An in vivo phytolectin induced skin test and T-cell mitogenicity

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Abstract

*Cucemis melo (C.M) Cucemis sativus (C.S) Citrullus vulgaris (C.V) and Citrullus colocynthus (C.C)* seed lectins were separated and partially characterized as judurate positive, have glucose polarity and agglutinate ovine washed 1% erythrocyte suspension. The dose determination for these phytolectin mitogenicity were found equal to a mg /50 gm chicks. The white leghorn Gallus domesticus two days old chicks were found as valid animal model for T cell mitogenicity as wing skin test. The C.M, C.S, C.N and C.C lectin solutions were found mitogenic in wing skin test model. C.S lectin was the best mitogen among the others. This mitogenicity was of simple positive linear type and it was dose dependent. Refadin and tuberculin were found as immunomodulant of cellular immunosuppressive characters. These immunomodulating potentials was dose dependent and of simple linear negative type. The developed test system was helpful as an in vivo test for evaluation of T-cell mitogenicity and cellular immunomodulation.

Introduction

The in vivo phytolectin induced T cell mitogenicity had been proved in Bat foot pad reaction (Christe et al., 2000); Zebra finch chicks wing test (Smits and Williams, 1999) house martin wing test (Christe et al., 2001); other different bird species as tree swallow, Northern pentil, American kestrel and thered legged partridge (Smits et al., 1999). In the present work an attempt was made to investigate the T lymphocyte mitogenicity by the dried seed lectins of: C. sativus, C. melo and C. colocynthus in two days old chicks of white leghorn (Gallus domesticus) using phytohaemagglutinin wing test.

Materials and Methods

1. Lectins

The lectins were separated from dry seeds of the study plants as in Shnawa et al. (2004).

2. Control Mitogens

2-1: Phytohaemagglutinin (PHA)10% solution in a purified form from Iraqi Center for Cancer and Medical Genetics.

2-2: *Vibrio mitichinchovii* from Checoslovak culture collection LPS: The *V. mitichinchovii* LPS was prepared as in Kwapnisk, (1970).

Study Menu

Phytohaemagglutinin Avian skin test system
Lectin Separation Standard lectin

Two days old White Leghorn Chicks Conditioning And Housing

Standarization , modification & development of the system

3. The mitogenic dose:

For each of the test phytolectins, this dose was determined by phytolectin skin test in the bird wing using graded increasing doses of the phytolectin

4. Test system:

Two hundred and fifty of two days old white leghorn *G. domesticus* chicks were the test system for the test lectins. These chicks were brought from the commercial poultry plants in the vicinity of Babylon governorate.
1. Evaluation of skin test in poultry.
2. Evaluation of use of antimitotic substance.
3. The addition of the concept of Mitotic index, to the original model, to ascertain mitogenicity
4. The addition of Lymphoblast formation %, to the original model.
5. Development of the test system to study immunomodulation.

- **Antimitotic Agent:** The antimitotic agent was used in 100 μg/m of the body weight of chick (4 mg/kg) (Kaied, 2002).

- **Mitogenicity:** PHA skin test was made for the separated lectins and controls (Smits and Williams, 1999; Smits et al., 1999).

- **Immunomodulants:** Refadin in concentrations of 50, 40, 30, 20, 10, 5 mg/50 gm were injected intramuscularly (IM), 2 hr before phytolectin injections for wing skin test in the test chicks. Tuberculin, however, was used in 3, 2, and 1 unit was intramuscularly injected (IM) to the test chicks 2 hr before the lectin injections for skin test (Shnawa and Al-Shahery, 2001; Lendsey et al., 1980; Al-Khafaji, 2005).

- **Scoring Results:** For each of the treatments, five replicates were made. Skin test results were scored as erythema and induration in mm and as a mean of five readings. Mitogenicity versus blastogenicity were scored as percentages to the total lymphocyte numbers on two replicate stained film of cardiac blood on an average of 10 microscopic fields (Kaied, 2002).

No. of Dividing lymphocytes

\[ \text{Mitogenicity} \% = \frac{\text{No. of Dividing lymphocytes}}{\text{Total No. of lymphocytes}} \times 100 \]

\[ \text{Blastogenicity} \% = \frac{\text{No. of lymphoblasts}}{\text{Total No. of lymphocytes}} \times 100 \]
Statistics Linear regression analysis for the analysis of the data was done as in (Dawood and Alyas, 1991).

