly affected immune system seems to be bioactive.
fraction of this plant. However, the mechanism of action could be unfolded only after detailed investigations whereby the extract modulates the immune system however; the extract contains compounds which had Immunomodulatory activity. Besides, to isolate the active constituents and clarify its mechanism of action will be our auxiliary objective.

REFERENCES: