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RESEARCH PAPER

ANTICANCER ACTIVITY OF METHANOLIC LEAF EXTRACT OF *MORINDA TINCTORIA* ROXB. AGAINST EHRLICH ASCITES CARCINOMA IN MICE

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The methanol extract of the leaves of *Morinda tinctoria* Roxb. (MEMT) was studied for its anticancer activity using *in vitro* and *in vivo* cancer models. MEMT was investigated for its short-term cytotoxicity on EAC tumor cells by trypan blue dye exclusion method and *in vitro* cytotoxicity on NIH 3T3, A549, Hep2 and HepG2 cells by MTT assay. *In vivo* anticancer activity was studied on EAC tumor-bearing mice. Anticancer activity was assessed by monitoring the mean survival time, the percentage increase in life span, the effect on haematological parameters, antioxidant enzyme levels and solid tumor volume. 5-Fluorouracil (5-FU, 20 mg/kg/*i.p.*) was used as a standard. The extract showed potent *in vitro* cytotoxicity against each of the tested tumor cell lines, but it was found to be harmless to normal cells. MEMT at the dose of 200 and 400 mg/kg, significantly increased the mean survival time (P<0.001), exerted a protective effect on the hemopoietic system (P<0.05 – 0.001), prevented lipid peroxidation and restored the antioxidant enzymes *catalase, superoxide dismutase, glutathione peroxidase* and *glutathione-S-transferase* in the liver of tumor control animals (P<0.001). It also significantly reduces the solid tumor volume (P<0.01). The results showed a significant anticancer and cytotoxic effect of MEMT against EAC and human cancer cell lines, and thus supported the ethnomedical use of *Morinda tinctoria.*

Key words: *Morinda tinctoria* Roxb, MTT assay, Anticancer activity, EAC tumor cells, 5-Fluorouracil.

INTRODUCTION

Over the past few years, cancer has remained a serious reason behind death and also the number of people suffering from cancer is continuous to expand. Hence, a major portion of the present pharmacological research is dedicated to antitumor drug design customized to suit new molecular targets. As a result of the large propensity of plants, that synthesize a range of structurally diverse bioactive compounds, the medicinal plants could be a potential supply of chemical constituents with cytotoxicity and other pharmacological activities (Kim *et al* 2005; Indap *et al* 2006; Jain *et al* 2011; Chowdhury *et al* 2012; Deb *et al* 2013; Rashid *et al* 2014; Agarwal *et al* 2015; Shrestha *et al* 2016; Viana *et al* 2017). India could be a wealthy source of medicinally important plants and a number of plant extracts are employed in numerous systems of medicines like Ayurveda, Siddha and Unani to cure a variety of diseases but only some of them are scientifically explored. The wealthy and numerous plant sources of India are possible to produce effective antitumor agents. Plant-derived natural phytochemicals like flavonoids, terpenoids and alkaloids have received important attention in recent years as a result of their varied pharmacologic properties as well as cytotoxic and cancer chemopreventive effects. One among the most effective approaches in the explore for antitumor agents from plant sources is that the choice of plants supported ethnomedical leads (Kintzios, 2006). *Morinda tinctoria* Roxb (Family: Rubiaceae) is a small to medium size tree which is distributed throughout India (**Figure 1**).

Fig. 1. Image of plant *Morinda tinctoria* with medicinal properties

Many parts of this plant have been used to treat various ailments. The ripe fruits of *Morinda tinctoria* are used for respiratory disorders, arthritis, cancer, gastric ulcer and heart diseases, relieve pain, purify the blood and stimulate the immune system, an effective antioxidant, acts as an antibiotic resistant (Wang *et al* 2002). Literature survey revealed that this plant exhibited potent antimicrobial (Janakiraman *et al* 2012), anti-inflammatory (Sivaraman and Muralidharan, 2010), antidiabetic (Pattabiraman and Muthukumaran, 2011), hepatoprotective (Surendiran and Mathivanan, 2011), antiradical (Desai *et al* 2010), antihyperglycemic and antidiabetic (Palayan and Dhanasekaran, 2009), *In vitro* free radical scavenging (Sreena *et al* 2011) and cytoprotective activity (Sivaraman and Muralidharan, 2011). Therapeutically active iridoid glycoside was also isolated from *Morinda tinctoria* (Roxb.) roots (Dipita *et al* 2012). Based on the ethnopharmacological literature, in the present work, *Morinda tinctoria* was selected to prove scientifically its anticancer property in the experimental animal model by using transplantable tumors.