Results

I. Mitogenicity responses

The standard phytohaemagglutinin was found to be mitogenic; at 0.25 mg/ml (table 1) and in (table 2), *V. mitichinchovii* LPS induces mitogenic response of 0.51 at conc 0.25. The mitogenic dose determination showed that the minimal mitogenic conc was one mg/50 gram chick body weight (table 3). There was gradual increase of T lymphocyte mitogenicity as the lectin concentrations was increased as in C.S , C.C , C.M and CN lectins . The relationship was suggestive for linear correlation . The linear simple regression analysis were showing, simple linear positive correlations. The skin reaction was ranged from 0.8 to 1.6, 0.7 to 1.2, 0.9 to 1.8, 0.8 to 1.2 for C.M, C.C, C.S and CN lectins respectively . The mitotic index was ranged from 0.049 to 0.374 , 0.075 to 0.278 , 0.212 to 0.418 and 0.157 to 0.287 for C.M, C.C, C.S and CN accordingly, the blastogenic responses were ranged from 0.016 to 0.0715, 0.7 to 0.32, 0.02 to 0.096 and 0.01-25 to 0.063 respectively. The skin reaction the mitogenicity and blastognicity were rather analogous to that of PHA 2.5 mg/50 gram chick body weight (table 2).

Table (1): The skin reaction mitotic index and lymphoblast % of newly hatched Leghorn chicks to test lectins and LPS

<table>
<thead>
<tr>
<th>Lectin or mitogen</th>
<th>Skin Reaction</th>
<th>Mitotic Index</th>
<th>Lymphoblast %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con. mg/ml</td>
<td>m m induration</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4±0.373</td>
<td>0.096±031</td>
</tr>
<tr>
<td>C.S</td>
<td>3.648</td>
<td>0.7±0.199</td>
<td>0.431±014</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.6±0.244</td>
<td>0.387±0.422</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>Y=82.51+ (46.99)X</td>
<td>Y=102.88+(72.76)X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r=-0.54</td>
<td>r=-0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td>1.0</td>
<td>1.1 ±0.244</td>
<td>0.025 ± 0.0022</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.70±0.199</td>
<td>0.032 ± 0.0015</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>1.90±0.186</td>
<td>0.05 ± 0.0029</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y=2.0 +(0.91 ) X</td>
<td>Y=4.78±(-9.001 ) X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r=-0.99</td>
<td>r=0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHA</td>
<td>1.0</td>
<td>1.0±0.11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.2±0.122</td>
<td>0.125 ± 0.0023</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>2.0±0.055</td>
<td>0.037 ± 0.0026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y=1.451 +(6.0)X</td>
<td>Y=0.1394(18.8) X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r=-0.99</td>
<td>r=0.92</td>
</tr>
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<td></td>
</tr>
</tbody>
</table>
II – Influence of Refadin and Tuberculin on Mitogenicity

It was evident that there were a gradual decrease in C.S lectin mitogenicity wing skin test as the concentration of Refadin and tuberculin were increased (Table 3). The dose response relationship was suggestive for simple linear type for Refadin as in the followings:
The C.S lectin at 3.648 mg showed skin test of 1.8. The preconditioning with 50, 40, 30, 20 and 10 mg of Refadin followed by 0.1 ml of Cs the lectin showed skin test 1.9, 2, 2.1, 2.9 and 2.5 for the abovementioned concentration respectively. The mitogenicity was parallel to the studied Refadin concentrations which were 0.525, 0.547, 0.573, 0.79, and 0.886. The blastogenic responses were 0.016, 0.025, 0.039, 0.047 and 0.05 for 50, 40, 30, 20, at 10 mg Refadin accordingly; thus Refadin at concentration of 20, 10 mg induce increase in skin reaction & mitogenicity. The tuberculin at con. 3 units (U) suppress the blastogenicity and mitotic index. The preconditioning of chicks with 3, 2 and 1 unit tuberuein following by C.S lectin skin test gave skin reaction values as 1.8 , 2.1 and 2.2 for 3, 2 and 1 tuberculin unit , likewise, mitogenicity was 0.484, 0.726, 0.83 for 3,2,1 unit of tuberculin respectively. These mean values were higher than those for PHA and LPS and equivalent to that of C.S alone.
The mitogenicity and blastogenicity was increased as the con of tuberculin was decreased. Tuberculin at the concentrations of 2 U and I U induce an increase in skin test & mitogenicity as compared to C.S alone. Conversely; at those concentrations blastogenicity appeared to be suppressed as compared to C.S alone.

