Toxicological Evaluation of Annotemoyin-1
Isolated from Annona squamosa Linn. on Long Evan's Rats

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Abstract: The potential toxicity of annotemoyin-1 isolated from the seeds of Annona squamosa Linn., collected from the relevant areas of Bangladesh was evaluated on Long Evan's rats. Annotemoyin-1 (100 µgm and 200 µgm) was administered daily for 14 days and the effects on body weight, hematological and biochemical parameters of the blood and histopathological parameters of heart, kidney, lungs and liver were studied. There was no significant difference between weight gain in rats receiving annotemoyin-1 and control rats. The changes of hematological and biochemical parameters were statistically insignificant. No abnormalities were found in the histopathological parameters of heart, kidney, lungs and liver of the experimental groups of rats when compared with control groups of rats. From this study, it was inferred that annotemoyin-1 (100 µgm and 200 µgm) over 14 days, had no toxic effect on rats.

Key words: Sub-acute toxicity, annotemoyin-1, Annona squamosa Linn

INTRODUCTION

Annona squamosa Linn., is a fruit bearing small tree and is found to grow everywhere in Bangladesh (Ghani, 1998, Prain, 1965). It is locally credited with medicinal importance and has found uses in folk medicine as a cure for various diseases. The root is considered as a drastic purgative. An infusion of the leaves is considered efficacious in prolapsus ani of children. The crushed leaves are sniffed to overcome hysteria and fainting spells, they are also applied on ulcers and wounds. The ripe fruits of this plants are applied to malignant tumors to hasten suppuration. The dried unripe fruit powder is used to destroy vermin. The seeds are acrid and poisonous. Powdered seeds and also powdered dried fruits serve as fish poison and insecticides. A paste of the seed powder has been applied to the head to kill lice. If applied to the uterus, it induces abortion. Heat-extracted oil from the seeds has been employed against agricultural paste. It is also used for destroying worms in the wounds of cattle (Kirtikar and Basu, 1993). The compound annotemoyin-1 has been isolated from the chloroform extract of the seeds of Annona squamosa and shows significant cytotoxic anti-tumor, anti-bacterial and anti-fungal activity (Parvin, 2002). The purpose of this study was to evaluate the toxicological effect of annotemoyin-1 isolated from Annona squamosa.

MATERIALS AND METHODS

Plant materials: The matured seeds of the plant Annona squamosa Linn., were collected during the month of September-October, 2000 from the adjoining areas of Rajshahi district and were identified by the Bangladesh National Herbarium (Specimen No. 29, 544). The seeds were sun dried and pulverized in to a coarse powder.

Extraction and isolation: The plant materials were extracted in cold with absolute alcohol and then fractionated with pet-ether and chloroform. The chloroform soluble fraction was subjected to column chromatography and the pure compound annotemoyin-1 was isolated by solvent washing followed by preparative thin layer chromatography (PTLC) and was characterized by Mass, 1H-NMR, 13C-NMR, HMBC, 1H-1H COSY 45° (Center for Phytochemistry, Southern Cross University, Australia), and IR and UV spectroscopy (Rajshahi University). The spectroscopic data were identical with the reported data for annotemoyin-1 isolated from the plant Annona atemoya by Duret et al. (1996).

Animals: For sub-acute toxicity studies, sixteen adult male Long Evan's rats (Animal Resources Branch of the International Center for Diarrhoeal Research, Bangladesh) were weighed and placed into four groups each group containing four rats. The rats were kept in numbered iron
Table 1: Effect of compound Annotemin-1 on body weight of rats after intraperitoneal administration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (µg)</th>
<th>Body weight (gm)</th>
<th>% Change</th>
<th>Calculated t value</th>
<th>t value at 5% level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>of rats</td>
<td>Before drug treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=4, M±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>100 µl of vehicle</td>
<td>103.7±3.86</td>
<td>+5.78</td>
<td>+2.26</td>
<td>2.447</td>
</tr>
<tr>
<td>B</td>
<td>200 µl of vehicle</td>
<td>112.5±1.34</td>
<td>+16.49</td>
<td>+4.69</td>
<td>2.447</td>
</tr>
<tr>
<td>C</td>
<td>100 µg of Annotemin-1</td>
<td>109.7±3.56</td>
<td>+5.78</td>
<td>+2.26</td>
<td>2.447</td>
</tr>
<tr>
<td>D</td>
<td>200 µg of Annotemin-1</td>
<td>112.5±1.34</td>
<td>+16.49</td>
<td>+4.69</td>
<td>2.447</td>
</tr>
</tbody>
</table>