EXPERIMENTAL

Materials

5-Fluorouracil (5-FU) was obtained from Dabur Pharmaceutical Ltd (New Delhi, India). Trypan blue, thiobarbituric acid, trichloroacetic acid, ethylenediaminetetra acetic acid (EDTA), RPMI-1640 media and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertazolium bromide (MTT) were procured from HiMedia (Mumbai, India). Dimethyl sulfoxide and methanol were obtained from Loba Chemie (Mumbai, India). All other chemicals used were of analytical grade.

Collection and extraction

Leaves of *Morinda tinctoria* was collected from Tiruchengode, Namakkal District, Tamil Nadu, India and identified by Dr. G.V.S. Murthy, Botanical Survey of India, Coimbatore, Tamilnadu, India and a voucher specimen was preserved in our laboratory for future reference (No.: SVCP/NPL/MT-0124). The plant material was shade dried, coarsely powdered and extracted with 80% methanol at room temperature for 72 h. After extraction, the extract was filtered, concentrated under vacuum and stored in a desiccator until further use.

Phytochemical analysis

The extract was screened for the presence of various phytochemical constituents employing standard procedures (Wagner *et al* 1984; Harborne, 1984). Conventional protocol for detecting the presence of steroids, alkaloids, tannins, flavonoids, glycosides etc, was used.

In vitro cytotoxicity studies

Tumor cells and inoculation

Stock cells of Normal Mouse Embryonic Fibroblast (NIH 3T3), Human Lung Carcinoma (A549), Human Laryngeal Epithelial Carcinoma (Hep2), Human Liver Cancer cells (HepG2) were obtained from National Centre for Cell Sciences (Pune, India). The cultures were maintained in Dulbecco's modified eagles medium (DMEM) containing 10 % inactivated calf serum and were grown in 25 cm² tissue culture flasks (Tarson Products Ltd, Kolkatta, India) until confluent and used for cytotoxic assays. EAC cells were obtained from Amala Cancer Research Centre (Thrissur, Kerala, India). The cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation. Tumor cells aspirated from the peritoneal cavity of mice were washed with normal saline and were used for further studies.

Preparation of suspensions and solutions

For cytotoxicity assays, the extract was dissolved in dimethyl sulfoxide (DMSO) and the volume made up to 10 ml to obtain a 1000 μg/ml stock solution. Serial two-fold dilutions were made using DMSO to get lower concentration. MEMT was suspended in distilled water using sodium carboxymethyl cellulose (CMC, 0.3%) and administered orally to the animals with the help of an intragastric catheter to study *in vivo* antitumor activity.

Short term cytotoxic activity

Short term cytotoxicity study of MEMT was determined by using trypan blue dye exclusion method (Kumar *et al* 2011). Briefly, 0.1 ml of the cell suspension contains 1×10^6 cells/ml was exposed to 0.1 ml of various concentrations of MEMT (500 – 31.25 μg/ml) and incubated at 37°C for 3 h. After 3h, the trypan blue dye exclusion test was performed to determine the percentage cytotoxicity and the IC_{50} value was calculated.

Antiproliferative activity

Stock cells of Normal Mouse Embryonic Fibroblast (NIH 3T3), Human Lung Cancer cells (A549), Human Laryngeal Epithelial Carcinoma (Hep2), Human Liver Cancer cells (HepG2), were cultured in RPMI-1640 and DMEM supplemented with 10% calf serum, penicillin (100 IU/ml) and streptomycin (100 μg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with 0.2% trypsin and 0.02% EDTA in PBS. The antiproliferative assay was carried out by adding 0.1 ml of cell suspension containing 10,000 cells to each well of a 96-well microtitre plate (Tarson, Kolkatta, India) and fresh medium containing various concentrations of the extract was added at 24 h after seeding. Control cells were incubated without the extract and with DMSO. The plates were incubated at 37°C in a humidified atmosphere with 5% CO_2 for a period of 72 h. The percentage growth inhibition was determined by the MTT assay method and IC_{50} value was calculated (Kumar *et al* 2011).

