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Toxicological Evaluation of Annotemoyin-1 Isolated from *Annona squmosa* 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

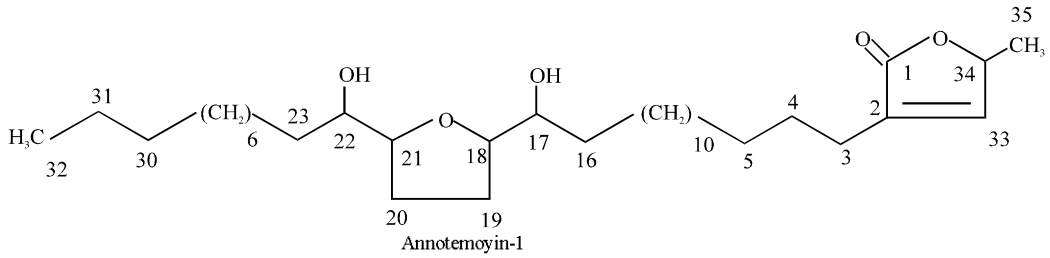
Annona squamosa Linn., is a fruit bearing small tree and is found to grow everywhere in Bangladesh (Ghani, 1998, Prain, 1963). It is locally credited with medicinal importance and has found uses in folke medicine as a cure for various diseases. The root is considered as a drastic purgative. An infusion 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 poisoinous. 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, ^{1}H-NMR, ^{13}C-NMR, HMBC, ^{1}H-^{1}H 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



Annotemoyin-1

Table 1: Effect of compound Annotemoyin-1 on body weight of rats after intraperitoneal administration

Groups of rats	Dose (i.p) μg rat^{-1} day^{-1}	Body weight (gm) Before drug treatment (n=4), M_1±SD_1	Body weight (gm) Before drug treatment (n-4), M_2±SD_2	% Change	Calculated t value	t value at 5% level of significance
A	100 μl of vehicle	103.75±3.96	109.75±3.56	+5.78	+2.26	2.447
B	200 μl of vehicle	112.125±1.34	130.625±5.56	+16.49	+6.49	2.447
C	100 μg of Annotemoyin-1	109.50±1.80	111.25±1.09	+1.59	+2.33	2.447
D	200 μg of Annotemoyin-1	114.00 ±1.58	115.00±1.87	+0.877	+0.58	2.447

Table 2a: Effect of compound Annotemoyin-1 on hematological profile of control and experimental rat at dose 100 μgm rat^{-1} day^{-1}

	Control rats M_1±SD_1			Experimental rats M_2±SD_2		
Hematological parameters	1st day	7th day	14th day	1st day	7th day	14th day
RBC count (million/cu.mm)	4.62±0.10	4.92±0.08	4.97±0.010	4.92±0.19	4.8±0.15	4.97±0.08
WBC count (thousand/cu.mm)	6.74±0.14	6.82±0.17	6.85±0.22	6.67±0.23	6.9±0.22	6.87±0.25
Neutrophil	48.14±1.47	48.0±1.40	46.0±0.70	62±1.8	63.5±1.1	64.25±2.0
Lymphocyte	47.75±2.04	47.25±1.08	50.75±0.82	31.75±2.4	31.5±1.1	32±1.4
Monocyte	4.0±0.76	4.25±0.43	4.0±0.70	3.25±0.82	2.5±0.7	2.25±0.82
Eosinophil	4.75±0.43	4.25±0.85	4.00±1.0	3.0±0.70	3.0±0.70	3.0±0.70
Platelet count (no/cu.mm)	351250±2407	355000±1274	360000±6965	302500±1089	305000±8660	310000±187
Haemoglobin%	12.85±0.36	13.17±0.28	13.77±0.08	13.53±0.48	13.73±0.24	14.4±0.42
E.S.R. (mm/1st h)	11.25±1.29	11.75±1.29	11.75±0.82	13.75±1.08	14.75±0.82	14.5±0.50

Table 2b: Effect of compound Annotemoyin-1 on hematological profile of control and experimental rat at dose 200 μgm rat^{-1} day^{-1}

