Full Length Research Paper

Antimetastatic and antiproliferative activity of methanolic fraction of *Jatropha curcas* against B16F10 melanoma induced lung metastasis in C57BL/6 mice

R. Balaji[1*], N. Rekha[1], M. Deecaraman[1] and L. Manikandan[2]

[1]Department of Industrial Biotechnology, Dr. M. G. R. Educational and Research Institute, Maduravoyal, Chennai-600095, Tamil Nadu, India.
[2]Department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700032, India.

The methanolic fraction of *Jatropha curcas* was studied for its anti-metastatic activity using B16F10 melanoma cells in C57BL/6 mice. Simultaneous administration of methanolic fraction at doses 100 and 200mg/kg, p.o significantly (p < 0.01) inhibited the metastatic colony formation of the melanoma in lungs by 47.54 and 69.52% respectively, with increase in the survival rate of the metastatic tumour bearing animals, as compared to the untreated control animals. Lung collagen hydroxyproline content was highly elevated in the control animals, which was reduced by the simultaneous administration of methanolic fraction at the tested dose levels. The level of lung hexosamines and uronic acid content was also elevated in the control animals. Administration of methanolic fraction of *J. curcas* at tested dose levels 100 and 200 mg/kg, p.o significantly reduced the elevated level of hexosamine and uronic acid content, when compared to that of vehicle treated control animals. Levels of serum sialic acids and γ-glutamyltranspeptidase that are markers of neoplastic proliferation were also reduced in the methanolic fraction treated animals as compared to the higher levels in the control animals. Histopathological analysis of the lung tissues also correlated with these findings. The *in vitro* cytotoxic activity of methanolic fraction of *J. curcas* on B16F10 melanoma cells was studied using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) assay and the IC_{50} was found to be 24.8 µg/ml. Thus, simultaneous administration of methanolic fraction of *J. curcas* at the tested dose levels were effective in inhibiting the metastasis of B16F10 melanoma cells and possessed significant anti-metastatic and antiprolifertaive activity.

Key words: *Jatropha curcas*, B16F10 melanoma cells, metastasis, antiprolifertive.

INTRODUCTION

Metastasis is one of the major causes of mortality in cancer patients, although surgical removal of tumours can enhance and prolong survival (Mays and Nelson, 1999; Rusthoven et al. 1996). The events that lead to the metastasis are (a) detachment from the primary tumour, (b) circulation through blood and lymphatics, (c) adhesion to the extracellular matrix, (d) invasion after the proteolytic cleavage of the basement membrane, and (e) adhesion and growth at the new site (Poste and Fidler, 1980). The tumour invasion of a basement membrane is also an important step, which involves the adhesion of tumour cells to the extracellular matrix (ECM) components followed by the degradation of the ECM (Cavallaro and Christofori, 2001). The most significant turning point in cancer is the establishment of distant metastasis. The patient can no longer be cured by local therapy alone at this point (Liotta and Kohn, 2003). The number of melanoma cases worldwide is increasing faster than any other cancer (Diepgen and Mahler, 2002). The estimates suggested a doubling of melanoma incidence every 10 - 20 years (Garbe et al., 2000). Despite improved survival rates, the death rate from cutaneous metastatic

*Corresponding author. E-mail: balajipharmacology@gmail.com Tel: +91-04423782176 , -23782186, -23782085. Fax: +91-044237831 165.

melanoma (CMM) continues to climb as a result of exponential increases in incidence, making it a major public health problem for the foreseeable future (Lens and Dawes, 2004). Melanoma now accounts for approximately 4% of all cancers diagnosed in the United States. When diagnosed early in the course of the disease, melanoma is readily cured by simple wide surgical excision. However, once melanoma metastasizes, no treatment currently available reliably affects the course of disease. At the time of autopsy, the lung, liver, brain and lymph nodes are the most common sites of metastasis. Patients with pulmonary metastasis have a median survival period of 8 - 10 months (Morton et al., 2003).

Any agent or drug which can interfere with any of these steps can significantly reduce the metastatic potential and can be useful in the inhibition of tumour metastasis. Several attempts have been made to inhibit tumour metastasis preventing the formation of tumours and tumour invasion using herbs (Ha et al., 2004; Yang et al., 2003). Plant derived pharmacologically active compounds are found to be a proven source of useful antitumour compounds. It is abundantly clear that natural product lead compounds emanating from drug discovery programmes have been of exceptional value in mechanistic studies involving biological components of relevance in the control of cancer. Thus, plant derived natural products have long been and will continue to be an important source for anticancer drug development. *Jatropha curcas* Linn (Family: Euphorbiaceae), is an evergreen shrub, indigenous to America, but cultivated in most parts of India.

This evergreen plant is common in waste places throughout India, especially on the Coromandel Coast and in Travancore; in the Southern parts it is cultivated chiefly for hedges in the Konkan and also in Malay Peninsula (Caius, 1986). Leaves are regarded as antiparasitc, applied to scabies; rubefacient for paralysis, rheumatism; also applied to hard tumours (Duke, 2001). Compounds that have been isolated from *J. curcas* leaves include the flavonoids apigenin and its glycosides vitexin and isovitexin, the sterols stigmasterol, β -D-sitosterol and its β -D-glucoside (Subramanian et al. 1971; Khafagy et al., 1977; Chhabra et al., 1990; Saxena et al., 2005).

The methanolic extract of leaves of *J. curcas* was reported to have anticrustcean activity, DNA intercalating effect and also anti-viral activity (Gupta et al., 1996; Khan et al., 1991). The butanolic extract of dried leaves of *J. curcas* and decoction of the dried root bark was reported to have antispasmodic activity (Kambu et al., 1990). An ethanolic extract of the defatted leaves and twigs of *J. curcas* show antileukemic activity (Hufford and Oguntimein, 1978).