In vivo anticancer studies

Animals

Healthy male Swiss albino mice (20-25 g) were utilized throughout the study. They were housed in standard microlon boxes and were given standard laboratory diet and water *ad libitum*. The study was conducted after obtaining Institutional Animal Ethical Committee (IAEC) clearance (Proposal No: SVCP/IAEC/ 01/2016-17 dt 12.06.2016) and the animals were handled as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines of Good Laboratory Practice.

Acute toxicity studies

The oral acute toxicity study of MEMT was carried out in Swiss albino mice using the OECD Guidelines (Acute Oral Toxicity 423; OECD, 2001). The animals received MEMT starting at 2 g/kg orally by gavage. The animals were observed for toxic symptoms and mortality continuously for first 4 h after dosing. Finally, the number of survivors was noted after 24 h and these animals were then maintained for further 13 days with observations made daily.

Effect of MEMT on mean survival time

Healthy Swiss albino mice were divided into four groups (I-IV) each group consisting of six animals. All the animals were inoculated with EAC cells (1×10^6 cells/mouse) on day '0' and treatment with MEMT started 24 h after inoculation. Group I served as tumor control which received the vehicle (CMC, 0.3%). Group II animals were treated with the standard drug 5-fluorouracil (5-FU, 20 mg/kg) by intraperitoneal route. Group III and IV received the plant extract at the dose of 200 mg/kg and 400 mg/kg by the oral route, respectively. All the treatments were given for nine days. The mean survival time and percentage increase in life span were calculated (Kumar *et al* 2011).

$$\% \text{ increase in life span} = [T - C / C] \times 100$$

where:
T = No. of days the treated animals survived
C = No. of days the control animals survived

Effect of MEMT on haematological studies

In order to detect the influence of MEMT on the haematological status of tumor-bearing animals, a comparison was made among four groups (n = 6) of mice on the 14th day after inoculation. Group I served as normal control which received the vehicle (CMC, 0.3 %). Group II served as tumor control. Group III and IV were treated with the plant extract at the dose of 200 mg/kg and 400 mg/kg by oral route for nine days (Kumar *et al* 2011). Blood was drawn from each mouse by retro-orbital plexus method after anaesthetized slightly with anaesthetic ether. The hematological parameters like the total red blood cell (RBC), the white blood cells (WBC), the lymphocytes (LYM), the hematocrit (HCT), the hemoglobin (HGB), and the MID cells (less frequently occurring and rate cells correlating to monocytes, eosinophils, basophils etc.) were determined using a blood automatic analyzer (Celldyn, Abbot Inc. USA).

After blood collection, animals were sacrificed by cervical dislocation. The liver from each mouse was excised and rinsed in ice-cold normal saline solution. A 10 % *w/v* liver homogenate was prepared in ice-cold 10% KCl solution and was centrifuged for 15 min at 4°C. The supernatant, thus obtained was used for the estimation of lipid peroxidation (LPO) (Devasagayam and Tarachand, 1987), catalase (CAT) (Sinha, 1972), superoxide dismutase (SOD) (Marklund and Marklund, 1974), glutathione peroxidase (GPx) (Rotruk *et al* 1973) and glutathione S-transferase (GST) (Habig *et al* 1974).

Effect of MEMT on solid tumor

Mice were divided into three groups and each group consisting of six animals. All the animals were injected EAC cells (2×10^6 cells/mouse) into the right hind limb of the animals intramuscularly. Group I served as tumor control. Group II and III were treated with MEMT at the dose of 200 mg/kg and 400 mg/kg by the oral route, respectively, for five alternative days. From the 15th day onwards, tumor diameter was measured every fifth day and recorded up to 30 days by using vernier callipers. The tumor volume was calculated by using the formula $V = 4/3\ \pi r^2$, where 'r' is the mean of r_1 and r_2 which are the two independent radii of the tumor mass (Kumar *et al* 2011).

Statistical analysis

All the values were expressed as mean ± SEM. The data were statistically analyzed by one-way ANOVA, followed by Tukey's multiple comparison test and data for solid tumor volume were analyzed by Dunnett test. P values <0.05 were considered significant.

RESULTS

Phytochemical analysis

Phytochemical studies of MEMT showed the presence of alkaloids, carbohydrates, steroids, proteins, saponins, fixed oils and fat, tannins, phenolic compounds, flavonoids, glycosides.

