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In vivo Cytotoxic Evaluation of a New Benzofuran Derivative Isolated from *Nyctanthes arbor-tristis* L. on Ehrlich Ascite Carcinoma Cells (EAC) in Mice

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The potential cytotoxic effect of a new benzofuran derivative, 4-hydroxy hexahydrobenzofuran-7-one isolated from ***Nyctanthes arbor-tristis*** Linn was evaluated on Ehrlich Ascite Carcinoma (EAC) cells in Swiss Albino mice. 4-Hydroxy hexahydrobenzofuran-7-one was administered in a daily dose of 20 mg kg^{-1} of mouse after inoculation of Ehrlich Ascite Carcinoma (EAC) cell to the mice. After 5 days, the growth inhibition of cell in percentage was determined in comparison with control mice and it was found that there was no significant difference between average no. of cells counted after treatment in mice receiving 4-hydroxy hexahydrobenzofuran-7-one and in control mice $(2.15\pm0.031)\times10^7$ vs $(3.79\pm0.019)\times10^7$ cells, respectively. The compound inhibited the cell growth by 43.27% only. 4-Hydroxy hexahydrobenzofuran-7-one (20mg kg^{-1} mouse day^{-1}) over 5 days had no significant cytotoxic effect in mice in dose of 20-mg kg^{-1} mouse for 5 days.

Key words: Cytotoxic, Benzofuran, Derivative, ***Nyctanthes arbor-tristis*** L. EAC, Mice

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Introduction

The plant ***Nyctanthes arbor-tristis*** L. is well known and widely distributed not only in Bangladesh but also in Indo-Pak subcontinent and South East Asia (Anonymous, 1983) having remarkable folk medicinal use. The flowers are used as stomachic, carminative, astringent to bowel, antibilious, expectorant, hair tonic and in the treatment of piles and various skin diseases. The bark is used for the treatment of bronchitis and snakebite (Kirtikar and Basu, 1993; Drury, 1973). It is evident from the existing works that the family Nyctanthaceae is an important source of biologically active compounds. So far literature surveyed, there was no sufficient comprehensive study of biological and phytochemical properties of the plant ***N. arbor-tristis*** L. The compound, 4-hydroxy hexahydrobenzofuran-7-one isolated from the chloroform extract of the flowers of ***N. arbor-tristis*** L. showed significant antibacterial, larvicidal (mosquito larvae) properties and also found toxic in brine shrimp lethality bioassay (Khatune, 2000). Therefore, it was subjected to anticancer (antitumor) screening on EAC cell to Swiss Albino mice.

The purpose of this study was to evaluate the cytotoxicity of the isolated 4-hydroxy hexahydrobenzofuran-7-one.

Materials and Methods

Plant materials

The fresh flowers of the plant ***N. arbor-tristis*** L. were collected during the month of September-October, (1998) from Rajshahi, Bangladesh. The plant was identified by Professor A.T.M Naderuzzaman, Department of Botany, University of Rajshahi and a voucher specimen number has been deposited in Bangladesh National Herbarium, Dhaka.

Extraction and isolation

The fresh flowers (500 gm) were taken in an aspirator and soaked in 2.5 liters rectified spirit at room temperature for 15 days with continuous changing the fresh flowers with the old ones every three days interval. The rectified spirit extract after evaporation of the solvent was fractionated with petroleum ether, chloroform, ethyl acetate and finally with methanol.

The compound was isolated from chloroform fraction and purified by column chromatography (Beckett and Stanlake, 1986) followed by preparative thin layer chromatography (PTLC) (Egon and Stahl, 1969) and obtained as colorless oily mass. The chemical structure of the compound was determined using [UV, IR (Beximco Pharmaceutical Ltd., Dhaka), ^{1}H-^{1}H COSY 90^{0}, ^{13}C-DEPT and long range correlation (Strathclyde University, Glasgow, London)] spectral analyses and was identified as 4-hydroxy hexahydrobenzofuran-7-one (Fig. 1).

