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Antitumor and *in-vitro* cytotoxic property of G*rewia asiatica* lim., against Erhlich's ascites carcinoma cells

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ABSTRACT

Background and purpose of the study: Grewia asiatica Linn is a medicinal plant and belongs to the family Tiliaceae. Various parts 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 diarrhea. The present study was aimed to evaluate antitumor and *in vitro* cytoxic activity of the methanolic extract of *Grewia asiatica* (MEGA).

Methods: The methanolic extract was prepared by soxhlet extraction. MEGA at the doses of 250 mg/kg and 500mg/kg body weight were administered orally for consecutive 10 days by taking tumor inoculation as day 0. At the end of the treatment schedule, blood parameters and increased life span were noted. MEGA was also assessed for *in vitro* cytotoxicity against four cancer cell lines using MTT assay method and the viability of the cells were determined by Trypan Blue Exclusion Method.

Results: MEGA at the tested doses showed anticancer activity against Ehrlich's ascites carcinoma (EAC) cells. Oral administration of 250 and 500mg/kg body weight of MEGA increased the life span of EAC ascitic tumour bearing mice by 41.22 and 61.16%, respectively. The viable and non viable cell counts also significantly improved after the MEGA treatment. *In vitro* cytotoxicity of MEGA against four cancer cell lines showed IC_{50} value of 53.70, 54.90, 199.5 and 177.8 µg/ml for HL – 60, K-562, MCF-7 and Hela cells respectively.

Major conclusion: The results of the present study are encouraging as the extract MEGA exhibit significant reduction in the tumor burden and caused prolongation of lifespan of the tumor hosts. These parameters suggest that the methanol extract of *Grewia asiatica* exhibits potential antitumor and *in vitro* cytotoxic activities.

KEYWORDS

Grewia asiatica;
Antitumor;
Cytotoxicity.

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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 diarrhea[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.

MATERIAL AND METHODS

Plant material

The leaves of *Grewia asiatica* were collected from Jorhat, Assam, India. The plant material was identified by the Botanical Survey of India, Shibpur, Kolkata 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 (60-80^{0}C), chloroform and methanol 90% in a soxhlet extractor apparatus. The petroleum ether (60-80^{0}C), 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. 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 $\pm$ 3 gm with dose of 250 mg and 500 mg/kg body weight.

Chemicals

Cisplatin was purchased from Dabur India Ltd., New Delhi. 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 and the four human cell lines namely HL-60, K-562, MCF-7 and Hela were obtained from Chittaranjan National Cancer Institute, Kolkata.

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 concentrating of serum and antibiotics as stated. Cells were grown at 37^{0}C 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 modal.

Ascites tumor model

Animals were divided into four groups of five animals in each group. All the animals were injected intraperitoneally (ip) 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) i.p was used as standard refer-

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ence 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[6], 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^{0}C, 50 µl of MTT solution (6mg in 5ml) was added to each well and the plates were incubated at 37^{0}C for 4 hrs. In MTT assay, plates were read at 540nm in the microtitre plate reader (BIORAD). 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

In -vitro cytotoxicity

The MEGA showed significant cytotoxic effect against the tested human cancer all 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 (Figure 1 and Figure 2).

TABLE 1 : Effect of MEGA on various cancer cell lines

S.NO	Cancer Cell Lines	MTT Assay	Trypan blue exclusion assay
		MEGA (IC_{50}, µg/ml)	MEGA (IC_{50}, µg/ml)
1	HL – 60	53.70	89.12
2	K – 562	54.90	51.11
3	MCF – 7	199.5	85.11
4	Hela	177.8	128.8

