ANTITUMOR AND *IN-VITRO* CYTOTOXIC ACTIVITY OF *GREWIA ASIATICA* LINN. AGAINST EHRLICH'S ASCITES CARCINOMA CELL LINES

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Summary

Antitumor and *in-vitro* cytoxicity activity of the methanolic extract of *Grewia asiatica* (MEGA) (Family: Tiliaceae) has been assessed. MEGA showed anticancer activity against Ehrlich's ascites carcinoma (EAC) cell lines. Intraperitoneal administration of 250 and 500mg/kg of MEGA increased the life span of EAC ascitic tumor bearing mice by 41.22% and 61.06%, respectively. Significantly improved results were also found in the viable and non viable cell counts after the MEGA treatment. MEGA was assessed for *in-vitro* cytotoxicity activity against four cancer cell lines and showed 50% cytotoxicity to HL – 60, K-562, MCF-7 and Hela cells *in-vitro* at concentrations of 53.70µg/ml, 54.90µg/ml, 199.5µg/ml and177.8µg/ml, respectively. MEGA was found to be active in preventing the EAC induced ascites tumor development in mice in a dose dependent manner. *In-vitro* cytotoxicity assays show good activity against all the human cell lines.

Keywords: *Grewia asiatica*, antitumor, cytotixicity

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Introduction

Grewia asiatica Linn. is a medicinal plant and belongs to the family Tiliaceae, is known as phalsa in Assamese. It is a small tree, which is indigenous to tropical countries such as India, Malayasia and Sri Lanka. The ethnopharmacological claims for *Grewia asiatica* includes the use of leaf decoction for treating throat infections. Various part of the plant has been reported for possessing several therapeutic utilities like rheumatism, pustular eruptions, appetizer, aphrodisiac, and in curing inflammation, heart and blood disorder, fevers and diarrhoea (1, 2). Previous studies on the plant have led to the isolation of Grewinol, a keto-alcohol from the flowers (3) and a lactone (4). Literature survey reveals that no work was done with the leaves and the aerial part of the plant in evaluating the antitumour activity.

So the present investigation was carried out with the leaves and aerial part of the plant to evaluate the anticancer activity of the MEGA against EAC in Swiss albino mice and its cytotoxic activity against four human cancer cell lines – acute myeloblastic leukemia (HL – 60), chronic myelogenic leukemia (K – 562), cervical epithelial carcinoma (Hela) and breast adenocarcinoma (MCF-7). This study primarily concerned with investigating the possible antineoplastic activity of this plant as indicated by its use for throat disorders and other associated diseases.

Materials and methods

Plant Material

The leaves of *Grewia asiatica* were collected from Jorhat, Assam, India in the month of March, 2003. The plant material was identified by the Botanical from Jorhat, Assam, India. The plant material was identified by the Botanical Survey of India, Shibpur, Kolkata, India and a voucher specimen (GA – 04) was preserved in our laboratory for future references. The leaves were dried in shade and mechanically crushed and successively extracted with petroleum ether (60-80ºC), chloroform and methanol 90% in a soxhlet extractor apparatus. The petroleum ether (60-80ºC), chloroform and methanolic extracts were concentrated under reduced pressure to obtain a semi-solid mass and the yield was 9.5%, 12.4% and 15.8% respectively. The methanolic extract was taken and presolubilized in 0.9% NaCl for the study.

Animals

Male Swiss albino mice were purchased from a local authorized animal center in Kolkata, India. They were kept for acclimatization in our animal laboratory under specified conditions with access to food and water *ad libitum*. The antitumor studies were carried out in the mice weighing 20 + 3 gm with dose of 250mg and 500mg/k.g.body weight. The in vivo studies were carried at the Division of Pharmacology and Pharmaceutical Chemistry Laboratories, Department of Pharmaceutical Technology, Jadavpur University, India and the *in-vitro* cytotoxicity studies were carried out at Chittaranjan National Cancer Institute (CNCI) Kolkata, India.

Chemicals

Cisplatin was purchased from Dabur India Ltd., New Delhi, India. All other chemicals and reagents were of analytical grade.

Cancer Cell Lines

Ehrlich's ascites carcinoma (EAC) cell lines were obtained from Indian Institute of Chemical Biology (IICB), Kolkata, India and the four human cell lines namely HL-60, K-562, MCF-7 and Hela were obtained from Chittaranjan National Cancer Institute, Kolkata, India.

Acute myeloblastic leukemia (HL-60) and chronic myelogenic leukemia (K-562) were maintained in RMPI 1640 supplemented with 15% heat inactivated fetal bovine serum and streptomycin (10mg/ml). Breast adenocarcinoma (MCF-7) and cervical epithelial carcinoma (Hela) cells were maintained in MEM supplemented with similar concentration of serum and antibiotics as stated. Cells were grown at 37^0C in a humidified atmosphere of 5% Co_2 / 95% air.