The leaf extract has been confirmed to possess disinfectant/antiparasitic activity (Beyioku et al., 1998). The extracts of dried leaves, dried latex and dried seeds of *J. curcas* have been investigated and proved to have antibacterial activity (Grosvenor et al., 1995). Therefore based on the above facts, that no scientific study have been carried out in the leaf of *J. curcas* regarding the antimetastatic activity, the present study has been carried out to investigate and evaluate the effect of methanolic fraction of *J. curcas* against B16F10, a highly metastatic melanoma cell line induced metastasis in C57BL/6 mice.

MATERIALS AND METHODS

Chemicals

Fetal Bovine Serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), penicillin–streptomycin (PS) and glutamine were purchased from GIBCO laboratories, Grand Island, NY. Doxorubicin, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetra zolium bromide; Thiazolyl blue), Glycyl glycine and γ-glutamyl-4-nitroanilide were purchased from Sigma St. Louis, USA. Hydroxyproline, N-acetyl neuraminic acid (NANA), Glucosamine hydrochloride and Glucuronic acid were purchased from SISCO Research Laboratory, Mumbai, India. All other reagents were of analytical reagent grade.

Animals

Six to eight weeks old male C57BL/6 mice were purchased from National Institute of Nutrition, Hyderabad, India. The animals were housed in well-ventilated cages kept in air-controlled rooms during the experiment. They were fed with normal mouse chow (Sai Durga Feeds, Banglore, India) and water *ad libitum*. All the animal experiments were performed according to the rules and regulations of Animal Ethics Committee, Government of India.

Cells

B16F10, a highly metastatic melanoma cell line was purchased from National Centre for Cell Sciences, Pune, India. Cells were maintained in DMEM containing 2 mM L- glutamine, supplemented with 10% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin and incubated in a humidified atmosphere with 5% CO_2 at 37°C.

Extraction

The plant material of *J. curcas* leaf used for the investigation was collected from Nursery of Central Research Institute (Siddha), Arumbakkam, Chennai–106. The plant was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai and a voucher specimen is kept in the herbarium.

The leaves were dried under shade and powdered in a mechanical grinder. The powdered material was extracted with methanol by soxhlet extraction. The methanol extract was concentrated using rotary evaporator and dried under vacuum. As the methanolic extract contains different type of phytochemicals, it was fractionated by hexane, pet ether, chloroform, ethyl acetate and methanol. The various fractions were tested against a panel of cell lines representative of tumors from a variety of tissue types. Preliminary screening of the different solvent fractions of *J. curcas* for cytotoxicity was studied against murine B16F10 melanoma cells and human cancer cell lines Lung (NCIH460), Colon (HCT116), Liver (HepG2) and Cervical (HeLa) using MTT assay method.

The methanolic fraction of *J. curcas* was found to be active with better cytotoxic activity in the panel of tested cancer cell lines when compared to that of other fractions of *J. curcas*. Further preliminary phytochemical tests have been carried out for all the fractions (Data not shown in the manuscript). The methanolic fraction was found to be positive for the presence of flavonoids (Harborne, 1973; Trease and Evans, 1996). So the methanolic fraction was used for further investigation.

MTT assay

Growth of B16F10 melanoma cells in the presence of Methanolic Fraction of *J. curcas* (MFJC) was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) assay. This assay was performed as described in the modified method of Mosmann (1983). Cells were seeded in 96 well microculture plate at 2×10^3 cells per well and allowed to adhere overnight. Cells were exposed to 0, 2.5, 5, 10, 50, 100 and 200 µg/ml of MFJC for 48 h. MTT was prepared at a concentration of 5 mg/ml in sterile phosphate buffered saline system (PBS). A 20 µl aliquot of the stock solution of MTT was added to each well. After 3 h of incubation at 37°C, 150 µl of DMSO was added to each well in order to dissolve the formazan crystals. Optical absorbancy were measured at 570 nm using a 96 well Spectramax Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). The results obtained were calculated and presented as a percentage of control values.

In vivo study

Highly metastatic B16F10 melanoma cells (5×10^5 cells/ 0.2 ml PBS) was injected to each animal via lateral tail vein on day 0 (Kobayashi et al., 1998). The animals were divided into four groups, comprising twelve animals in each group. An additional group of the same strain of animals (n = 6) were also kept without any treatment or tumour and used for the estimation of normal levels of different biochemical parameters. Group I was administered 2% v/v aqueous Tween 80, which served as control. In Group II and III MFJC at 100 and 200 mg /kg was administered orally to animals, simultaneously with the induction of metastasis and continued for 14 days. Group IV served as standard, which received Doxorubicin 4 mg/kg, i.p (Injections were on days 0, 4, 8 and 12 from tumour inoculation). Six animals from each group were sacrificed after 15 days of tumour induction, the lungs were dissected out and blood was collected. The number of blackish metastatic colonies present over the entire surface of the five lobes of the lung was counted under a dissecting microscope. Then the lung tissue was subjected to the estimation of collagen hydroxyproline (Bergman and Loxley, 1970), hexosamines (Elson and Morgan, 1933) and uronic acid (Bitter and Muir, 1962). The serum sialic acid levels of all the animals were determined according to the procedure of Skoza and Mohos (1976). The serum α-glutamyltranspeptidase (γ-GT) (Szasz, 1976) levels were also assayed and expressed as units per litre.

Effect of methanolic fraction of *J. curcas* on the survival rate of metastatic tumour bearing animals

Six animals of each group from the above experiment were observed for their survival rate. The mortality of each animals were observed and the percentage increase in life span (% ILS) was calculated from the formula:

% ILS = [(T- C/C)] × 100

Where T represents the number of survival days of treated animals

and C represent the number of survival days of control animals.

Histopathology of lungs

Lung tissues were fixed in 10% formaldehyde, dehydrated and embedded in paraffin wax. 4 µm sections were then stained with hematoxylin and eosin (H & E) mounted in DPX and examined under a microscope.

Statistical analysis

Statistical analysis results are presented as Mean ± S.D. Statistical evaluation of data was performed by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical test

Preliminary phytochemical screening of the methanolic fraction of the *J. curcas* leaves revealed the presence of flavonoids.