	Control rats M_1±SD_1			Experimental rats M_2±SD_2		
Hematological parameters	1st day	7th day	14th day	1st day	7th day	14th day
RBC count (million/cu.mm)	4.8±0.07	5.25±0.11	5.07±0.08	4.95±0.11	5.05±0.05	5.0±0.08
WBC count (thousand/cu.mm)	7.22±0.10	7.15±0.10	7.32±0.01	6.97±0.108	7.07±0.14	7.3±0.12
Neutrophil	42.5±2.06	44.25±1.6	42.6±2.0	63.25±2.28	63.5±2.29	62.0±3.08
Lymphocyte	51±2.3	51.25±1.2	52.25±1.7	33.75±1.78	33.0±2.1	34.25±1.49
Monocyte	5.0±1.5	4.5±1.29	4.25±0.80	2.5±0.5	2.75±0.82	3.25±1.29
Eosinophil	4.25±0.5	4.25±1.5	4.00±1.5	3.10±0.04	3.0±0.1	3.0±0.07
Platelet count (no/cu.mm)	346250±6495	345000±13535	347500±5590	300000±7071	320000±1581	307500±14790
Haemoglobin%	12.75±0.96	13.15±0.28	13.75±0.08	13.30±0.22	13.62±0.02	14.15±0.49
E.S.R. (mm/1st hour)	11.25±1.29	11.5±1.1	10.5±1.1	13.5±1.118	13±1.22	12.5±1.18

Table 3a: Effect of compound Annotemoyin-1 on biochemical parameters of control and experimental rats at a dose of 100 μg rat^{-1} day^{-1}

Biochemical parameters	Control rats (Group A) n=4, M_1±SD_1	Experimental rats (Groups C) n=4, M_2±SD_2	% Change	Calculated t values	t values at 5% Level of significance
SGPT (IU L^{-1})	8.75±0.82	9.0±0.70	+2.85	+0.463	2.447
SGOT (IU L^{-1})	10.0±0.70	10.5±0.5	+5.0	+1.16	2.447
Bilirubin μg dl^{-1}	0.317±0.48	0.35±0.037	+10.41	+0.137	2.447
SALP IU L^{-1}	0.48±0.027	0.49±0.072	+2.08	+0.073	2.447
Creatinine	0.59±0.01	0.59±0.043	0	0	2.447
Blood urea (m.mol l^{-1})	17.75±0.82	16.0±1.58	-9.86	-1.96	2.447

Table 3b: Effect of compound Annotemoyin-1 on biochemical parameters of rats blood at a dose of 200 μg rat^{-1} day^{-1}

Biochemical parameters	Control rats (Group A) n=4, M_1±SD_1	Experimental rats (Groups C) n=4, M_2±SD_2	% Change	Calculated t values	t values at 5% Level of significance
SGPT (IU L^{-1})	8.75±0.82	9.25±0.829	+14.28	+2.44	2.447
SGOT (IU L^{-1})	10.0±0.7	11.0±0.7	+10.0	+2.02	2.447
Bilirubin μg dl^{-1}	0.317±0.48	0.38±0.05	+19.87	+2.04	2.447
SALP IU/L	0.48±0.027	0.54±0.041	+12.50	+1.874	2.447
Creatinine	0.59±0.01	0.57±0.033	-3.38	-2.49	2.447
Blood urea (m.Mol L^{-1})	17.75±0.82	17.50±1.80	-1.408	-1.24	2.447

M_1 and M_2 = Sample mean value; SD_1 and SD_2 = Standard deviations, n = Number of rats, + = Increase; - = Decrease

Table 4: Histopathological studies after treatment with compound of Annatomoyin-1 at a dose level of 100 μg rat^{-1} day^{-1} and 200 μg rat^{-1} day^{-1}

Group	Dose (i.p) μg rat^{-1} day^{-1}	Histopathological changes observed			
		Liver	Heart	Lung	Kidney
A	100 μl vehicle	-	-	-	-
B	200 μl vehicle	-	-	-	-
C	100 μg of Annotemoyin-1	-	-	-	-
D	200 μg of Annotemoyin-1	-	-	-	-

- 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: Annotemoyin-1 was dissolved in water with Tween-20 and administered intraperitonealy at two different doses (100 μgm rat^{-1} day^{-1} and 200 μgm 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 haemotoxylin 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 annotemoyin-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).

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