Antitumor Activity

Antitumor activity of the methanolic extracts of *Grewia asiatica* (MEGA) was determined using ascites tumor model. Animals were divided into four groups of five animals in each group. All the animals were injected intraperitoneally (i.p.) with 2 x 10^6 cells/ml viable EAC cells in phosphate buffer saline (aspirated from 15 days old EAC ascites tumor in mice). After 24 hrs of tumor inoculation, MEGA at a dose of 250 and 500mg/kg body weight was administered orally and this was continued for 10 consecutive days. The group administered with vehicle alone (0.9% w/v Nacl) was maintained as control. Cisplatin (2mg/kg b.w.) i.p was used as standard reference drug. The blood parameters and the ILS (increase in life span), tumor volume, tumor cells count, viable and non-viable cells, mean survival time of the control and tumor groups were noted and compared to that of that of standard Cisplatin. The ILS was determined using the formula % ILS = (1 –T/C) X 100 where T is the mean survival time of treated group and C that of control group (5).

Cytotoxicity Studies

Cytotoxicity assays were performed in 96 well microtiter plates, by procedure as described by Roy et al., 2002. Stock solution of the extracts were made in 1% DMSO and diluted with the medium to a final concentration of 0.5, 1, 10, 50, 100, 200 µg/ml in the plate. After 48 hrs of incubation at 37^0C, 50 µl of MTT solution (6mg in 5ml) was added to each well and the plates were incubated at 37^0C for 4 hrs. In MTT assay, plates were read at 540nm in the microtitre plate reader (BIORAD) (6). Similarly the viability of the cells were determined by Trypan Blue Exclusion Method. The percentage cytotoxicity was calculated after comparing with the untreated control.

Statistical Analysis

Experimental data were expressed as mean + SEM. Student's t test was applied for expressing the significance and $P< 0.05$ was considered as significant.

Results

The MEGA showed significant cytotoxic effect against the tested human cancer cell lines as represented in Table 1. The IC_{50} value of the MEGA by MTT was calculated by Regression analysis and was found to be 53.70 µg/ml in HL – 60 54.9 µg/ml in K-562, 199.5 µg/ml in MCF-7 and 177.8 µg/ml in Hela Cells respectively. The IC_{50} value of MEGA by and trypan blue exclusion assay was calculated by Regression analysis and found to be 89.12 µg/ml in HL-60, 51.11 µg/ml in K-562, 85.11 µg/ml in MCF – 7 and 128.8 µg/ml in Hela cells respectively. In the EAC studies, the MEGA treated group showed decrease in the viable cell count as compared with the EAC treated group.

Table 1.: Effect of MEGA on various cancer cell lines

Cancer Cell Lines	MTT Assay	Trypan blue exclusion assay
	MEGA (IC_{50}, µg/ml)	**MEGA (IC_{50}, µg/ml)**
HL – 60	53.70	89.12
K – 562	54.90	51.11
MCF – 7	199.5	85.11
Hela	177.8	128.8

Table 2.: Effect of methanol extract of *Grewia asacita* (MEGA) on Body weight, Mean survival time, ILS, Tumor volume, Packed cell volume, Viable and Nonviable tumor cell count in EAC Bearing mice.

Treatment	Dose (mg/kg)	Total body weight (g)	Mean survival time (days)	% Increase of life Span (ILS)	Tumor volume (ml)	Packed cell volume (ml)	Viable cell ($X10^4$ cells/ml)	Non Viable cell ($X10^4$ cells/ml)
EAC control (0.9% Nacl)	5 ml	22.12 ± 0.21	18.51 ± 0.38	-	3.29 ±0.01	2.83 ± 0.04	18.11 ± 0.02	10.9 ± 0.10
MEGA (250mg/kg) +EAC	250	20.03 ± 0.84	26.14 ± 0.41	41.22	2.50 ± 0.51	1.70 ± 0.51*	11.80 ± 0.07*	14.1 ±0.60*
MEGA (500mg/kg) + EAC	500	19.60 ± 0.94	29.83 ± 0.69	61.16	1.14 ± 0.23	1.20 ± 0.12*	9.82 ± 0.51*	16.5 ± 0.60*
Cisplatin + EAC	5	20.54 ± 1.24	35.78 ± 0.56	93.30	0.60 ± 0.20	0.20± 0.01*	1.25 ± 0.10*	21.56 ± .20*

EAC=2x 10^6 cells/mouse , *P<0.05 for the treated groups when compared with EAC, Values are mean ± SEM, n=5.

Discussion

It was reported that plant derived extracts showed cytotoxicity towards tumor cells and antitumor activity in experimental animals (7, 8). Prolongation of the life span with the MEGA treatment is a clear suggestive of the anticancer activity of the plant. Body weight of the tumor bearing mice was also found to be decreased with the MEGA administration. In the hematological studies, the hemoglobin content was decreased in EAC treated mice, whereas restoration and elevation of hemoglobin levels to more or less to the normal levels were observed in case of MEGA treated animals.

Furthermore, the elevated WBC levels were restored with the treated group (Table 2). Preliminary investigation with the methanolic fraction of this important plant demonstrates significant antitumour activity. The doses of 250 mg/kg b.w and 500mg/kg b.w, p.o. were selected based on the preliminary studies carried out. The MEGA was also found to be active against all the four human cells lines as observed from the cytotoxicity assays. Antitumor activity of this plant may be either though induction of apoptosis or by inhibition of neovascularisation (9, 10). The presence of various phytoconstituents in the plant may attribute the observed anticancer activity. Further investigation is being carried out for finding the phytochemical entities responsible for eliciting the effects.

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