TOXIC EFFECTS OF A NON-POLAR EXTRACT OF THE LEAVES OF ARTOCARPUS ALTILIS (PARK) FOLLOWING SUBACUTE ADMINISTRATION TO RATS


Department of Natural Product Research, Scientific Research Council, University of the West Indies, Mona, Faculty of Medical Sciences, Kingston, Jamaica

ABSTRACT

Intraperitoneal injection of hexane soluble compounds from the leaves of Artocarpus altillis lowered the following haematological parameters in rats: white blood cell counts, thrombocytes, and haemoglobin significantly (P < 0.05). Plasma analysis also revealed a significant lowering of total bilirubin, aspartate aminotransferase and alkaline phosphatase.

Histological examination of the liver, kidney and lung revealed extensive erythrocytic aggregation. Prominent peribronchial and perivascular lymphatic hyperplasia were seen in the lungs. Marked follicular hyperplasia were seen in the spleen. The present report has highlighted some of the toxic effects of the oil of the breadfruit leaves.

INTRODUCTION

Artocarpus altillis (Park.) breadfruit, is a tropical fruit which is a stable food for many people in the West Indies and the Pacific islands (Purseglove, 1968). Wong (1976) reported the folkloric usage of an infusion prepared from the young fruit rind, yellow leaf and latex by West Indians for alleviating oliguria, hypertension and pain, respectively.

It was only recently that scientists started investigating the medicinal, toxicological and pesticidal properties of extracts of A. altillis leaves. So far nematocidal, antifungal, negative chronotropic and haemolytic activities have been reported (Robinson et.al., 1990; Williams et.al., 1990; Williams 1991; Young et. al., 1992). Recent studies carried out by Young et.al. (1992 submitted) revealed extensive haemolysis, leading to massive intravascular haemolysis following acute intravascular administration of a medium polar extract. These findings have prompted us to investigate further the effects of these leaf extracts on several blood and plasma parameters.
MATERIALS AND METHODS

Preparation of Extract

Fresh leaves were collected from A. altillis and air dried in the laboratory for 2 weeks. Leaves weighing 150 g were crushed and extracted with hexane for 5 days. The extract was decanted and treated with activated charcoal at 1 g / 150 ml of extract to remove chlorophyl and other pigments. The extract was then filtered using a Whatman No. 201 filter paper and hexane removed in a rotary evaporator under reduced pressure leaving a dark brown oily residue. The concentrated oil was divided into 5 ml aliquots and placed in rotary evaporator under reduced pressure on a water bath set at 60°C until free of solvent odour.

The extract was mixed with dimethyl sulfoxide (DMSO) in a ratio of 1:1 (v/v) for administration into rats.

Bioassay

Administration of extract

Extract was administered to adult Wistar rats (300 - 320 g) intraperitoneally at a dose of 200 mg/Kg (0.2 ml) three times per week, on alternate days for two weeks. Control animals were treated with 0.2 ml of 50% DMSO in physiological saline. Five animals were used in each treatment.

Toxicity studies

Rats were sacrificed after 2 weeks by cervical dislocation and blood withdrawn via cardiac puncture. The values of the following haematological parameters: haemoglobin (Hb), red blood cell (RBC), lymphocytes (Ly) and mean concentration of haemoglobin per cell (MCHC) were determined using a Coulter Counter T-890. The concentration of the following plasma constituents: total proteins, albumin, total globulins, bilirubin, aspartate aminotransferase (AST) = glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase (ALK) were also determined in control and treated rat samples using automated methods as described by Singh et al., (1992).

The following organs were collected following sacrifice: liver, spleen, heart, kidney and lungs. Organs were fixed in 10% formaldehyde for 72 hours then embedded in paraplast. Sections of 10 um thickness were cut on a Reichert Microtome and stained in haematoxylin and eosin for routine histological examination.

Statistical analysis

Mean percentages of two treatments were separated using Student t-test (α 0.05) following Arc Sin transformation (Zar, 1974).
Williams, et al.: Toxic Effects of a Non-polar Extract of the Leaves of Artocarpus Altilis

RESULTS

Results presented in Table 1 revealed that the hexane soluble compounds of A. altilis leaves produced a significant (P < 0.05) lowering of total bilirubin, alkaline phosphatase (ALK) and aspartate aminotransferase (AST) contents in rats. There was significant correlated lowering of the levels of total protein and globulin (P < 0.05). The levels of albumin was not significantly altered.

The content of four haematological parameters: platelets, white blood cells, lymphocytes and haemoglobin were lowered significantly (P < 0.05) by the following percentages 50, 32.8, 30.0 and 11.2% respectively (Table 2). Red blood cell counts were lowered, but not significantly at P = 0.05.

Histological examinations of the kidney revealed extensive leakage of erythrocytes which aggregated into the renal interstitial spaces (Plate 1). Post mortem examinations of rats following sacrifice revealed splenomegaly in 75%. Examination of sections of the spleens revealed follicular hyperplasia.