Effect of MFJC on the lung tumour nodule formation

Earlier, it has been reported that B16F10 cells mainly form lung tumours (Engbrin et al., 2002). Administration of the methanolic fraction of *J. curcas* reduced pulmonary metastasis formation of B16F10 melanoma cells. Vehicle treated control animals developed massive number of tumour nodules on their lungs and the number of countable colonies were (264.67 ± 25.53). There was a significant ($P < 0.01$) reduction in the lung tumour nodule formation when the animals were simultaneously treated with MFJC at 100 and 200 mg/kg, p.o by 47.54 and 69.52% respectively in a dose dependent manner (Table 1). It was reduced to 30.66 ± 3.78 countable colonies by the doxorubicin treatment. The survival rate of the animals also increased to 43.14 and 66.25% by the simultaneous treatment with MFJC at 100 and 200 mg/kg, p.o respectively (Table 1). In doxorubicin treated group the survival rate of animals increased to 123.48%. In *in vitro* cytotoxic assay MFJC at tested concentrations (0 – 200 µg/ml) showed significant reduction in the proliferation of melanoma cells and the GI_{50} was found to be 24.8 µg/ml (Figure 1). Histopathology of the lung also showed marked reduction in tumour mass in the lungs of treated animals (Figure 2; a - e).

Effect of MFJC on the lung collagen hydroxyproline content

Effect of MFJC treatment on the lung biochemical parameters is shown in Table 2. The lung collagen hydroxy-

Table 1. Effect of methanolic fraction of *J. curcas* on lung colonization of B16F10 melanoma cells and survival of the animals[a].

Treatment	No. of metastatic colonies	% Inhibition	No. of days survived	%ILS[b]
Vehicle Control	264.67 ± 25.53	-	36.92 ± 5.1	-
MFJC 100 mg/kg, p.o	138.83 ± 8.66*	47.54	52.85 ± 6.2*	43.14
MFJC 200 mg/kg, p.o	80.67 ± 7.74*	69.52	61.38 ± 7.7*	66.25
Doxorubicin 4 mg/kg,i.p	30.66 ± 3.78*	88.41	82.51 ± 8.6*	123.48

Values are expressed as Mean ± SD. MFJC- methanolic fraction of *J. curcas*. [a] The lungs were dissected out and observed for metastasis on 15th day after induction of B16F10 melanoma (5 × 10^{5} cells). MFJC treatment (100 and 200 mg/kg) started simultaneously with tumour cell induction through the oral administration (14 doses at 24 h interval, p.o). [b]Increase in life span = (T-C/C) × 100, where T and C are the number of days survived by the treated and control (vehicle treated) group of animals respectively. *$P < 0.01$ statistically significant when MFJC treated groups and doxorubicin groups are compared with vehicle control. Values are expressed as Mean ± SD.
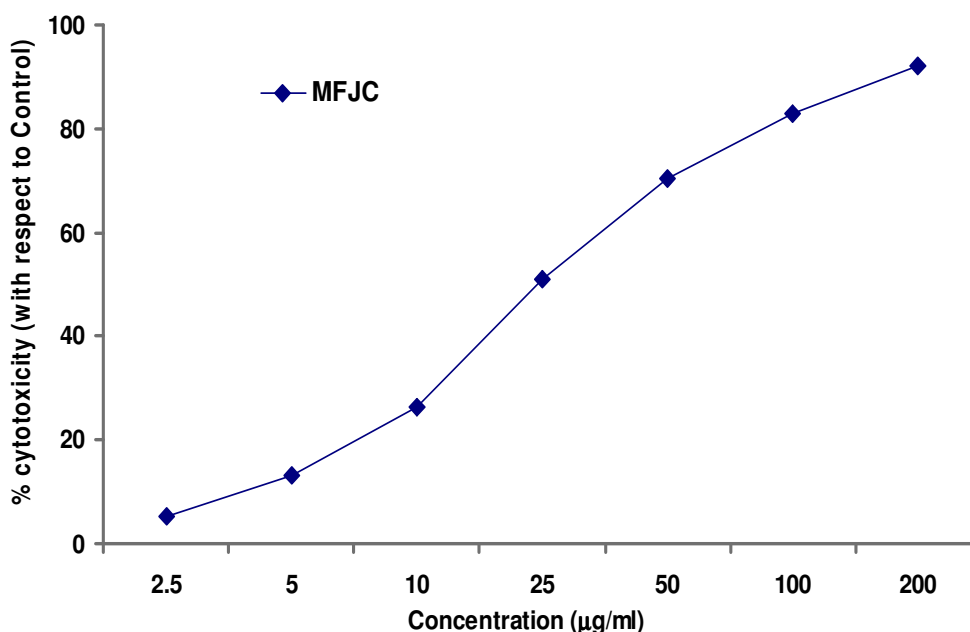

Figure 1. Effect of methanolic fraction of *J. curcas* on B16F10 melanoma cell proliferation. MFJC-methanolic fraction of *J. curcas*. B16F10 cells were treated with different concentrations of MFJC (0-200μg/ml) for 48h. Control cells were maintained in the medium for the indicated time period. Results are expressed as a percentage of vehicle-treated control and Mean ± S.D of three separate experiments.

proline content was drastically elevated in the control group compared to that of normal level indicating the fibrosis of lung tissue. This elevated level was significantly ($P < 0.01$) reduced in the animals treated with MFJC 100 and 200 mg/kg, p.o in a dose dependent manner indicating the decreased metastatic lung fibrosis, comparable with that of doxorubicin.

Effect of MFJC on the lung uronic acid content

Tumour bearing control animals showed elevated levels of uronic acid in the lung tissue when compared with that

of normal control (Table 2). Oral administration of MFJC at a dose of 100 and 200 mg/kg body weight significantly ($P < 0.01$) reduced these elevated levels in lung tissue. The results were found to be in a dose dependent manner, comparable with that of doxorubicin.

Effect of MFJC on the lung hexosamine content

There was an increased level of lung hexosamine content in the control tumour bearing animals, when compared with that of normal animals (Table 2). Treatment with MFJC at 100 and 200 mg/kg, p. o significantly ($P < 0.01$)

Table 2. Effect of methanolic fraction of *J. curcas* administration on the lung biochemical parameters of metastasis bearing animals.