Table 2a: Effect of compound Annotemin-1 on hematological profile of control and experimental rats at dose 100 µg rat⁻¹ day⁻¹

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Control rats (M±SD)</th>
<th>Experimental rats (M±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count (million/cu.mm)</td>
<td>4.6±0.40</td>
<td>0.10</td>
</tr>
<tr>
<td>WBC count (thousand/cu.mm)</td>
<td>6.7±0.24</td>
<td>0.14</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>48.3±1.47</td>
<td>1.47</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>47.5±2.04</td>
<td>2.04</td>
</tr>
<tr>
<td>Monocytes</td>
<td>4.5±0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>4.7±0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Platelet count (per cu.mm)</td>
<td>350±000</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 2b: Effect of compound Annotemin-1 on hematological profile of control and experimental rats at dose 200 µg rat⁻¹ day⁻¹

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Control rats (M±SD)</th>
<th>Experimental rats (M±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count (million/cu.mm)</td>
<td>4.6±0.40</td>
<td>0.10</td>
</tr>
<tr>
<td>WBC count (thousand/cu.mm)</td>
<td>6.7±0.24</td>
<td>0.14</td>
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<tr>
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<td>Eosinophils</td>
<td>4.7±0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Platelet count (per cu.mm)</td>
<td>350±000</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 3a: Effect of compound Annotemin-1 on biochemical parameters of control and experimental rats at a dose of 100 µg rat⁻¹ day⁻¹

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control rats (Group A) n=4, M±SD</th>
<th>Experimental rats (Group C) n=4, M±SD</th>
<th>% Change</th>
<th>Calculated t values</th>
<th>t values at 5% level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT (IU L⁻¹)</td>
<td>8.75±0.82</td>
<td>9.0±0.70</td>
<td>+2.85</td>
<td>+0.68</td>
<td>2.447</td>
</tr>
<tr>
<td>SGOT (IU L⁻¹)</td>
<td>10.0±0.70</td>
<td>10.5±0.5</td>
<td>+5.0</td>
<td>+1.16</td>
<td>2.447</td>
</tr>
<tr>
<td>Bilirubin µg dl⁻¹</td>
<td>0.3±0.04</td>
<td>0.3±0.04</td>
<td>+0.04</td>
<td>+0.37</td>
<td>2.447</td>
</tr>
<tr>
<td>ALP (IU L⁻¹)</td>
<td>0.4±0.27</td>
<td>0.4±0.27</td>
<td>+0.27</td>
<td>+0.73</td>
<td>2.447</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.5±0.01</td>
<td>0.5±0.01</td>
<td>0</td>
<td>0</td>
<td>2.447</td>
</tr>
<tr>
<td>Blood urea (mMol L⁻¹)</td>
<td>17.7±0.82</td>
<td>16.9±1.8</td>
<td>-0.86</td>
<td>-1.46</td>
<td>2.447</td>
</tr>
</tbody>
</table>

Table 3b: Effect of compound Annotemin-1 on biochemical parameters of rats blood at a dose of 200 µg rat⁻¹ day⁻¹

