RESEARCH COMMUNICATION

Effect of Homeopathic Medicines on Transplanted Tumors in Mice

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Abstract

Ultra low doses used in homeopathic medicines are reported to have healing potential for various diseases but their action remains controversial. In this study we have investigated the antitumour and antimetastatic activity of selected homeopathic medicines against transplanted tumours in mice. It was found that Ruta graveolens 200c and *Hydrastis canadensis* 200c significantly increased the lifespan of Ehrlich Ascites Carcinoma and Dalton's Lymphoma Ascites induced tumour-bearing animals by 49.7%, and 69.4% respectively. Moreover there was 95.6% and 95.8% reduction of solid tumour volume in Ruta 200c and Hydrastis 200c treated animals on the 31st day after tumour inoculation. Hydrastis 1M given orally significantly inhibited the growth of developed solid tumours produced by DLA cells and increased the lifespan of tumour bearing animals. Some 9 out of 15 animals with developed tumors were completely tumour free after treatment with Hydrastis 1M. Significant anti-metastatic activity was also found in B16F-10 melanoma-bearing animals treated with Thuja1M, Hydrastis 1M and Lycopodium1M. This was evident from the inhibition of lung tumour nodule formation, morphological and histopathological analysis of lung and decreased levels of γ-GT in serum, a cellular marker of proliferation. These findings support that homeopathic preparations of Ruta and Hydrastis have significant antitumour activity. The mechanism of action of these medicines is not known at present.

Key words: Alternative medicine - homeopathy - hydrastis – lycopodium - melanoma - metastasis - ruta - thuja

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Introduction

The use of alternative medicines and the exploration of their uses against cancer are gaining importance. Homeopathic medicines are being practiced as a major alternative system in various diseases including cancer. Although it is widely accepted among people, the effectiveness and the mechanism of these drugs are still controversial. In Homeopathy, the drugs used are in the form of dynamized preparations. These dynamized preparations are made by repeated agitation (succession). According to homeopathic theories these dynamized preparations have the same property of the mother tincture. Several experimental evidences support the effectiveness of homeopathic medicines in cancer. Effectiveness of homeopathic medicines Chelidonium and Lycopodium in ameliorating p-dimethyl aminoazobenzene induced and phenobarbital promoted hepatocarcinogenesis in mice was reported earlier (Biswas et al., 2005; Pathak et al., 2006). Prostate tumour xenografts were found to be significantly reduced by *Sabal serrulata* (MacLaughlin et al., 2006). Selected homeopathic remedies were reported to significantly slow the progression of cancer and reduce cancer incidence and mortality in Copenhagen rats injected with MAT-LyLu prostate cancer cells (Jonas et al., 2006). Hydrastis at different potencies was found to increase the life span of ascites tumour bearing animals (Maliekal, 1997). N'nitrosodiethylamine (NDEA) induced hepatocarcinogenesis in rats was found to be inhibited by Ruta 200c, Hydrastis 200c and Lycopodium 200c (Harikumar et al., 2007). In a clinical study, Pathak et al (2003) found Ruta 6 to inhibit glioma growth in brain cancer patients. Homeopathy has been reported as a supportive therapy in cancer (Rajendran, 2004).

Transplanted tumours in animals are effective methods to find out the efficacy of the drugs against cancer and several models have been suggested. In this study we have determined the action of homeopathic medicines – Ruta 200C, Hydrastis 200C, Thuja 1M, Lycopodium 1M and Hydrastis 1M against solid tumour formation as well as metastasis of tumour in animal models. Results indicated that some of the preparations are highly active in reducing tumour incidence, developed tumour volume as well as inhibition of metastasis.

Materials and Methods

Homeopathic medicines

Ruta 200c, Hydrastis 200c and Thuja 200c in ethanol were purchased from Willmar Schwabe, Germany.

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Thuja1M, Hydrastis1M and Lycopodium1M in water were a gift from Boiron laboratories, France. Successed water was purchased from Similia Homeo Laboratories, Aluva, India.

Chemicals

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Himedia Laboratories, Mumbai, India. Fetal Calf Serum was purchased from Biological Industries, Israel. All other reagents used were of Analytical Reagent grade.

Cells

Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cells were originally obtained from the Cancer Institute, Adayar, Chennai, and are being maintained in our laboratory in the peritoneal cavity of Swiss Albino mice.

B16F-10 melanoma cells, a highly metastatic cell line was obtained from the National Centre for Cell Science, Pune, India. The cells are maintained in DMEM supplemented with 10% Foetal Calf Serum and antibiotics. These cells were also grown subcutaneously as transplantable solid tumour in C57BL/6 mice.

