Immunostimulatory Effect of Aqueous Leaf Extract of Tectona grandis Linn. on Male Wistar Albino Rat

S.I. Egba, O.U. Njoku and C.D. Igwe

Abstract: Immunostimulators are agents designed to enhance the body’s resistance against infections and infectious agents. Studies have reported that plant extracts possess medicinal values including immunomodulatory properties. This study evaluated the immunostimulatory effect of different concentrations of aqueous leaf extracts of Tectona grandis in male wistar albino rats. The extract was administered orally at doses of 100 mg/kg and 300 mg/kg body weight daily to immune depressed rats divided into 4 groups of 4 rats each for 21 days. Immune depression was achieved by oral administration of 2% pyrogallol for 7 days prior to start of treatment with extract. Humoral and cellular immune responses of the animals challenged by sheep red blood cells (SRBCs) as well as haematological parameters were used to assess the immunostimulatory effect of the extract. Results obtained showed a significant decrease in mean paw diameter of test animals when compared to the negative control at both doses 100 mg/kg and 300 mg/kg body weight. The result of the humoral immune response showed a significant (p<0.05) increase in both primary and secondary antibody titres at 100 mg/kg and 300 mg/kg body weight in the test animals compared to the negative control. Results of packed cell volume (PCV), red blood cell (RBC), total white blood cell (TWBC) count and haemoglobin (Hb) concentration showed similar significant (p<0.05) increase in the test animals when compared with the negative control. Summarily, the results seem to suggest that aqueous leaf extract of Tectona grandis possess immunostimulatory properties and may be of immense benefit in subjects with immune depressed conditions.

Key words: Immunostimulatory • Tectona grandis • Delayed Type Hypersensitivity • Haematological And Pyrogallol

INTRODUCTION

The immune system is designed to protect the host from invading pathogens and to eliminate disease [1]. Modification of the immune response by pharmacological agents is most effective in therapy if begun before exposure to the antigen has an opportunity to generate a primary response. The function and the efficacy of the immune system may be influenced by many exogenous factors like food and pharmaceuticals, physical and psychological stress and hormones [2]. It has been shown that most pharmacological activities are related to the immunostimulatory and antioxidant properties of plant secondary metabolites [3]. Immunostimulatory therapy is now being recognized as an alternative to conventional chemotherapy for a variety of disease conditions, involving the impaired immune response of the host. Immunostimulators have been known to support T-cell function, activate macrophages and granulocytes and complement natural killer cells apart from affecting the production of various effector molecules generated by activated cells [4]. Medicinal plants with immunomodulatory effects provide alternative potential to conventional chemotherapy for a variety of diseases, especially in relation to host defense mechanism [5]. Medicinal plants serve as therapeutic alternatives, safer choices or in some cases as the only effective treatment [6]. Plants have been used extensively to increase body’s resistance against infection by potentiating immunity, re-establishing body equilibrium and conditioning of the
body tissues. Many plants which are used as traditional medicines are reported to have immunomodulatory properties. Some of these plants stimulate both cellular and humoral immunity.

_Tectona grandis_ Linn is a large deciduous tree belonging to family Verbenaceae and also known as Teak tree or Sagwan [7]. In ethnomedicine, roots were given in anuria and retention of urine. Bark is astringent, sweet, acrid and used in bronchitis. Wood of the plant is sedative, refrigerant, astringent, diuretic, used in headache, toothache and also subdues the inflammation and irritation of skin [8,9]. Leaves of the plant possess wound healing and antiviral activity. It has been reported that _Tectona grandis_ is effective in the treatment of typhoid fever, E-coli and Pseudomonas infections [10]. This could probably be due to its leucocyte mobilization activity. In the present study, attempt has been made to investigate the immunostimulatory potential of _T. grandis_ leaf extract.

**MATERIALS AND METHODS**

**Plant Material:** Fresh leaves of the plant _Tectona grandis_ were collected from the botanical garden of the University of Nigeria, Nsukka, Enugu State, Nigeria. The leaves were shade-dried for 5 days and then ground to powder from which extracts used in the study were prepared.

**Animals:** Healthy male Wistar albino rats weighing 80-120 grams were used for the study. All animals were housed in an animal house under normal room conditions. A commercial pellet diet and water were fed the animals ad libitum.

**Preparation of Plant Extract:** The dried powdered plant material leaves (10 grams) was dissolved in 100ml of distilled water and left to stand for 24 hours then filtered. The filtrate was concentrated and used for the study.

**Antigen Preparation:** Fresh blood was collected from sheep sacrificed in a local slaughter house and preserved in EDTA bottles. It was washed three times with normal saline via centrifugation. The suspension was adjusted to 1 × 10^8 SRBC/ml for immunization and challenge.

