

FUNDAMENTAL RESEARCH

Inhibition of Chemically Induced Carcinogenesis by Drugs Used in Homeopathic Medicine*

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Homeopathy is considered as one modality for cancer therapy. However, there are only very few clinical reports on the activity of the drugs, as well as in experimental animals. Presently we have evaluated the inhibitory effects of potentized homeopathic preparations against N'-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma in rats as well as 3-methylcholanthrene-induced sarcomas in mice. We have used Ruta, Hydrastis, Lycopodium and Thuja, which are commonly employed in homeopathy for treating cancer. Administration of NDEA in rats resulted in tumor induction in the liver and elevated marker enzymes such as gamma-glutamyl transpeptidase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and alkaline phosphatase in the serum and in liver. Concomitant administration of homeopathic drugs retarded the tumor growth and significantly reduced the elevated marker enzymes level as revealed by morphological, biochemical and histopathological evaluation. Out of the four drugs studied, Ruta 200c showed maximum inhibition of liver tumor development. Ruta 200c and phosphorus 1M were found to reduce the incidence of 3-methylcholanthrene-induced sarcomas and also increase the life span of mice harboring the tumours. These studies demonstrate that homeopathic drugs, at ultra low doses, may be able to decrease tumor induction by carcinogen administration. At present we do not know the mechanisms of action of these drugs useful against carcinogenesis.

Keywords: N'-Nitrosodiethylamine; hepatocellular carcinoma; sarcoma; homeopathy; Ruta; Thuja; Hydrastis; Lycopodium; phosphorus

Introduction

Hepatocellular carcinoma (HCC) is one of the major diseases affecting the liver. The etiology of HCC involves viruses, schistosomal infections and chemicals. Of the chemicals, which induce HCC, includes fungal toxins and poly aromatic hydrocarbons (Blum, 2005). The limited options and poor treatment success make HCC one of the leading causes of death. As HCC is difficult to treat several approaches are made towards its prevention (Jorge, 2005). Sarcoma is a term used to describe a whole family of cancers that arise in the body's connective tissues, which include fat, muscle, blood vessels, deep skin tissues, nerves, bones, and cartilage. It accounts for nearly 1-2% of all cancers in adults.

Homeopathy a nearly 200-year-old system of medicine works on the principle of "Similia similibus curentur" which means let likes be cured by likes

(Walach et al., 2005; Attarwala et al., 2006). Homeopathic medicines use ultra low doses of drugs for treatment. Dilutions are such that even the physical existence of a single molecule of the original compound is theoretically impossible. Hence the mechanisms of action of homeopathic drugs are different from other drugs useful in therapy. Homeo medicines are cheaper, easy to administer and are without any known side effects. There are only a few reports on the activity of homeopathic drugs in experimental cancers and cell cultures. Ruta 6 has been shown to selectively induce cell death in brain cancer cells and produces instability of telomeres (Pathak et al., 2003). Ruta 200c and the methanolic extract of Ruta graveolens extract was found to possess antitumor activity against Dalton's Lymphoma ascites and Ehrlich carcinoma ascites tumor cell induced ascites as well as solid tumors in mice (Preethi et al., 2006). Lycopodium at homeopathic dose was found to be hepatoprotective against CCl4 induced liver damage (Sur et al., 1990). Recently inhibition of

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carcinogenesis induced by azo dye in rat liver was seen by the administration of Chelidonium and Lycopodium (Biswas *et al.*, 2004; Pathak *et al.*, 2006). Thuja has been found to be effective against prostate cancer. Another homeo drug Hydrastis was reported to inhibit Dalton's Lymphoma Ascites induced solid tumor in mice (Maliekal, 1997).

In the present investigations we have evaluated the anticarcinogenic activity of some of the potentized homeopathic preparations using two different models. In the first model the effect of Ruta, Hydrastis, Lycopodium and Thuja at 200c potencies were tested against nitrosodiethylamine induced hepatocellular carcinoma in rats and in the second model the effect of Ruta 200c and phosphorus 1M against 3-methyl cholanthrene induced sarcoma in mice was studied.

Materials and Methods

Animals

Young female Wistar rats (5-6 week old, 180 ± 20g weight) and Swiss albino mice (6-8 week old, 25 ± 3g weight) were purchased from Small Animal Breeding Station, Kerala Agricultural University, Thrissur. They were housed in well-ventilated polypropylene cages under controlled temperature, and humidity, and were provided with normal mouse chow (Sai Durga Feeds and Foods, Bangalore) and water *ad libitum*. Animal experiments were conducted after getting prior permission from Institutional Animal Ethics Committee (IAEC) and as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

Chemicals

N'-Nitrosodiethylamine (NDEA), 3-methyl cholanthrene, glycyl-glycine and γ -glutamyl p-nitroanilide were purchased from Sigma-Aldrich Inc, St. Louis, USA. 2,4-Dinitrobenzene and reduced glutathione were obtained from SRL, Mumbai. All other reagents were used in the study were of analytical grade.