Treatment	Lung collagen hydroxy proline (μg/mg protein)	Lung uronic acid (μg/100 mg)	Hexosamines (mg/100 mg)
Normal	1.84 ± 0.42	36.41 ± 3.6	0.61 ± 0.1
Vehicle Control	18.27 ± 1.38	244.92 ± 15.1	5.14 ± 0.68
MFJC 100 mg/kg, p.o	10.16 ± 0.98*	152.39 ± 7.4*	2.46 ± 0.18*
MFJC 200 mg/kg, p.o	7.18 ± 0.82*	108.13 ± 9.1*	1.53 ± 0.16*
Doxorubicin 4 mg/kg, i.p	4.93 ± 0.28*	71.24 ± 6.8*	1.18 ± 0.05*

Values are expressed as Mean ± SD. MFJC- methanolic fraction of *J. curcas.* *P < 0.01 statistically significant when MFJC treated groups and doxorubicin groups are compared with vehicle control.
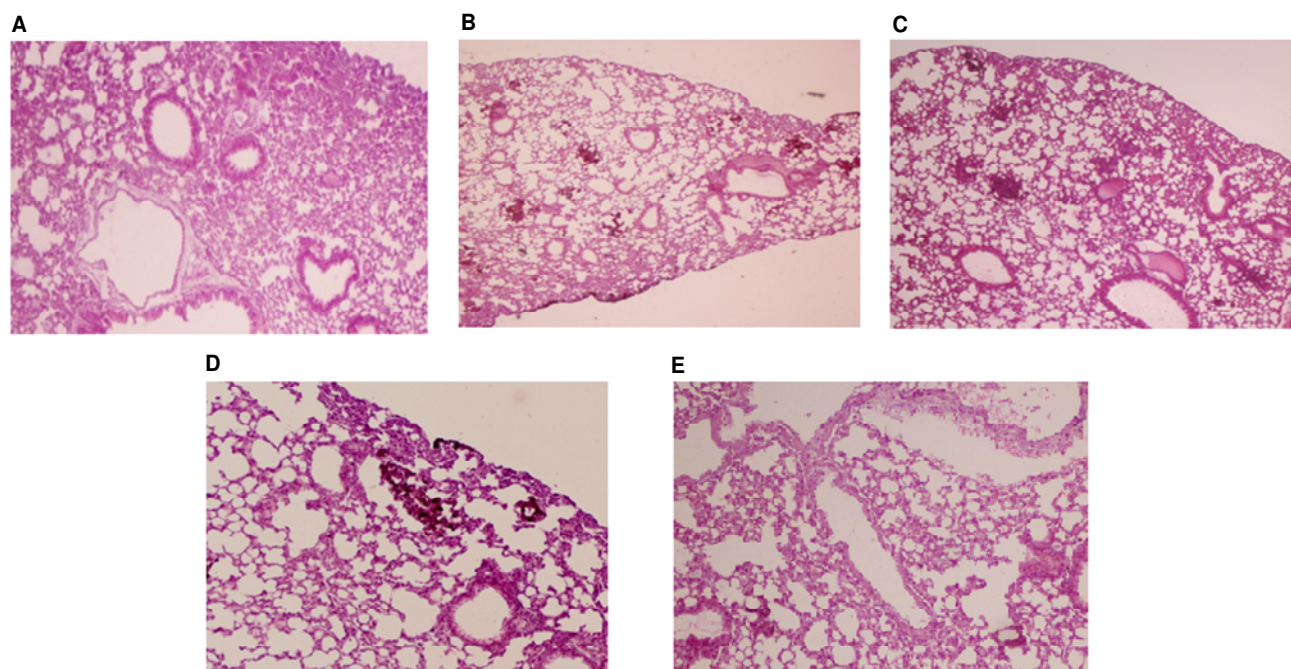

Figure 2. Histopathology of lung of metastatic tumour bearing animals (H and E – 100×). Lungs of the metastasis induced animals were fixed in formalin, and 4 μm sections were taken and stained with hematoxylin and eosin. A) Normal lung, B) Vehicle Control, C) MFJC – 100 mg/kg, p.o, D) MFJC – 200 mg/kg, p.o, E) Doxorubicin 4 mg/kg, i.p.

reduced the elevated lung hexosamine content indicating the decreased tumour burden. The results were comparable with that of doxorubicin.

Effect of MFJC on the serum sialic acid level

The serum sialic acid level was drastically elevated in the control group when compared to that of normal animals (Figure 3). MFJC (100 and 200 mg/kg, p.o) treated animals showed a significant (P < 0.01) reduction in the sialic acid content. The elevated sialic acid levels in the metastatic tumour bearing animals were significantly (p < 0.01) reduced to near normal upon treatment with doxorubicin.

Effect of MFJC on serum GGT level

The γ-GT activity in the control animals was very high as compared to the γ-GT level in the serum of the normal animals (Figure 4). The higher content of γ GT was significantly (P < 0.01) reduced to near normal, when the animals were simultaneously treated with MFJC at tested dose levels and the results were comparable with that of doxorubicin.