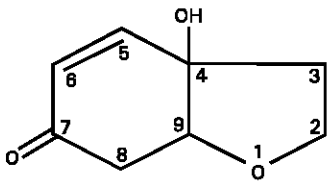

Fig. 1: 4-Hydroxy hexahydrobenzofuran-7-one

Animals

For evaluation of cytotoxicity study, an experiment was carried out with eight adult male Swiss Albino mice (collected from the Animal Resources Branch of the International Center for Diarrhoeal Research, Bangladesh) of 6-8 weeks of age were weighed (25±2 gm) and placed into two groups. The mice were kept in properly numbered iron cages individually and were supplied with basal diet (Hawk ***et al.***, 1954). The rats were acclimatized for 14 days before drug administration.

Transplantation of EAC cells

Ascitic fluids, drawn out from different tumor bearing Swiss Albino mouse, were diluted with normal saline and the viability of tumor cells were checked by trypan blue (0.4%) exclusion assay (Hawael ***et al.***, 1979). Cell samples showing above 90% viability were injected intraperitoneally (1 ml each) and were kept for 24 h for the multiplication of EAC cells.

Administration

4-Hydroxy hexahydrobenzofuran-7-one was dissolved in distilled water with dimethyl sulfoxide (DMSO) and administered intraperitoneally at a dose of 20 mg/kg mouse/day for 5 consecutive days to one group of four mice. The second group received only water and DMSO and served as the control.

Experimental procedure

A measured amount of fresh food was supplied daily at 10 h interval and the general condition of the body and the behavior of the animals were observed daily, throughout the study. For cytotoxicity study, total intraperitoneal cells were harvested in normal saline after death at 5th day. Total number of viable cells per animal of treated experimental groups were compared with those of control groups and the percentage of cell growth inhibition were calculated using standard procedures with the reagents supplied by Boehringer Manneim GmbH Diagnostica. Viable cells were counted by haemocytometer under a microscope at Sericulture Research Institute, Rajshahi, Bangladesh.

Statistical analysis

Results are presented as the M±s.d. Student's ***t***-test was used for comparison between the experimental and control groups. $P<0.05$ was considered to be statistically significant.

Results and Discussion

Table 1 shows the results of cytotoxicity of 4-hydroxy hexahydrobenzofuran-7-one on EAC cells. After 5 days of drug administration, the animals of both control and experimental groups were killed and total intraperitoneal cells were drawn out and examined under microscope. The

Table 1: Cell growth inhibition after daily intraperitoneal administration of 4-hydroxy hexahydrofuran-7-one 20 mg kg^{-1} mouse day^{-1} for 5 days in mice

	No. of cells counted			
Group	Mouse no. 1	Mouse no. 2	Mouse no. 3	Mouse no. 4
Control	3.60×10^7	3.71×10^7	3.89×10^7	3.95×10^7
Experimental	1.92×10^7	2.09×10^7	2.18×10^7	2.41×10^7

Group	Average no. of cells counted (M±SD)	Cell growth inhibited by NCS-2	% of cell growth inhibited by NCS-2
Control	$(3.79 \pm 0.019) \times 10^7$		
Experimental	$(2.15 \pm 0.031) \times 10^7$	1.64	43.27

M±SD= mean±standard deviation. There was no significant difference between the average no. of cells counted between control and experimental groups animals.

M= Simple mean value SD = Standard deviation.

compound that has the ability to suppress the growth of tumor cells more than 75% is considered to be highly anticarcinogenic. The compound 4-hydroxy hexahydrobenzofuran-7-one was found to inhibit the cell growth only 43.27%. Non significant difference was observed between the average no. of cells counted between experimental and control animals. Previously it was reported by Rahman (1999) that a flavone type compound, pectolinarigenin, isolated from the plant ***Clerodendrum indicum*** L. showed moderate anticarcinogenic (cell growth inhibition was 66.17%) effect against the same tumor (EAC) cells.

In our study, it was also observed that there were no abnormalities in the behaviour in either control or experimental animals indicating that 4-hydroxy hexahydrobenzofuran-7-one has no adverse effect on central nervous system.

From the study it is concluded that 4-hydroxy hexahydrobenzofuran-7-one has no significant cytotoxicity on EAC cells in mice at the dose and duration used in the study. Further investigation should be carried out with this compound in order to identify the pharmacological mechanism.

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