In vitro cytotoxicity studies

Short term cytotoxicity study

In short-term cytotoxicity study by Trypan Blue Dye Exclusion method, the IC_{50} value against EAC cell lines was found to be 194.57µg/ml.

Antiproliferative activity

In MTT assay, the percentage cytotoxicity progressively increased in a concentration-dependent manner. The plant extract showed moderate activity against the tested cancer cell lines but the IC_{50} value against normal cell line was found to be high which indicates the selective cytotoxicity of the extract towards the cancer cells (**Table 1**). The result also suggested that the extract is safer on normal cells.

Table 1. *In vitro* cytotoxicity studies on human cancer cell lines

Cell lines used	IC_{50} (µg/ml)
NIH 3T3 (Normal Mouse Embryonic Fibroblast)	267.76
A549 (Human Lung Cancer)	184.28
HepG2 (Human Liver Cancer)	164.51
Hep2 (Human Laryngeal Epithelial Carcinoma)	199.14

*Average of three determinations, three replicates,
IC_{50}: Drug concentration inhibiting 50% cellular growth following 72 h of drug exposure

Table 2. Effect of MEMT on mean survival time and increase in life span of EAC tumor-bearing mice

Design of Treatment	MST (in days)	Increase in life span (%)
Tumor Control	16.8 ± 0.65	–
5-FU, 20 mg/kg	33.2 ± 1.36^{a}	97.62
MEMT, 200 mg/kg	21.8 ± 1.25^{b}	29.76
MEMT, 400 mg/kg	31.9 ± 2.27^{a,b}	89.88

n = 6; Data were expressed as Mean ± SEM; ^{a}P<0.001 *vs* Tumor Control; ^{b}P<0.001 *vs* 5-FU
Data were analysed by using one-way ANOVA followed by Tukey-Kramer Multiple comparison test

In vivo anticancer studies

Acute toxicity studies

In acute toxicity studies, animals treated with MEMT did not show any toxic symptoms or mortality when dosed up to 2000 mg/kg body weight by the oral route. This indicated that the extract was safe at the tested dose level. Hence $1/10^{th}$ (200 mg/kg) and $1/5^{th}$ (400 mg/kg) of this dose were selected for the *in vivo* studies.

Effect of MEMT on survival time

The effect of MEMT on survival time of tumor bearing mice is shown in **Table 2**. The mean survival time and percentage increase in life span were significantly increased in extract treatment groups when compared to tumor control group. The results obtained were comparable with that of the standard 5-Fluorouracil.

Effect of MEMT on Haematological parameters

Haematological parameters of tumor bearing mice on the day 14 were showed significant changes when compared to normal mice (**Table 3**). The total WBC count and the haematocrit (HCV) were found to increase with a reduction in the haemoglobin content and RBC count. At the same time interval, MEMT (200 and 400 mg/kg) treatment could change these parameters near to normal. Maximum alternation occurred in the MEMT treatment at the dose of 400 mg/kg.

Table 3. Effect of MEMT on haematological parameters of EAC tumor-bearing mice

Parameters	Normal	Tumor control	MEMT 200	MEMT 400
Hemoglobin (g/dl)	12.56±0.58	6.46±0.34[a]	9.2±0.38[a,e]	11.74±0.45[d]
RBC (M/ul)	7.16±0.22	4.04±0.09[a]	4.54±0.28[a,e]	6.22±0.26[c,d]
WBC (K/ul)	4.6±0.15	8.4±0.4[a]	5.6±0.27[d]	4.9±0.17[d]
Hematocrit (HCT) (%)	17.7±0.59	30.16±1.76[a]	20.4±0.95[d]	17.08±0.69[d]
Lymphocytes (%)	62.4±4.1	18.36±1.9[a]	48.6±1.3[b,d]	55.12±2.45[d]
MID cells (%)	14.16±1.6	62.72±2.7[a]	22.6±2.36[d]	21.92±1.88[d]

n = 6; Data were expressed as mean ± SEM; [a]P<0.001, [b]P<0.01, [c]P<0.05 when compared with Normal; [d]P<0.001, [e]P<0.05 when compared with Tumor Control. The data were analysed by using one-way (ANOVA) followed by Tukey-Kramer multiple comparison test.