Histopathological analysis of lung

The hematoxylin and eosin stained sections of lung tissues are shown in Figure 2 A-E (×100). The lung from
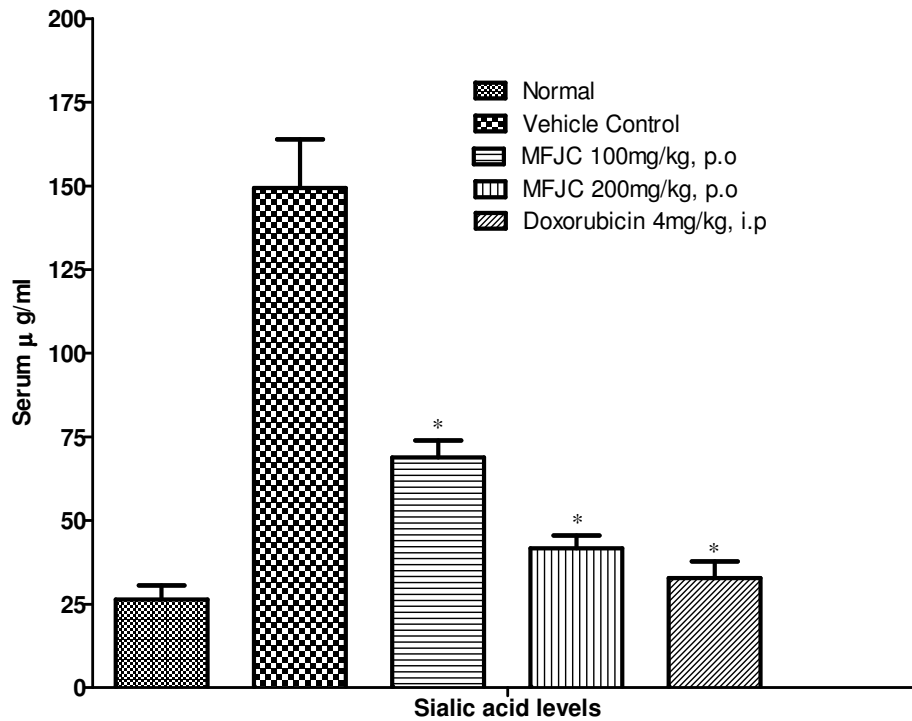
Figure 3. Effect of methanolic fraction of *J. curcas* administration on serum sialic acid levels of metastasis induced animals. Values are expressed as Mean ± SD. MFJC-methanolic fraction of *J. curcas*. *P < 0.01 statistically significant when MFJC treated groups and doxorubicin groups are compared with vehicle control.
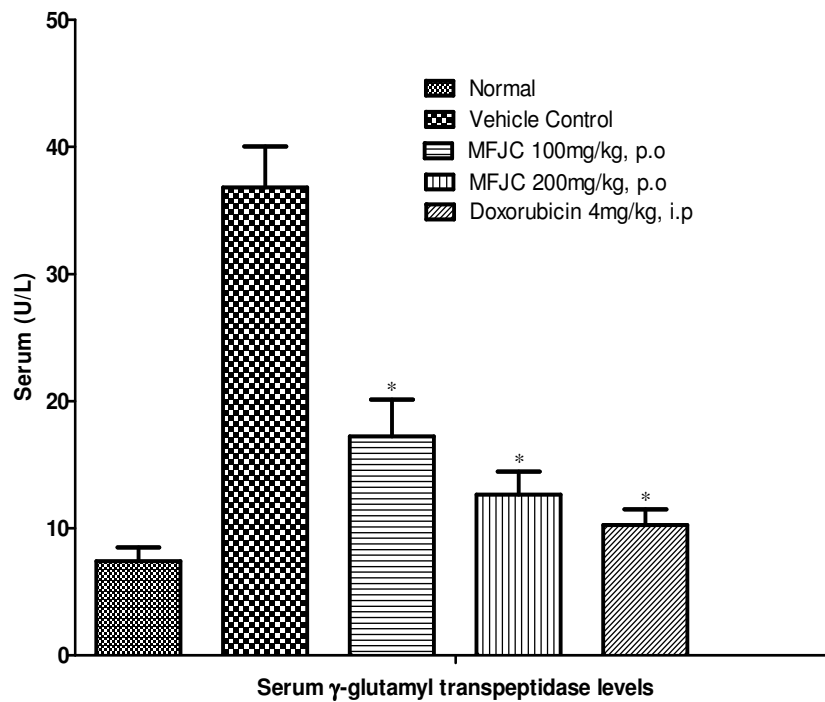

Figure 4. Effect of methanolic fraction of *J. curcas* administraton on serum γ-glutamyl transpeptidase levels of metastasis induced animals. Values are expressed as Mean ± SD. MFJC- methanolic fraction of *Jatropha curcas*. *P < 0.01 statistically significant when MFJC treated groups and doxorubicin groups are compared with vehicle control.

healthy normal animal shows normal architecture of lungs with bronchioles, alveoli and interstitium (Figure 2a). The lungs of vehicle control animals (Figure 2b) showed massive tumour growth around the bronchioles and infiltration of metastatic colonies of melanoma in the interstitium of the lung. Increased fibrosis reduces alveolar space, which leads to reduction in vital capacity of the lung. Simultaneous administration of MFJC at 100 and 200 mg/kg, p.o showed significant reduction in tumour mass and regeneration of alveolar passage with ciliated columnar epithelial cells (Figure 2c and d). Lungs of the Doxoubicin treated animals were almost similar to the healthy normal lung (Figure 2e).

MFJC- methanolic fraction of *J. curcas*

B16F10 cells were treated with different concentrations of MFJC (0 – 200 μg/ml) for 48 h. Control cells were maintained in the medium for the indicated time period. Results are expressed as a percentage of vehicle-treated control and Mean ± S.D of three separate experiments.

[a]The lungs were dissected out and observed for metastasis on 15th day after induction of B16F10 melanoma (5×10^5 cells). MFJC treatment (100 and 200 mg/kg) started simultaneously with tumour cell induction through the oral administration (14 doses at 24 h interval, p.o).

[b]Increase in life span = (T-C/C) × 100, where T and C are the number of days survived by the treated and control (vehicle treated) group of animals respectively.

*$P < 0.01$ statistically significant when MFJC treated groups and doxorubicin groups are compared with vehicle control.

DISCUSSION AND CONCLUSION

B16F10 melanoma cells are highly metastatic and form colonies of tumour nodules in the lungs when administered through tail vein, which in turn promote lung fibrosis and collagen deposition. In the earliest post-injection time points, the majority of cells find themselves in the pulmonary tissue, but some are also localized in other organs. After 14 days, only the lungs contain B16 cells, now seen as tumour nodules and none of the other tissues show the establishment of tumours (Fidler, 1970). In the present study, we analyzed the anti-metastatic activity of methanolic fraction of *J. curcas* in B16F10 melanoma induced lung metastasis. Oral administration of MFJC at 100 and 200 mg/kg, p.o resulted in marked reduction in the metastasis by B16F10 melanoma. Exuberant collagen deposition within the lung is a key marker of fibrosis (Phan, 2003). The lung fibrosis was evaluated by estimating the lung collagen hydroxyproline content, because during lung fibrosis collagen is deposited massively in the alveolus of lungs.