Animals

Inbred Swiss Albino mice and C57BL/6 male mice (4-6 weeks old) were purchased from the National Institute of Nutrition (Hyderabad, India), housed in well-ventilated cages and provided with normal mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. The experiments were performed according to the rules and regulations of the CPSCEA, Government of India.

Determination of effect of homeopathic medicines on ascites tumour development in mice.

Three groups of Swiss albino mice (8 animals/ group) were used for the experiment. Ascites tumours were induced in animals by injecting $1x10^6$ Ehrlich Ascites Carcinoma cells to the peritoneal cavity. Group I served as untreated control with EAC cells alone. Group II was treated with Ruta 200c 10mL/dose/animal orally. Group III was treated orally with Hydrastis1M (100µL/dose/animal). A lowered dose was used for Ruta 200 c as this is prepared in ethanol. Drug administration was started 24 hrs after tumour inoculation and continued everyday for 10 consecutive days.

In another set of experiments, ascites tumour in mice was induced by injecting $1x10^6$ Dalton's lymphoma cells and the experiment was conducted as for Ehrlich ascites carcinoma cells.The death pattern of the animals due to tumour burden was noted everyday and the percentage of increase in lifespan was calculated using the formula T-C/C x 100 were 'T' and 'C' are the average number of days the animals survived in treated and the untreated group respectively.

Determination of effect of homeopathic medicines on solid tumour development.

DLA cells ($1x10^6$/animal) were injected subcutaneously to the hind limbs of 3 groups (8 animals/ group) of Swiss albino mice to develop as solid tumour. Group I served as untreated control. Group II received Ruta 200c (10µL) orally and Group III received Hydrastis 200c (10µL) orally. Drug administration was started from the first day of tumour inoculation and continued for 12 consecutive days. Initial diameter of the hind limb was noted using vernier calipers. The tumour diameter was measured from the 7th day and continued on every 3rd day for 31 days. The tumour volume was calculated using the formula $V= 4/3\pi r1^2r2$, where r1 and r2 are the radius of the tumour at two different sites (Kuttan et al., 1985).

Determination of the effect of homeopathic medicine Hydrastis 1M on developed tumour

Thirty numbers of adult Swiss albino mice were injected on the hind limb with DLA cells (1 million) in 0.1 ml phosphate buffered saline. After 30 days, when tumour had developed to volume of 2cc they were divided into 2 groups (15 animals/ group). Hydrastis1M (100µL) was given orally for 20 consecutive days for the first group. Second group of animals were treated with potentiated water prepared in glass bottle. Diameter of tumour was measured every 3rd day using vernier caliper and volume was calculated using the formula $V= 4/3\pi r1^2r2$. Death of the animals due to tumour burden in each group was noted and increase in lifespan was calculated as given above.

Determination of the effect of homeopathic medicine Thuja 1M, Hydrastis 1M and Lycopodium 1M on the metastasis of B16F-10 melanoma cells.

Male C57BL/6 mice weighing 20-25 g were separated into 5 groups of 10 animals per group. Metastasis was studied by injecting B16F10 melanoma cells (10^6 cells / animal) via the lateral tail vein. Group I was kept as untreated control. Group II, III, IV and V received the succussed water, Thuja1M, Hydrastis1M and Lycopodium1M (100µL/day/animal) respectively for 10 consecutive days from the day of tumour cell inoculation. The animals were sacrificed 21 days after tumour inoculation. Lungs were excised and lung tumour nodules were counted. The lungs were used for histopathological analysis. The blood was collected and the serum was analyzed for γ-glutamyl transpeptidase levels, a marker for cell proliferation.

Statistical analysis

The results are expressed as mean ± SD. Statistical evaluation of the data was done by ANOVA followed by Dunnet's test (Post-hoc) using Graphpad In Stat 3 software package.

Results

Effect homeopathic medicine Ruta 200c and Hydrastis 1M on survival rate of ascites tumour bearing animals.

The treatment with homeopathic medicines- Ruta 200c and Hydrastis1M significantly increased lifespan of ascites tumour bearing animals induced by EAC cells. Administration of Ruta 200c increased the lifespan of tumour bearing animals by 49.7% whereas the Hydrastis 1M increased the lifespan by 69.4%. When the Ruta 200c

Table 1. Effects of Ruta 200c and Hydrastis 1M on Lifespan of Ascites Tumour Bearing Animals.

Treatment	EAC cells		DLA cells	
	Mean survival (days)	Percentage increase in lifespan	Mean survival (days)	Percentage increase in lifespan
Control	14.3 ± 1.5	---	18.5 ± 1.9	---
Ruta 200c	21.5 ± 4.1*	49.7%	27.3 ± 6.9**	47.7%
Hydrastis1M	24.1 ± 4.8***	69.4%	ND	

*p<0.05, **p<0.01, ***p<0.001 ND = Not determined

was administered to DLA tumour bearing animals, the increase in lifespan was found to be 47.7% (Table 1).