Effect of MEMT on antioxidant parameters

The levels of lipid peroxidation in liver tissue were significantly increased in EAC tumor control group as compared to the normal group. After administration of MEMT at different doses to EAC tumor-bearing mice, the levels of lipid peroxidation were significantly reduced as compared to tumor control groups. Inoculation with the tumor cells drastically increased the GST and GPx content in both tumor control groups as compared with normal group. Administration of MEMT at the tested doses decreased GST and GPx levels as compared with the tumor control group. The levels of superoxide dismutase (SOD) in the livers of the EAC tumor-bearing mice decreased significantly when compared with normal group. After administration of MEMT at the tested doses, increased levels of SOD as compared with the tumor control groups were observed. The catalase (CAT) levels in EAC tumor control group decreased as compared with normal group. Treatment with the MEMT increased the catalase levels as compared to that of tumor control groups. The results obtained are presented in the **Table 4**.

Table 4. Effect of MEMT on lipid peroxidation and antioxidant enzyme levels of EAC tumor-bearing mice

Design of treatment	LPO	SOD	CAT	GPx	GST
Normal	7.6 ± 0.39	0.48 ± 0.01	31.19 ± 1.94	13.4 ± 1.02	0.14 ± 0.007
Tumor control	29.60 ± 1.31[a]	0.13 ± 0.04[a]	12.25 ± 1.34[a]	41.3 ± 1.29[a]	0.32 ± 0.01[a]
MEMT 200	18.7 ± 2.03[a,d]	0.22 ± 0.11[a,d]	21.73 ± 1.24[c,d]	22.8 ± 0.56[a,d]	0.27 ± 0.04[a,d]
MEMT 400	11.2 ± 1.01[a,d]	0.38 ± 0.04[b,d]	26.4 ± 1.31[d]	19.10 ± 0.51[c,d]	0.16 ± 0.04[d]

n = 6; Data were expressed as mean ± SEM; [a]P<0.001; [b]P<0.01; [c]P<0.05 *vs* Normal; [d]P<0.001 *vs* Tumor control; Data were analysed by Tukey-Kramer multiple comparison test; LPO, moles of MDA/min/mg protein; SOD, units/min/mg protein; CAT, mole of H_2O_2 consumed/min/mg protein; GPx, moles of GSH oxidized/min/mg protein; GST, moles of CDNB conjugation formed/min/mg protein.

Effect of MEMT on solid tumor volume

The solid tumor volume of EAC tumor-bearing mice was presented in **Table 5**. The solid tumor volume was gradually increased in tumor control group. The extract treatment significantly reduces the tumor volume in a dose-dependent manner when compared to tumor control groups.

Table 5. Effect of MEMT on solid tumor volume of EAC tumor-bearing mice

Design of treatment	Solid Tumor Volume (cm^3)					
	5th day	10th day	15th day	20th day	25th day	30th day
Tumor control	0.60 ±0.03	0.91±0.13	1.27±0.15	1.74±0.16	1.81±0.36	2.59±0.31
MEMT 200	0.51±0.03	0.84±0.05	0.96±0.06	1.37±0.11	1.62±0.12	2.03±0.11
MEMT 400	0.38±0.02^a	0.61±0.02^b	0.72±0.05^a	1.22±0.02^a	1.63±0.04	1.88±0.04^b

n = 6; Data were expressed as mean ± SEM. ^{a}P<0.01; ^{b}P<0.05 when compared to Tumor Control,
The data were analyzed by using one-way (ANOVA) followed by Dunnet's test.

DISCUSSION

Cancer is a disease of misguided cells that have a high potential of excess proliferation without apparent relation to the physiological demand of the process. It is the second largest cause of death in the world. Plants belonging to the genus *Morinda* and several of their constituents have shown potent anticancer properties in many models based on the studies conducted throughout the world. Based on these observations, in the present study, the MEMT was evaluated for its *in vitro* cytotoxicity and *in vivo* antitumor properties.

The reliable criteria for judging the value of any anticancer drug is the prolongation of life span, the disappearance of leukemic cells from the blood and reduction of solid tumor volume (Marklund *et al* 1982). Transplantable tumor cells such as EAC are rapidly growing cancer cells with aggressive behaviour. The tumor implantation includes a local inflammatory reaction, with increasing vascular permeability, which results in an intense ascetic fluid accumulation. The ascitic fluid is vital for tumor augmentation since it constitutes a direct nutritional source for cancer cells (Shimizu *et al* 2004). Our results show an increase in life span accompanied by a reduction in WBC count in MEMT treated mice. The plant extract also inhibited the accumulation of ascitic fluid in the peritoneal cavity of the tumor-bearing animals. These results clearly demonstrated the antitumor effect of MEMT on EAC tumor cells.