Fifteen to thirty percent of collagen is hydroxyproline (Voet and Voet, 1995) and accumulation of extracellular matrix, especially collagen, will reduce the pulmonary function. There was an elevated level of lung collagen hydroxyproline content in metastasis bearing animals. Treatment with MFJC at 100 and 200 mg/kg, p.o, caused significant reduction in the accumulation of collagen, as estimated from the hydroxyproline content. These findings correlate with the reduction in the number of lung tumour nodules in the treated groups. Histopathological analysis of lungs correlates with these findings. Hyaluronan (HA) is a nonsulfated, linear glycosamino-glycan, consisting of repeating units of (β,1-4)-D-glucu-ronic acid-(β,1-3)-N-acetyl-D-glucosamine (Brinck and Heldin, 1999) and is one of the chief components of the extracellular matrix, contributes significantly to cell proliferation and migration and may also be involved in the progression of some malignant tumours (Stern, 2004). The acidic and basic modifications of monosac-charides yield uronic acids (glucuronic acid) and amino sugars (hexosamines) and they form a vital part in many structural polysaccharides and glycosaminoglycans (GAG) found in the ECM. Tumour cells can induce the host stromal cells to supplement the matrix components necessary for the growing tumour (McKinnell et al., 1998).

Concentration of HA is elevated in several cancers regardless of the tumour grade (Hautmann et al., 2000; Setala et al., 1999). The degradation products of hyalu-ronic acid (HA), oligoHA, are also known to stimulate endothelial-cell proliferation and to promote neovascula-rization associated with angiogenesis which in turn promotes tumour metastasis and tumour directed angiogenesis (West et al., 1985; Slevin et al., 2002). There was an elevated level of these monosaccharides in the control metastatic tumour bearing animals which clearly indicates the active growth and proliferation of tumour cells. Administration of methanolic fraction of *J. curcas* significantly reduced the uronic acid and hexosamine content in the tumour bearing animals. The inhibitory effect of methanolic fraction of *J. curcas* on proliferation of B16F10 melanoma cells (*in vitro*) strongly supports these results. Hexosamine has an important role in the synthesis of sialic acid, a family of acetylated derivatives of N-acetyl neuraminic acid, occurs as a terminal component of carbohydrate chain of glycol-proteins and gangliosides. It forms structural component of glycolipids present on the surface of tumour cells (Voet and Voet, 1995; Erbil et al., 1985). Various studies have reported the significance of sialic acid as a tumour marker in various cancers including melanoma. Aberrant glycosylation processes in tumour cells contribute to the biosynthesis of certain oligosaccharides; hence, malign-nant or transformed cells contain increased sialic acid residues on their surfaces (Yogeeswaran, 1981; Hulbert et al., 1979).

Sialic acid levels also served to monitor treatment of

cancer. Cancer cells that can metastasize often have a lot of sialic acid-rich glycoproteins. This helps these late-stage cancer cells enter the blood stream (Baxi et al., 1990; Tewarson et al., 1993; Patel et al., 1997; Bathi et al., 2001). In the present study, sialic acid level was drastically increased in the control metastatic tumour bearing animals when compared to that of normal animal. The elevated sialic acid levels were significantly reduced in the animals treated with methanolic fraction of *J. curcas*. Serum γ-glutamyltranspeptidase (GGT) is also a marker of neoplastic proliferation. A close association between the metastatic ability of B16 melanoma sub lines and expression of membrane-associated GGT has been reported previously (Prezioso et al., 1993). GGT cata-lyses intracellular GSH break down and provide energy to the tumour cells by γ-glutamyl cycle (West et al., 1985). High level of GGT was found in the serum of control metastatic tumour bearing animals when compared with that of normal animals. Administration of methanolic fraction of *J. curcas* at 100 and 200 mg/kg, p.o signi-ficantly reduced the serum GGT level. All these results correlated with the inhibition of lung tumour nodules and the increase in life span of metastatic tumour bearing animals, when treated with methanolic fraction of *J. curcas*. Histopathological analysis also supports these findings. Cancer metastasis, which involves multiple processes and various cytophysiological changes, is a primary cause of cancer death. Currently available therapeutic drugs have limited effects on metasatic tumours. Therefore, there is an urgent need for novel therapeutic approaches to treat tumour metastasis. Search for effective agent from plant resources, such as flavonoids and another phytochemicals, for treatment of cancer metastasis has become one of the top priorities in cancer research. Flavonoids, a family of phytochemical compounds, are widely distributed in foods of plant origin such as vegetables, fruits and in medicinal plants, display a wide range of pharmacological properties including antioxidative, anti-inflammatory, antiproliferative, anti-mutagenic, anti-carcinogenic and anti-cancer effects (Kuo, 1997; Birt et al., 2001; Ren et al., 2003; Yamamoto and Gaynor, 2001; Li et al., 2007).

Accumulating evidence from *in vitro* and animal studies suggest that some plant flavonoids possess potent cancer chemoprevention activities, anti-invasive activities *in vitro* and antimetastasis activities in animal models (Taniguchi et al., 1992; Caltagirone et al., 2000; Iwashita et al., 2000). Recently more research has been focused on the role of flavonoids in cancer prevention because epidemiological investigations suggest that increased intake of fruits and vegetables are associated with the reduced risks of certain cancers. In preliminary phyto-chemical studies the methanolic fraction of *J. curcas* used in this study showed positive results for the presence of flavonoids, which correlates with earlier reports. Thus, the antimetastatic activity may be in part due to the presence of these flavonoids. Further studies have to be conducted to find out the exact mechanism of action of the phyto-

chemical(s) in inhibiting the cascade of event of metastasis.