The lungs showed marked perivascular and bronchial lymphatic hyperplasia obliterating the normal architecture of the bronchial walls. Evident also was erosion of the smooth muscle lining the bronchioles.

Examination of liver sections showed enlargement of the central veins along with red blood cells aggregations.

DISCUSSION

Reduction in the levels of bilirubin and immunoglobulins suggest that the extract is capable of affecting the reticulo endothelial and the immune system. Similarly, reduction in the values of AST and ALK indicate the inhibitory actions of the extract on these enzymes.

The extensive aggregation of erythrocytes found in the kidney and liver suggest a reduction in the viscosity of the blood passing through these organs. The sticking of red blood cells could be due to alterations in the properties of the erythrocytes surfaces. These erythrocytic aggregations also indicates a possible reduction in the microcirculatory efficiency throughout these organs (Bowman and Rand, 1980).

The significant lowering of the following haematological parameters: platelets, white blood cells, and haemoglobin suggest a case of toxicity similar to that found in benzene poisoning (Cornish, 1975).

The extract reduced the number of red cells, which were extensively aggregated. These conditions are seen in autoimmune haemolytic anaemia. The extract seems to reduce some bone marrow precursors which resulted in the lowering of leucocyte and thrombocyte numbers. The 75% splenomegaly found in treated animals could be linked to an enlarged splenic platelet pool (Castle, 1982). The increase in splenic germinal centres is characteristic of an immune response.
Prominent peribronchial and perivascular lymphatic hyperplasia were present in the lungs. This could be related to an allergic reaction either to the oil or its metabolitic products (Singh, et. al., 1992). Evident also was erosion of the bronchial smooth muscle which is similar to those produced by ingested rape, Brassica napus forage (Kingsbury, 1975). It is very likely that the extract caused alteration of lung phospholipid fatty acid profile (Archer et.al., 1987) leading to a possible disruption of the pulmonary surfactant, and possibly affecting the compliance and capacity of the lung.

ACKNOWLEDGEMENT

We are grateful to Dr. M. Green (Department of Pathology), Prof. M. West (Department of Pharmacology) and Prof. R. Young (Department of Physiology) for reading the manuscript and providing suggestions for improvement.

REFERENCES


Table 1 Data on the effects of extracts of *Artocarpus altilis* on some plasma parameters in rats.

<table>
<thead>
<tr>
<th></th>
<th>TP $^1$ (g/L)</th>
<th>Alb $^2$ (g/L)</th>
<th>Glob $^3$ (g/L)</th>
<th>Tbil $^4$ (umol/L)</th>
<th>ALK $^5$ (IU/L)</th>
<th>AST $^6$ (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>72.1±</td>
<td>31.2±</td>
<td>41.3±</td>
<td>11.8±</td>
<td>234.0±</td>
<td>354.2±</td>
</tr>
<tr>
<td></td>
<td>1.2a</td>
<td>0.7a</td>
<td>1.4a</td>
<td>1.5a</td>
<td>31.9a</td>
<td>37.6a</td>
</tr>
<tr>
<td>TREATED</td>
<td>62.6±</td>
<td>29.8±</td>
<td>32.8±</td>
<td>7.8±</td>
<td>48.6±</td>
<td>187.8±</td>
</tr>
<tr>
<td></td>
<td>2.6a</td>
<td>1.3a</td>
<td>4.3b</td>
<td>2.5b</td>
<td>3.2b</td>
<td>4.3b</td>
</tr>
</tbody>
</table>

1. TP = Total Protein; 2. Alb. = Albumin; 3. Glob = Globulin
4. Tbil = Total Bilirubin; 5. ALK = Alkaline phosphatase; 6. AST = Aspartate aminotransferase.

Means in a column with same letters are not significantly different from each other (P < 0.05).
Table 2 Data on the effects of *Artocarpus altilis* on some haematological parameters in rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CONTROL</th>
<th>TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>16.37±</td>
<td>14.5±</td>
</tr>
<tr>
<td>RBC (x10¹²/L)</td>
<td>8.42±</td>
<td>7.51±</td>
</tr>
<tr>
<td>Platelets (x10⁹/L)</td>
<td>162.7±</td>
<td>82.7±</td>
</tr>
<tr>
<td>WBC (x10⁹/L)</td>
<td>15.3±</td>
<td>10.33±</td>
</tr>
<tr>
<td>Ly (x10⁹/L)</td>
<td>9.9±</td>
<td>6.9±</td>
</tr>
<tr>
<td>MCHC (x10⁹/L)</td>
<td>33.1±</td>
<td>33.9±</td>
</tr>
</tbody>
</table>

6. MCHC = Mean Concentration of Haemoglobin per Cell

Means in a column with same letters are not significantly different from each other (P<0.05).
Plate 1. Hematoxylin-Eosin stained section of the kidney fixed in formol-saline, note (a) aggregation of erythrocytes into vascular space x 480.