The most common problems encountered in cancer chemotherapy are bone marrow suppression and anaemia (Marklund *et al* 1982). Anaemia is found frequently in cancer patients. Similar results were observed in the present study in animals of the EAC tumor control group. This is mainly due to a reduction in RBC or haemoglobin production and this may occur either due to the iron deficiency or to hemolytic or other myelopathic conditions. Treatment with MEMT brought back the haemoglobin content, RBC and WBC counts near to normal. This indicates that the extract has a significant protective effect on the hemopoietic system.

Excessive production of the free radicals leads oxidative stress, which results in damage to macromolecules such as lipids, and can encourage lipid peroxidation *in vivo.* Malondialdehyde, the end product of lipid peroxidation has been reported to be higher in tumor tissue than in non-diseased organ. Glutathione, a powerful inhibitor of the neoplastic process, plays a vital role as an endogenous antioxidant system that originates particularly in high concentrations in liver and is known to have key functions in the protective process. The free radical scavenging system, superoxide dismutase and catalase are present in all oxygen metabolising cells and their function is to provide a defence against the potentially damaging reactive of superoxide and hydrogen peroxide (Sinclair *et al* 1990). The decrease in SOD action in tumor-bearing animals, which might be due to loss of Mn-SOD activity in cancer cells and loss of mitochondria, leading to a reduction in total SOD activity in the liver. Inhibition of SOD and catalase activities as a result of cancer growth was also reported (Marklund *et al* 1982). Treatment with MEMT in different dose levels significantly increased the SOD and catalase levels in a dose-dependent manner. Plant-derived extracts containing antioxidant principles such as flavonoids, phenolic compounds and tannins showed cytotoxicity towards cancer cells and anticancer activity in experimental animals (Marklund *et al* 1982; Li and Oberley, 1997). Anticancer activity of these antioxidants is either through induction of apoptosis or by inhibition of angiogenesis. The lowering of lipid peroxidation, GST, GPx and increase in levels of SOD and catalase in MEMT treated group indicates its potential as an inhibitor of cancer induced intracellular oxidative stress.

In EAC tumor-bearing animals, there was a regular and hurried increase in ascetic fluid volume. Ascitic fluid is the direct dietary source for tumor growth and it meets the nutritional requirements of tumor cells (Shimizu *et al* 2004). MEMT treatment decreased the volume of solid tumor and increases the life span of the tumor-bearing animals. Hence it may conclude that MEMT, by a direct cytotoxic effect or by decreasing the nutritional fluid volume and arresting the tumor cell growth. The present study revealed that the extract was cytotoxic towards EAC cell lines and it was also found to be potent cytotoxic against human cancer cell lines. The cytotoxic potency of the extract was confirmed by the *in vitro* cytotoxic assay methods against animal cancer cells lines and human cancer cell lines.

The extract exhibits potent cytotoxicity against all the tested cancer cell lines. At the same time, the IC_{50} for the normal cell line was found to be high when compared to cancer cells, which indicated that the extract is having cytotoxicity against the cancer cells, but it is safe for normal cells.

Phytochemical studies indicated the occurrence of flavonoids, saponins, tannins and phenols in MEMT. Many such compounds are known to possess potent antitumor properties (Kintzios, 2006). The extract of *Morinda tinctoria* is rich in flavonoids and saponins. Flavonoids have been found to possess anti-angiogenic, anti-mutagenic and anti-malignant effect (Brown, 1980; Hirano *et al* 1989). Moreover, they have a chemo-preventive role in cancer through their effects on signal transduction pathway in the cell proliferation and the inhibition of neo-vascularization (Weber *et al* 1996; Fotsis *et al* 1997). Antitumor and cytotoxic properties of the extract may be due to these phytochemical constituents.

CONCLUSION

The present study demonstrates the potent cytotoxic and antitumor properties of methanolic leaf extract of *Morinda tinctoria*. Further studies to characterize the active principles and to elucidate the mechanism of action are in progress.

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