**Treatment Regimen:** Animals were divided into four groups each having four rats. Group I (normal control) received oral administration of water via gavage. The dose volume for this group was 0.3ml. Group II (negative control) received gavage administration of 2% pyrogallol 100mg/kg body weight p.o. Group III received the plant extract at a dose of 100mg/kg body weight (bw) and pyrogallol solution. This group was referred to as the low dose group. The group IV animals received also oral administration of the plant extract of 300mg/kg bw and pyrogallol solution. This group was referred to as the high dose group. All administrations were done daily for 21 days.

**Sheep Red Blood Cell (SRBC)-Induced Humoral Antibody (HA) Titer:** To specifically assess effects on antibody formation, groups of four rats per treatment were immunized with 0.1ml of sheep red blood cell suspensions (1.0 × 10^7 SRBC/ml) intraperitonially. The day of immunization was referred to as day 0. Seven days later (day +7) the rats were challenged by injecting 0.1ml of 1.0 x 10^8 SRBC suspension into the left hind foot pad of the rats. Blood samples were collected from all the animals separately by ocular puncture using glass capillary tubes on day +7 (after challenge) for measurement of primary antibody titer and on day +14 for measurement of secondary antibody titer.

Antibody levels were determined by the method described by Agrawal et al. [8]. After allowing the collected blood to clot, serum was isolated and 25 microL was placed into one well of a 96-well micro titer plate. Serial two-fold dilutions of the serum were made using 25 microL normal saline each time of transfer across the plate. To the 25 microL diluted serum in each well, 25 microL of 1% v/v SRBC suspension in normal saline was added. The micro titer plate was maintained at room temperature for 1 hour and the content then examined visually for haemagglutination. The value of the highest serum dilution showing haemagglutination was defined as the antibody titer for the given Rat.

**Sheep Red Blood Cell-Induced Delayed Hypersensitivity (DTH) Response:** A modified method of Switi et al. [6] was used to analyze effects on DTH responses in the treated rats. Daily treatment with _Tectona grandis_ leaf extract per oral began 14 days prior to the challenge. The negative and normal control rats received water respectively each day. On day 1 all rats were immunized. After 14 days of gavage treatment, 0.1ml of SRBC solution injected subcutaneously into their right hind footpad, the thickness of each rat’s footpad was measured just before the challenge using a Schneltaster caliper that could measure to a minimum unit of 0.01mm. The rats were then challenged by injecting 0.1ml of SRBC solution intraperitonially into their left hind footpad (Deemed time 0). Footpad thickness was the re-measured after 24hrs. The difference between the thickness of the left footpad
just before and 48 hours after challenge (in mm) was taken as a measure of DTH.

**Carbon Clearance:** The method described by Dash et al. [9] was used to analyze phagocytic activity by the white blood cells in rats. For each treatment regimen, a total of 16 rats were utilized. Daily treatment with *Tectona grandis* leaf extract (p.o.) occurred for 14 days prior to the assessment of in situ phagocytic activity. The negative and normal control groups received pyrogallol and water respectively, daily. A colloidal carbon ink suspension was injected via the tail vein into each rat 48 hours after the final treatment. From each rat, blood samples (25ml) were then withdrawn from the retro-orbital plexus under mild ether anesthesia, immediately after the injection and then 15 minutes thereafter. Each blood sample was lysed with 2 ml of 0.1% acetic acid and the absorbance of the resulting solution evaluated at 675 nm. The phagocytic index \( K \), was calculated using the following equation:

\[
K = \frac{(\log \text{OD}_1 - \log \text{OD}_2)}{t_1 - t_2}
\]

Where \( \text{OD}_1 \) and \( \text{OD}_2 \) are the optical densities at time \( t_1 \) and \( t_2 \) respectively.

Total white blood cell (TWBC) count, red blood cell (RBC) count, haemoglobin estimation and packed cell volume (PCV) was determined using standard haematological technique as described by Ochei and Kolharktar [10].

**Statistical Analysis:** Statistical analysis of the data obtained from the experiment was performed using the one way analysis of variance (ANOVA) followed by post HOC LSD test. The significance in the difference was accepted at \( p > 0.05 \). The results are expressed as mean ± SD (standard deviation).

**RESULTS AND DISCUSSION**

Table 1 shows a dose dependent significant increase in humoral antibody formation compared to the negative control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Primary</th>
<th>Secondary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0.67 ± 1.155</td>
<td>4.00 ± 3.464</td>
</tr>
<tr>
<td>Normal Control</td>
<td>4.00 ± 0.000</td>
<td>42.67 ± 18.475</td>
</tr>
<tr>
<td>100mg/kg</td>
<td><em>6.00 ± 2.360</em></td>
<td><em>12.00 ± 5.654</em></td>
</tr>
<tr>
<td>300mg/kg</td>
<td><em>10.00 ± 4.228</em></td>
<td><em>24.00 ± 11.347</em></td>
</tr>
</tbody>
</table>