REFERENCES

Bathi RJ, Nandimath K, Kannan N, Shetty P (2001). Evaluation of glycoproteins as prognosticators in head and neck malignancy. Indian. J. Dent. Res. 12: 93-99.

Baxi BR, Patel PS, Adhavaryu SG (1990). A report on clinical importance of serum glycoconjugates in oral cancer. Indian. J. Clin. Biochem. 5: 139-144.

Bergman I, Loxley R (1970). The determination of hydroxyproline in urine hydrolysate. Clin. Chim. Acta. 27: 347-349.

Beyioku AF, Oyibo WA, Anuforom BC (1998). Disinfectant/antiparasitic activities of *Jatropha curcas*. East. Afr. Med. J. 75: 508-511.

Birt DF, Hendrich S, Wang W (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol. Ther. 90(2-3): 157-177.

Bitter T, Muir HM (1962). A modified uronic acid carbazole reaction. Analyt. Biochem. 62(4): 330-334.

Brinck J, Heldin P (1999). Expression of recombinant hyaluronan synthase (HAS) isoforms in CHO cells reduces cell migration and cell surface CD44. Exp. Cell. Res. 252(2): 342-351.

Caius JF (1986). The Medicinal and Poisonous Plants of India. Scientific Publishers, Jodhpur. pp. 212-216.

Caltagirone S, Rossi C, Poggi A, Ranelletti FO, Natali PG, Brunetti M, Aiello FB, Piantelli M (2000). Flavonoids Apigenin and Quercetin Inhibit Melanoma Growth and Metastatic Potential. Int. J. Cancer. 87(4): 595-600.

Cavallaro U, Christofori G (2001). Cell adhesion in tumour invasion and metastasis: loss of the glue is not enough. Biochim. Biophys. Acta. 1552: 39-45.

Chhabra SC, Mahunnah RLA, Mshiu EN (1990). Plants used in traditional medicine in Eastern Tanzania. III. Angiosperms (Euphorbiaceae to Menispermaceae). J. Ethnopharmacol. 28(3): 255-83.

Diepgen TL, Mahler V (2002). The epidemiology of skin cancer. Br. J. Dermatol. 146(61): 1-6.

Duke JA (2001). Handbook of Medicinal Herbs. CRC Press, London. pp. 253-5.

Elson LA, Morgan WTJ (1933). A colorimetric method for determination of glucosamine and chondrosamine. J. Biochem. 27: 1824-8.

Engbrin JA, Hoffman M.P, Karmand AJ, Kleinman HK (2002). The B16F10 cell receptor for a metastasis promoting site on Laminin-1 is a heparan sulfate / chondroitin sulfate containing proteoglycan. Cancer Res. 62: 3549–3554.

Erbil KM, Jones JD, Klee GG (1985). Use and limitations of serum total and lipid-bound sialic acid concentrations as markers for colorectal cancer. Cancer. 55(2): 404-409

Fidler IJ (1970). Metastasis: quantitative analysis of distribution and fate of tumour emboli labeled with 125I-5-iodo-2′-deoxyuridine. J. Natl. Cancer. Inst. 45: 773-782.

Fidler IJ, Hart IR (1982). Biological diversity in metastatic neoplasm: origins and implications. Science. 217: 998-1001.

Garbe C, McLeod GR, Buettner PG (2000). Time trends of cutaneous melanoma in Queensland, Australia and Central Europe. Cancer. 89: 1269-1278.

Grosvenor PW, Supriono A, Gray DO (1995). Medicinal Plants from Riau province, Sumatra, Indonesia. Part II: antibacterial and antifungal activity. J. Ethnopharmacol. 45: 97-111.

Gupta MP, Monge A, Karikas GA, Cerain A, Solis PN, Leon E, Trujillo M, Suarez O, Wilson F, Montenegro G, Noriega Y, Santana AI, Correa M, Sanchez C (1996). Screening of Panamanian medicinal plants for brine shrimp toxicity, crown gall tumour inhibition, cytotoxicity and DNA intercalation. Int. J. Pharmacog. 34: 19-27.

Ha KT, Kim JK, Lee YC, Kim CH (2004). Inhibitory effect of Daesungki-Tang on the invasiveness potential of hepatocellular carcinoma through inhibition of matrix metalloproteinase-2 and -9 activities. Toxicol. Appl. Pharmacol. 200: 1-6.

Harborne   JB (1973). Phytochemical method. A Guide to Modern

techniques to Analysis, 2nd Edn. Chapman and Hall, New York. pp. 10-75.

Hautmann SH, Lokeshwar VB, Schroeder GL, Civantos F, Duncan RC. Friedrich MG, Soloway MS (2001). Elevated tissue expression of hyaluronic acid and hyaluronidase validate HA-HAase urine test for bladder cancer. J. Urol. 165: 2068-2074.

Hufford CD, Oguntimein BO (1978). Non-polar constituents of *Jatropha curcas.* Lloydia. 41: 161-65.

Hulbert KBS, Karim AK, Elizabeth LA, Fernando AS (1979). Serum sialic acid and sialyltransferase as monitors of tumour burden in malignant melanoma patients. Cancer. Res. 39: 5036-5042.

Iwashita K, Kobori M, Yamaki K, Tsushida T (2000).Flavonoids inhibit cell growth and induce apoptosis in B16 melanoma 4A5 cells. Biosci. Biotechnol. Biochem. 64(9): 1813-1820.

Kambu K, Tona L, Kaba S, Cimanga K, Mukala N (1990). Antispasmodic activity of extracts proceeding of plant anti-diarrhea traditional preparations used in Kinshasa, Zaire. Ann. Pharm. Fr. 48: 200-208.

Khafagy SM, Mohamed YA, Abdel Salam NA, Mahmoud ZF (1977). Phytochemical study of *Jatropha curcas*. Planta Med. 31(3): 274-277.

Khan M, Jain DC, Bhauni RS, Zaim M, Thakur RS (1991). Occurrence of some antiviral sterols in *Artemisa annua*. Plant. Sci. 75: 161-165.