Table (2): The skin reaction, mitotic index and lymphoblast % of newly hatched Leghorn chicks for the test lectins and control mitogens.

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Conc mg/ml</th>
<th>Skin test</th>
<th>Mitotic index</th>
<th>lymphoblast %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.S</td>
<td>3.648</td>
<td>1.8 ± 0.373</td>
<td>0.418 ± 0.058</td>
<td>0.096 ± 0.1026</td>
</tr>
<tr>
<td></td>
<td>1.825</td>
<td>1.2 ± 0.353</td>
<td>0.23 ± 0.031</td>
<td>0.047 ± 0.016</td>
</tr>
<tr>
<td></td>
<td>0.912</td>
<td>0.9+0.244</td>
<td>0.212 ± 0.072</td>
<td>0.02 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>Y = -1.82 + 3.03X</td>
<td>r=0.96</td>
<td>Y = 0.164 +36.13X</td>
</tr>
<tr>
<td>C.M</td>
<td>5.565</td>
<td>1.6 ± 1.323</td>
<td>0.374 ± 0.063</td>
<td>0.0715 ± 0.036</td>
</tr>
<tr>
<td></td>
<td>2.78</td>
<td>1.2±0.199</td>
<td>0.108 ± 0.73</td>
<td>0.025 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.8 ± 0.199</td>
<td>0.049 ± 0.078</td>
<td>0.016 ± 0.021</td>
</tr>
<tr>
<td></td>
<td>1.39</td>
<td>Y=59000.7 +</td>
<td>6989X</td>
<td>X=r=0.86</td>
</tr>
<tr>
<td>C.V</td>
<td>1.673</td>
<td>1.2±0.12</td>
<td>0.287 ± 0.007</td>
<td>0.63 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>0.836</td>
<td>1.0±0.35</td>
<td>0.248 ± 0.001</td>
<td>0.23± 0.005</td>
</tr>
<tr>
<td></td>
<td>0.418</td>
<td>0.8±0.12</td>
<td>0.157 ± 0.002</td>
<td>0.012± 0.0041</td>
</tr>
<tr>
<td></td>
<td>r=0.98</td>
<td>Y= -19477.7 +</td>
<td>19477.7+6989X</td>
<td>r=0.988</td>
</tr>
<tr>
<td></td>
<td></td>
<td>63212=01X</td>
<td>r=0.98</td>
<td>Y=r=0.991</td>
</tr>
<tr>
<td>C.C</td>
<td>1.115</td>
<td>1.2±0.112</td>
<td>0.278 ± 0.008</td>
<td>0.32±0.0008</td>
</tr>
<tr>
<td></td>
<td>0.557</td>
<td>1.0± S</td>
<td>0.115 ± SE</td>
<td>0.26±0003</td>
</tr>
<tr>
<td></td>
<td>0.278</td>
<td>0.7±0.199</td>
<td>0.075 ± 0.199</td>
<td>0.7 ± 0.0005</td>
</tr>
<tr>
<td></td>
<td>r=0.953</td>
<td>Y=0.009+1.61X</td>
<td>0.04+391X</td>
<td>Y=0.023+2.84X</td>
</tr>
</tbody>
</table>
### Table (3):

The dose dependent immunomodulating effect of Refadin (A) and Tuberculin (B) on phytohaemagglutinin skin reaction, mitotic index and lymphoblast % of white Leghorn 2 days old chicks.