Effect of simultaneous treatment of homeopathic medicine Ruta 200c and Hydrastis 200c on solid tumour development induced by DLA cells.

A significant reduction in solid tumour volume was found in both Ruta 200c and Hydrastis 200c treated animals when compared to that of control animals. In Ruta 200c treated group there was 54.8% reduction in tumour volume on day 19th .On 31st day, reduction in tumour volume was 95.6% (Figure 1). In Hydrastis 200c treated group the reduction in tumour volume was 53.1% on day 19th and on 31st day the decrease in tumour volume was 95.8% (Figure 1).

Effect of homeopathic medicine Hydrastis 1M on DLA -

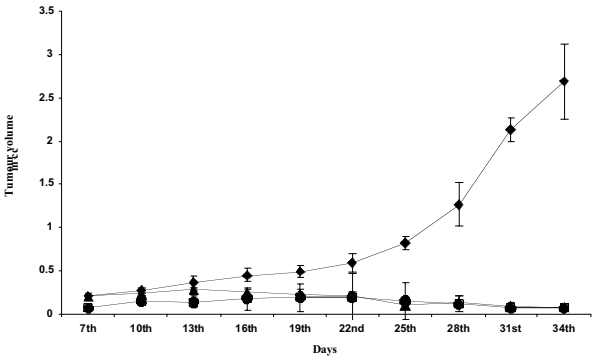

Figure 1. Effect of Ruta 200c (squares) and Hydrastis 200c (triangles)on Solid Tumour Progression with Ehrlich Ascites Carcinoma cells (diamonds for control)

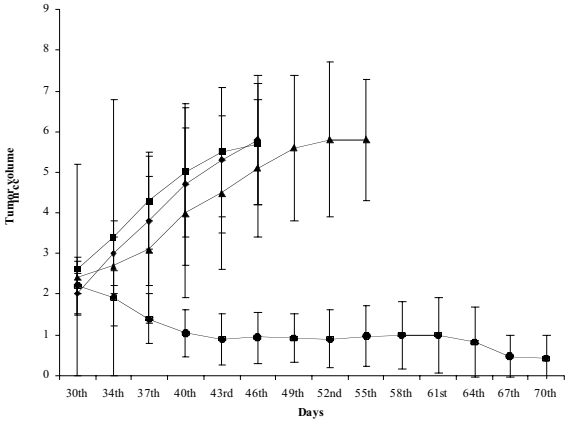

Figure 2. Effect of Hydrastis 1M on Development of Solid Tumors with Dalton's Lymphoma Ascites Cells (circles for fully and triangles for partial responders)

Table 2. Effect of Homeopathic Medicines on Lung Tumour Nodules and Serum γGT Activity

Treatment	No. of nodules per lung	Serum GGT (U/L)
Control	>250	36.14 ± 1.80
Distilled water	>250(massive)	33.23 ± 1.36
Thuja 1M	110.5 ± 14.3***	5.29 ± 0.45***
Hydrastis 1M	183.4 ± 16.2***	32.90 ± 1.70***
Lycopodium 1M	146.5 ± 20.2***	11.53 ± 1.20***

***p<0.001

induced developed solid tumour

There was significant reduction in tumour volume of the developed tumours in the animals treated with Hydrastis1M (Figure 2). It was found that 9 out of 15 animals did not increase their tumour after starting drug treatment. 6 out of 15 animals increased tumour volume inspite of Hydrastis treatment. In the control group all the animals increased their tumour volume and died by 55 days. . But in treated group only 4 animals died by 70 days and 6 animals within 95 days while all other animals remained tumour free up to 120 days and thereafter (Figure 3).

Antimetastatic effect of homeopathic medicines Thuja 1M, Hydrastis 1M and Lycopodium 1M

The morphology of lung of control group showed numerous lung nodules as black spots due to melanin deposition. In the animals treated with succussed water also there was significant melanin deposition. In the animals treated with Thuja 1M the lung morphology was similar to that of normal. There was also significant inhibition of metastasis and melanin deposition in the Lycopodium 1M and Hydrastis 1M treated groups.