Table 2 shows a dose dependent significant increase in DTH response compared to the negative control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MEAN ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0.467 ± 0.207</td>
</tr>
<tr>
<td>Normal Control</td>
<td>0.953 ± 0.438</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>0.410 ± 0.021</td>
</tr>
<tr>
<td>300mg/kg</td>
<td>0.155 ± 0.601*</td>
</tr>
</tbody>
</table>

Table 3 shows a significantly \( p < 0.05 \) increased phagocytic activity in Rats treated with different doses of the extract compared to negative control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MEAN ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Clearance</td>
<td>0.067 ± 0.058</td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.133 ± 0.000</td>
</tr>
<tr>
<td>Normal Control</td>
<td>0.113 ± 0.000*</td>
</tr>
<tr>
<td>300mg/kg</td>
<td>0.11 ± 0.000*</td>
</tr>
</tbody>
</table>

Table 4 shows a significant stimulation of the haematopoietic cells by the plant extract at low and high doses of 100 mg/kg and 300 mg/kg body weight.

**DISCUSSION**

In this study, the immunostimulatory activity of aqueous extract of *Tectona grandis* was investigated using rats as experimental model. Goldsby et al. [11] had earlier reported immunostimulatory effect of the ethanolic bark extract of the plant. The humoral response was measured as primary and secondary antibody titers in sheep red blood cells (SRBC) sensitized rats; the cell mediated immune response was measured by a delayed hypersensitivity response.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TWBC</th>
<th>RBC</th>
<th>HB</th>
<th>PCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>2333.33 ± 0.416</td>
<td>143.33 ± 11.547</td>
<td>11.000 ± 0.400</td>
<td>32.00 ± 2.000</td>
</tr>
<tr>
<td>Normal Control</td>
<td>4833.33 ± 0.472</td>
<td>193.33 ± 5.774</td>
<td>12.933 ± 0.416</td>
<td>38.67 ± 1.155</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>3250.53 ± 0.532</td>
<td>185.67 ± 7.07*</td>
<td>12.000 ± 0.283*</td>
<td>38.00 ± 2.121*</td>
</tr>
<tr>
<td>300mg/kg</td>
<td>3400.67 ± 0.147*</td>
<td>255.33 ± 6.36*</td>
<td>14.000 ± 0.910*</td>
<td>42.00 ± 2.828*</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. \( P < 0.05 \) is considered to be significant difference compared to the negative control (n=4). *denotes significant result.
type hypersensitivity (DTH) response in SRBC sensitized rats. The neutrophil recruiting and phagocytic activity of reticulo-endothelial system was measured by the rate of carbon clearance. Equally, the effect of the extract on hematological parameters was also investigated.

In this study, anti-SRBC antibody titers during both primary and secondary responses were found significantly (p<0.05) increased in the aqueous extracts treated rats compared to the untreated control. The activation of the humoral response by aqueous extract of *Tectona grandis* leaf that was noted here could indicate that there was an increased responsiveness of macrophages/B-lymphocytes subsets in these hosts [12].

The rate of *in situ* carbon particle clearance is frequently used as a measure of reticulo-endothelial system (RES) competency. Specifically, a faster removal of the particles is correlated with an enhanced phagocytic activity of RES cellular components. In this study, treatment with the plant extract activated the rate of carbon clearance observed in the treated groups compared to the untreated group [13].

Cell mediated immunity (CMI) involves effectors mechanism carried out by T lymphocytes and their products (lymphokines). CMI responses are critical to defense against infectious organisms, infection of foreign grafts, tumor immunity and delayed type hypersensitivity (DTH) reactions. DTH is known to be initiated by reaction between anti-specific T-cells and the antigen which results in the release of lymphokines that affect a variety of cell types, especially macrophages [14]. The significant (P<0.05) decrease in the DTH responses observed in rats treated with the plant extract suggests anti-inflammatory activity.

Since the plant extract treatments were seen here to give rise to increased circulating antibody titers specifically against the SRBC, it would be expected then that there would be increases induced in levels of one or more of the cell types involved in the humoral response to this antigen.

In this study, the results also showed a significant (p<0.05) increase in the PCV of the rats treated with the extracts compared to the control at both concentrations of 100mg/kg and 300mg/kg. The increase in PCV suggests an increased production of majority of the cells involved in the immune system which are produced from common haemopoietic stem cells found in the bone marrow [15]. Increased production of the immune cells could generally imply an enhanced immune system function.

This study also showed an increase in the red blood cell count and haemoglobin concentration in the extract-treated rats indicating an increased activity of the stem cells of the bone marrow.

**CONCLUSION**

In conclusion, the immunostimulatory potential of *Tectona grandis* leaf extract is established on the basis of its effect on humoral antibody titre, phagocytosis, delayed type hypersensitivity reaction and increased immune cell production.

**REFERENCES**