Kobayashi M, Kobayashi H, Pollard RB, Suzuki F (1998). A pathologic role of Th2 cells and their cytokine products on the pulmonary metastasis of murine B16 melanoma. J. Immunol. 160(12): 5869-5873.

Kuo SM (1997). Dietary flavonoid and cancer prevention: evidence and potential mechanism. Crit. Rev. Oncog. 8(1): 47-69.

Lens MB, Dawes M (2004). Global Perspectives of Contemporary Epidemiological Trends of Cutaneous Malignant Melanoma. Br. J. Dermatol. 150(2): 179-185.

Li Y, Fang H, Xu W (2007). Recent advance in the research of flavonoids as anticancer agents. Mini. Rev. Med. Chem. 7(7): 663-678.

Liotta LA, Kohn EC (2003). Invasion and metastasis. In: Kufe DW, Pollock RE, Weichselbaum RR, Bast Jr RC, Gansler TS, Holland JF, Frei III E (eds) Holland Frei Cancer Medicine 6. BC Decker Inc. Hamilton, Canada. Chapter 122.

Mays SR, Nelson BR (1999). Current therapy of cutaneous melanoma. Cutis. 63: 293-298.

McKinnell RG, Parchment RE, Perantoni AO, BurryPierce G (1998). The biological basis of cancer. Cambridge University Press, UK.

Morton DL, Essner RE, Kirkwood JM, Wollman RC (2003). Malignant melanoma. In: Kufe DW, Pollock RE, Weichselbaum RR, Bast Jr RC, Gansler TS, Holland JF, Frei III E (eds) Holland Frei Cancer Medicine 6. BC Decker Inc: Hamilton, Canada, Chapter 10.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth. 65: 55-63.

Patel PS, Raval GN, Patel MM, Balar DB, Patel DD (1997).Importance of glycoproteins in human cancer. Indian. J. Biochem. Biophys. 34: 226-233.

Phan SH (2003). Fibroblast phenotypes in pulmonary fibrosis. Am. J. Respir. Cell Mol. Biol. 29 (3): S87-S92.

Poste G, Fidler IJ (1980). The pathogenesis of cancer metastasis. Nature. London. 283: 139-146.

Prezioso JA, Wang N, Duty L, Bloomer WD, Gorelik E (1993). Enhancement of pulmonary metastasis formation and gamma-glutamyltranspeptidase activity in B16 melanoma induced by differentiation *invitro.* Clin. Exp. Metastasis. 11: 263-274.

Ren W, Qiao Z, Wang H, Zhu L, Zhang L (2003). Flavonoids: Promising anticancer agents. Med. Res. Rev. 23(4): 519-534.

Rusthoven JJ, Quirt IC, Iscoe NA, McCulloch PB, James KW, Lohmann RC, JensenJ, Burdette-Radoux S, Bodurtha AJ, Silver HK, Verma S, Armitage GR, Zee B, Bennett K (1996). Randomized, double-blind, placebo-controlled trial comparing the response rates of carmustine, dacarbazine, and cisplatin with and without tamoxifen in patients with metastatic melanoma. National Cancer Institute of Canada Clinical Trials Group. J. Clin. Oncol. 14: 2083-2090.

Saxena S, Sharma R, Rajore S and Batra A (2005). Isolation and identification of flavonoid "Vitexin" from *Jatropha curcas* L. J. Pl. Sci. Res. 21: 116-117.

Setala LP, Tammi MI, Tammi, RH, Eskelin, MJ, Lipponen, PR, Argen, UM, Parkkinen, J, Alhava, EM, Kosma, VM (1999). Hyaluronan expression in gastric cancer cells is associated with local and nodal spread and reduced survival rate. Br. J. Cancer. 79: 1133-1138.

Skoza L, Mohos S (1976). Stable thiobarbituric acid chromophore with dimethyl sulphoxide. Application to sialic acid assay in analytical de-o-acetylation. J. Biochem. 159: 457-462.

Slevin M, Kumar S, Gaffney J (2002). Angiogenic Oligosaccharides of Hyaluronan Induce Multiple Signaling Pathways affecting Vascular Endothelial Cell Mitogenic and Wound Healing Responses. J. Biol. Chem. 277: 41046-41059.

Stern R (2004). "Hyaluronan catabolism: a new metabolic pathway". Eur. J. Cell. Biol. 83(7): 317-325.

Subramanian SS, Nagarajan S, Sulochana N (1971). Flavonoids of some euphorbiaceous plants. Phytochem. 10(10): 2548-2549.

Szasz G (1976). Reaction rate method for g-glutamyl transpeptidase activities in serum. Clin. Chem. 22: 2031-2055.

Taniguchi S, Fujiki H, Kobayashi H, Go H, Miyado K, Sadano H, Shimokawa R (1992). Effect of (7) epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. Cancer. Lett. 65: 51-54.

Tewarson SL, Mittal VP, Singh M, Gupta GP (1993). Serum sialic acid-an important cancer marker. Indian. J. Cancer. 30: 125-131.

Trease GE, Evans WC (1996). Phenols and Phenolic glycosides In: Trease and Evans Pharmacognosy, 13th Edn. Balliere, Tindall, London. pp. 832-833.

Voet D, Voet JG (1995). Biochemistry. New York: John Wiley and Sons. pp. 157-258.

West DC, Hampson IN, Arnold F, Kumar S (1985). Angiogenesis induced by degradation products of hyaluronic acid. Science. 228:1324-6.

Yamamoto Y, Gaynor RB (2001). Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. J. Clin. Invest. 107(2): 135–142.

Yang S, Chen J, Guo Z, Xu XM, Wang L, Pei XF, Yang J, Underhill CB, Zhang L (2003). Triptolide inhibits the growth and metastasis of solid tumours. Mol. Cancer. Ther. 2: 65-72.

Yogeeswaran G (1981). Metastatic potential is positively correlated with cell surface sialylation of cultured murine cell lines. Science. 212: 1514-1516.