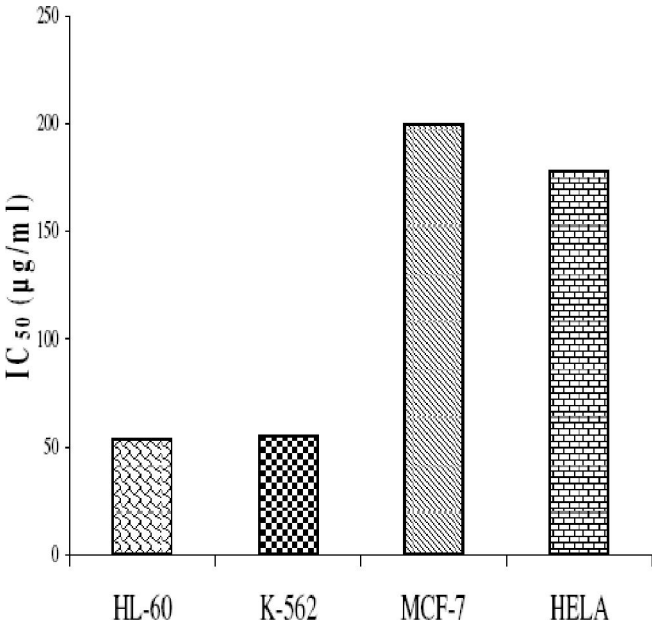

Figure 1 : Effect of MEGA on various cancer cell lines (MTT Assay)

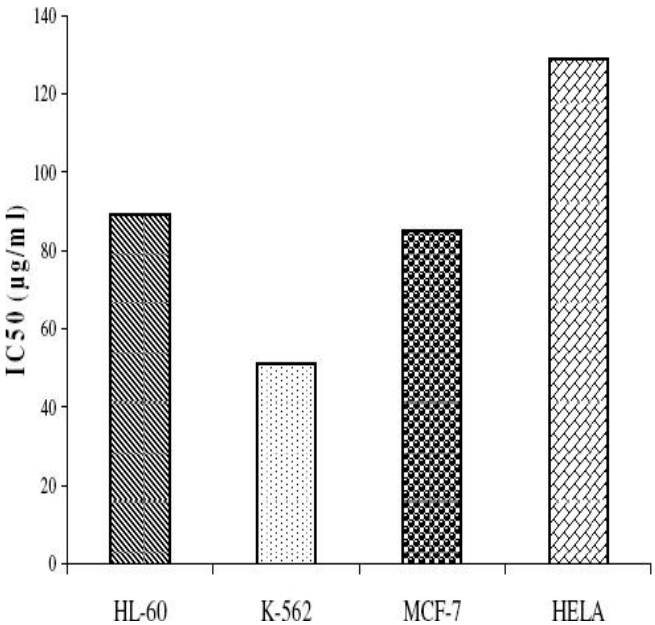

Figure 2 : Effect of MEGA on various cancer cell lines (Trypan Blue Assay)

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)	5ml	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	73.30	0.60±0.20	0.20±0.01*	1.25±0.10*	21.56±0.20*
MEGA (500mg/kg) + Cisplatin + EAC	500+5	20.11 ± 1.14	38.58± 1.01	78.67	0.54± 0.27	0.21±0.01	1.21±0.21	24.47±0.51

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

Antitumor activity

From the studies above it was noticed that the non-viable cells count was more in MEGA treated animals at dose of 500 mg/kg body weight as compared to the EAC treated ones. Treatment with the MEGA at the dose of 500mg/kg body weight prolonged the life span (increased by 61.16%) of EAC tumor bearing mice. The standard reference drug (Cisplatin 5mg/kg body weight) exhibited 73.3% ($p<0.001$). But the treatment with MEGA and Cisplatin combination showed 78.67 % increase in the life span. Body weight of the tumor bearing mice was also found to be decreased with the MEGA administration. In the haematological parameters, the haemoglobin content was decreased in EAC treated mice, whereas restoration and elevation of haemoglobin levels were observed in case of MEGA treated animals. Restoration of elevated WBC levels was seen with the treated group. (TABLE 2)

One of the major criteria of judging good anticancer drugs is that it should be able to prolong the life and decrease the leucocyte count. Reduced volume of tumor and increased life span also indicated decrease of cell division. The anaemia associated with carcinoma was also restored to normal levels when compared to the control group as indicated by the increased RBC count and hemoglobin level. These indicate that MEGA has no toxic effects on the haematological system[7-9].

DISCUSSION

Exploration of ethnopharmacology and traditional medicine is increasingly gaining momentum, accompanied by increased laboratory research into the pharmacological properties of the bioactive ingredients and their ability to treat different diseases. 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 obtained from the cytotoxicity assays. Further investigation is being carried out for finding the phytochemical entities responsible for eliciting the effects.

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