The effect of homeopathic medicines on tumour nodule formation after inoculation with B16F-10 melanoma cells is given in Table 2. Both control as well as animals treated with succussed water had massive tumour nodule in their lungs, which were more than 250. Animals treated with homeopathic medicines had less number of nodules per lung, which in the case of Thuja1M was 110.5, Hydrastis1M- 183.4 and Lycopodium1M-146.5 indicating that administration of homeopathic medicines significantly inhibited lung tumour nodule formation. Percentage decrease in the tumour nodules was

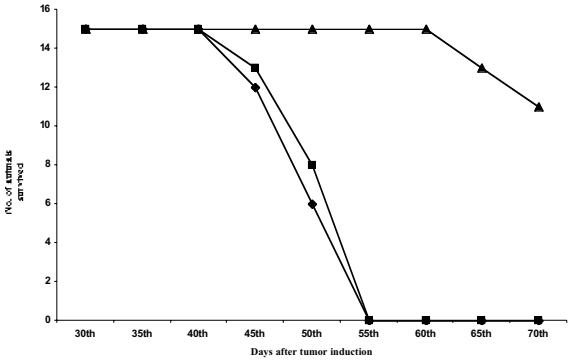

Figure 3. Effect of Hydrastis 1M I (triangles) on the Survival of Animals with Developed Tumours

55.8% in the case of Thuja1M, 26.6% in the case of Hydrastis1M and 14.4 % in the case of Lycopodium1M (Table 2).

Serum level of γ-glutamyl transpeptidase (GGT) activity which increases during cell proliferation was 36.14 units in untreated animals. (Table 2) which was almost similar to that of animals treated with successed water alone (33.23 units). Administration of Thuja1M reduced the γ-glutamyl transpeptidase activity to 5.29 units, which is almost closer to the normal level. Lycopodium1M treated animals also had lowered g-glutamyl transpeptidase activity (11.53 units) and in Hydrastis1M treated group the activity was found to be 32.9 units.

Histopathology of the lungs of control animals and the animals treated with succussed water showed massive tumours in the intra-alveolar regions of the lungs. In the case of animals treated with Thuja1M and Lycopodium1M the alveolar region of the lungs was almost free of tumours.

Discussion

Homeopathic medicines are being used to treat various diseases including cancer. Various homeopathic drugs have undergone clinical trials in human patients and were reported to be effective against various diseases (Rastogi et al., 1993; Banerji and Banerji, 2001; Pathak et al., 2003; Rajendran, 2004). Invitro and invivo analysis to elucidate the possible mechanism of action of the homeopathic drugs have been reported and a hypothetical conclusion that the drugs acts at molecular level through the activation/ inactivation of particular genes (Khuda-Bukhsh, 1997).

In present study it was found that potentiated Ruta and Hydrastis possessed significant antitumour activity in both ascites as well as solid tumour models. Moreover Hydrastis 1M was found to produce significant tumour reducing activity on developed solid tumours. The treatment with Hydrastis 1M significantly increased the lifespan of developed tumour bearing animals. The mechanism of inhibition of solid tumour is not known at present.

There is a cascade of events leading to the metastasis of tumours (Fidler et al., 1978; Poste and Fidler, 1980). Multiple mechanisms of genes and proteins are involved in the tumour cell metastasis. In our study it was found that the homeopathic medicines such as Thuja 1M inhibited metastasis of B16F-10 melanoma cells significantly, which is evident from the morphological analysis as well as from histopathological sections. Formation of lung nodules and deposition of melanin pigment were found to be inhibited in the drug treated animals. It has been already reported that there is a direct link between the metastatic ability of B16F-10 melanoma cells and the expression of membrane associated gamma glutamyl transpeptidase enzyme (Prezioso et al., 1993). The γ-GT activity in the homeopathic medicine-treated animals was found to be significantly lowered than that of control and the vehicle treated animals, which gives biochemical evidence for the effectiveness of homeopathic medicines in inhibiting metastasis.

The mechanism of action of potentiated drugs is not known at present. It was found that Ruta 6 induces damage to cancer cells while not affecting the normal cells. Hence the preferential action of Ruta 6 on glioma cells was reported through loss of telomeric DNA, followed by cell cycle arrest at G2/M phase, induction of endomitosis and fragmentation of DNA leading to death (Pathak et al., 2003). We have reported that some of the mother tinctures and potentiated drugs could inhibit growth of cultured cells also some of the potentiated drugs are capable of inducing apoptosis and Carcinosinum 200c was found to express p53, a proapoptotic gene in DLA cells (Sunila et al., 2007). Ruta 200c was found to induce chromosomal aberrations invivo in bone marrow cells after long term feeding (unpublished data). Therefore these drugs may be acting directly on DNA. Other possible mechanism includes immunomodulatory action of homeopathic medicines (Ramachandran et al., 2007) and through the activation or suppression of complex genes (Khuda-Bukhsh, 1997), which are involved in the progression of cancer. However Thangapazham et al., (2006a, 2006b) could not find any alteration in cell proliferation or mRNA expression after incubation with homeopathic drugs. More studies are needed in order to elucidate the mechanism of action of homeopathic medicines drugs.

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