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control rats (Group A) n=4, M±SD</th>
<th>Experimental rats (Group C) n=4, M±SD</th>
<th>% Change</th>
<th>Calculated t values</th>
<th>t values at 5% level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT (IU L⁻¹)</td>
<td>8.75±0.82</td>
<td>9.25±0.82</td>
<td>+14.28</td>
<td>+2.44</td>
<td>2.447</td>
</tr>
<tr>
<td>SGOT (IU L⁻¹)</td>
<td>10.0±0.70</td>
<td>11.0±0.7</td>
<td>+10.0</td>
<td>+2.02</td>
<td>2.447</td>
</tr>
<tr>
<td>Bilirubin µg dl⁻¹</td>
<td>0.3±0.04</td>
<td>0.3±0.04</td>
<td>+0.04</td>
<td>+0.37</td>
<td>2.447</td>
</tr>
<tr>
<td>ALP (IU L⁻¹)</td>
<td>0.4±0.27</td>
<td>0.5±0.27</td>
<td>+0.27</td>
<td>+0.73</td>
<td>2.447</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.5±0.01</td>
<td>0.5±0.01</td>
<td>0</td>
<td>0</td>
<td>2.447</td>
</tr>
<tr>
<td>Blood urea (mMol L⁻¹)</td>
<td>17.7±0.82</td>
<td>17.5±1.8</td>
<td>-0.86</td>
<td>-1.46</td>
<td>2.447</td>
</tr>
</tbody>
</table>

M₁ and M₂ = Sample mean value; SD₁ and SD₂ = Standard deviations; n = Number of rats; + = Increase; - = Decrease
Table 4: Histopathological studies after treatment with compound of Annotemoin-1 at a dose level of 100 µg rat⁻¹ day⁻¹ and 200 µg rat⁻¹ day⁻¹

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (µg)</th>
<th>Histopathological changes observed</th>
<th>Liver</th>
<th>Heart</th>
<th>Lung</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg rat⁻¹ day⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>100 µl vehicle</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>200 µl vehicle</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>100 µg of Annotemoin-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>200 µg of Annotemoin-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Means no change

Cages individually and were supplied with a basal diet (Hawk et al., 1954). The rats were kept under observation for 14 days before drug administration.

**Administration:** Annotemoin-1 was dissolved in water with Tween-20 and administered intraperitoneally at two different doses (100 µg rat⁻¹ day⁻¹ and 200 µg rat⁻¹ day⁻¹) for 14 consecutive days to two different respective groups. The 1st and 2nd groups received only water and served as control groups.

**Experimental procedure:** A measured amount of fresh food was supplied daily at a fixed time and the general well-being and behavior of the animals was observed daily, throughout the study. For the hematological study, the blood was drawn from the tail vein of four groups before drug administration, at 7 days and after the animals were sacrificed at the end of the experiment, to estimate total and differential blood count, platelet count and percent Hb. For the biochemical study, blood was collected after death at 14th day from the jugular veins of each of the animals. Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Serum alkaline phosphatase (SAP), urea, uric acid and creatinine were determined using standard procedures and reagents supplied by Boehringer Mannheim GmbH Diagnostica (King et al., 1934, Reitman et al., 1957, Fawcett et al., 1960, Coulomb et al., 1963). Histopathological studies of the liver, kidney, heart and lung were performed using a haematoxylin and eosin stain and D.P.X. mounting fluid. The samples were observed under a microscope at the Department of Pathology, Rajshahi Medical College Hospital, Rajshahi, Bangladesh.

**Statistical analysis:** Results are presented as the mean±SD. Student’s t test was used for the comparison between the experimental and control group. A p<0.05 value was considered statistically significant.

**RESULTS AND DISCUSSION**

**Gross general observation:** Rats of different groups showed no signs of tremor, convulsion and reflex abnormalities. No muscular numbness of the hind and fore legs, salivation or diarrhea was observed. However, the body weights of all the rats were increased after the administration of the compound which was statistically insignificant (Table 1).

**Hematological profiles:** Table 2a and b show the hematological profiles of the experimental rats. All hematological parameters were found to be within the normal limits in both experimental and control animals. Therefore, the compound had no toxic effect on hematological parameters.

**Biochemical parameters of blood:** The results shown in Table 3a and b indicated no significant difference for all biochemical parameters between experimental and control animals indicating that annotemoin-1 had no adverse effects on liver and kidney functions.

**Histopathological studies:** After 14 days of drug administration, the animals of both control and experimental groups were killed and the liver, kidney, lungs and heart were examined histopathologically under microscope. No abnormalities were shown in the cellular structure (Table 4).

**ACKNOWLEDGMENT**

The author wish to acknowledge the Department of Pathology, Rajshahi Medical College, Rajshahi, Bangladesh for the pathological test.

**REFERENCES**