<table>
<thead>
<tr>
<th>Test modulant</th>
<th>Skin Reaction mm</th>
<th>Mitotic Index</th>
<th>Lymphoblast %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Refadin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>1.9 ± 0.099</td>
<td>0.52 ± 0.009</td>
<td>0.016 ± 0.0021</td>
</tr>
<tr>
<td>40 mg/ml</td>
<td>2.0 ± 0.157</td>
<td>0.547 ± 0.011</td>
<td>0.025 ± 0.0015</td>
</tr>
<tr>
<td>30 mg/ml</td>
<td>2.1 ± 0.99</td>
<td>0.573 ± 0.009</td>
<td>0.039 ± 0.002</td>
</tr>
<tr>
<td>20 mg/ml</td>
<td>2.4 ± 0.099</td>
<td>0.79 ± 0.004</td>
<td>0.47 ± 0.00125</td>
</tr>
<tr>
<td>10 mg/ml</td>
<td>2.5 ± 0.157</td>
<td>0.886 ± 0.002</td>
<td>0.5 ± 0.0002</td>
</tr>
<tr>
<td></td>
<td>Y=160.14 + (-59.7) X</td>
<td>r = -0.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y=90.21 + (70.65) X</td>
<td>r=0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y=34.602 +(-333.35)X</td>
<td>r=-0.41</td>
<td></td>
</tr>
<tr>
<td>(B) Tuberculin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 U</td>
<td>1.8 ± 0.373</td>
<td>0.484 ± 0.007</td>
<td>0.03 ± 0.001</td>
</tr>
<tr>
<td>2 U</td>
<td>2.1 ± 0.244</td>
<td>0.726 ± 0.005</td>
<td>0.05 ± 0.0002</td>
</tr>
<tr>
<td>1 U</td>
<td>2.2 ± 0.199</td>
<td>0.87 ± 0.016</td>
<td>0.06 ± 0.0004</td>
</tr>
<tr>
<td></td>
<td>Y= 11.38 + (-4.61) X</td>
<td>r=-0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y=5.73 + (-5.48) X</td>
<td>r=-0.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y=5.017 +(-5.12) X</td>
<td>r=-0.98</td>
<td></td>
</tr>
<tr>
<td>PHA</td>
<td>0.25 mg/ml</td>
<td>2.0</td>
<td>0.571</td>
</tr>
</tbody>
</table>
Discussion

The separated phytolectins from the test plant seeds were found mitogenic at milligram levels (Table 1), such finding may be due to partial purity of these preparations, since more concentration needed in partial purity (Shnawa et al., 2004; Sadana 1998). The mitogenicity of the test phytolectins were found arranged from higher to lower as: C.S, C.M, C.V, C.C (Table 2). The best of which was C.S mitogenic lectin (This result matched with the results of Oppenheim, 1976). There were linear simple correlation between concentration (dose) and mitogenicity (Table 1,2) such findings were in parallel to that of (AL-Giebori 2003). Thus, the two days old chick of white leghorn G. domesticus appeared to be valid laboratory animal model for testing the in vivo T-cell mitogenicity as that for other avian models (Christe et al., 2001, Smits et. al., 1999) and bat foot pad reaction (Christe et al., 2000) meantime, this model is being proved as an emerged avian model for testing immunomodulating potential of materials (table 3). Refadin and tuberculin were found to be as a dose dependent cellular immunomodulants, since at low concentration, they were acting as potentiating agents, where as at high concentration, they were of immunosuppressive potentials (AL-Giebori, 2003; Kackmer and Mikula, 1999, Lendsey et al. 1980; Roth and Flaming 1999). The immuno suppressive effect at high concentrations of Refadin and tuberculin can be attributed to T cell mitogenic arrests (Munner et.al., 1988; Kackwer and Mikula., 1999) in conclusion one may state:

1. C. sativus seed lectin was of best mitogenic activity
2. Confirming that two days old chicks of white Leghorn G. domesticus is an in vivo model for mitogenicity of T lymphocyte.
3. Presenting a new avian model for testing cellular immunomodulant.
4. Both T cell mitogenecity & cellular immunomodulating responses were dose dependant and of linear simple correlation, especially when C.sativum

<table>
<thead>
<tr>
<th></th>
<th>LPS 0.25 mg/ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1.9</td>
<td>0.51</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>C.S</td>
<td>3.648</td>
<td>1.8</td>
<td>0.418</td>
<td>0.096</td>
</tr>
</tbody>
</table>
seed lectin was used as a test lectin.

References


68:57- 79.