Homeo drugs

Ruta 200c, Lycopodium 200c, Thuja 200c, and Hydrastis 200c in ethanol were obtained from Willmer-Schawbe, Germany. Phosphorus 1M in water was a gift from Boiron Laboratories, France. The vehicle alcohol was a gift from Similia Laboratories, Aluva, India. Sterile water (30c in glass) was purchased from Kerala State Homeopathic Co-operative Pharmacy, Alappuzha, India.

Effect of selected potentized homeo drugs on the induction of hepatocellular carcinoma by NDEA

Wistar rats were divided into following groups: Group I, Normal, without any treatment (n=6); II, Control, NDEA alone (n=15), III. Vehicle (ethanol) + NDEA (n=15); IV Ruta 200c + NDEA (n=15); V Lycopodium 200c + NDEA (n=15); VI, Thuja 200c + NDEA (n=15).

Drug administration

NDEA (0.02%) was prepared fresh in distilled water, every day and animals from group II-VI were given NDEA at a dose of 2.5mL/rat/dose, 5 days a week for 20 consecutive weeks by oral gavage. This dosage was found to produce liver cancer in rats within 20 weeks (Rajeshkumar and Kuttan, 2000). All the homeo drugs were administered once daily at a dose of 50 μ L/animal/dose, 5days in a week for the same period by gavage.

Administration of NDEA and homeo drugs was stopped at the 20th week and animals were kept under observation for another 9 weeks and then sacrificed under light ether anesthesia. Gross necropsies of animals were performed to assess visible morphological changes. Blood was collected from each animal through heart puncture into heparinized and non-heparinized tubes for separating plasma and serum.

Parameters assessed

a) Survival rate, morphology and weight of the liver
Survival rate of animals in each group was monitored every day. Livers from each animal were excised after sacrifice, washed in ice-cold saline (0.9%) and observed for tumour nodules and other orphological abnormalities. Weight of each liver was recorded and was expressed as liver weight/100g body weight.

Enzyme analysis in the liver and blood. A 25% homogenate of the liver tissue was prepared in cold Tris- HCl buffer (0.1M, pH 7.4). Protein content was determined by the method of Lowry *et al.*, 1951; γ -glutamyl transpeptidase activity was assayed by the method of Tate and Meister, 1974 Total Bilirubin (Malloy and Elyen, 1937), alkaline phosphatase (ALP) (Kind and King, 1954), glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) (Reitman and Frankel, 1957) were assayed using commercially available kits (Span Diagnostics, Gujarat, India). Levels of reduced glutathione (GSH) were estimated in packed RBCs by the method of Moron *et al.*, 1979. Estimation of γ -glutamyl transpeptidase, Total bilirubin, ALP, SGOT and SGPT in the serum were assayed as described previously.

Histopathological analysis. A portion of the excised liver was fixed in 10% buffered formalin for sectioning followed by staining with hematoxylin-eosin for histopathological evaluation.

Effect of potentized homeo drugs on 3-methyl cholanthrene (3MC) induction of sarcomas

Swiss albino mice were divided into following groups: Group I, Control, water + 3-MC (n=20); II, Ruta 200c + 3-MC (n=20); III, Phosphorus 1M + 3-MC (n=20). 3-MC was dissolved in DMSO. To the shaven ventral surface of each mouse 3-MC was administered at a dose of 200µg/animal/dose subcutaneously (s.c). All the animals were treated with homeo drugs next day after the initial injection of 3-MC. Water and Phosphorus 1M were given 100µL/ dose/day and Ruta 200c at a dose of 10µL/day/dose. Treatment continued for 6 days in a week for consecutive 20 weeks through oral gavage. The animals were then observed for onset of sarcoma and the survival time for 30 weeks.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Significance levels of comparison of differences were determined by one-way ANOVA followed by post- hoc Bonferroni's multiple comparison test using Graphpad Instat software.

Results

Effect of selected potentized homeo drugs on the induction of hepatocellular carcinoma by NDEA

The morphological analysis of the livers of control animals showed a number of tumor nodules on the surface with variable shapes, normal morphology was completely lost, necrotic mass was seen at some places, and some tumor nodules showed irregular protuberances from their surfaces. The livers of vehicle treated animals also exhibited same morphological characters as the controls. The livers of Ruta treated animals showed fewer incidences of tumor nodules. They retained the normal morphology of the liver with small necrotic masses seen in the large lobe of some animals. The livers of Lycopodium, Hydrastis and Thuja treated animals showed decreased number of tumor nodules when compared with that of control animals.

The effect of administration of homeo drugs on the survival rate of hepatocellular carcinoma harboring animals is shown in Table 1. There was no significant change in the survival of animals at 29th week treated with homeo drugs. The administration of NDEA significantly elevated the liver weights of all the animals as compared with normal untreated animals.

Administrations of homeo drugs were ineffective in controlling the increased the liver weight induced by the NDEA (Table 1).

Table 1. Effects of Selected Homeo Drugs on the Survival and Liver Weights of NDEA-treated Animals

Group	Number of animals surviving	Liver weight (g)/100g body weight
Normal	6/6	2.34 ± 0.38
Control (NDEA alone)	11/15	5.55 ± 2.67*
Vehicle (Ethanol)	12/15	6.06 ± 2.15*
Ruta	13/15	5.15 ± 0.96*
Lycopodium	13/15	5.13 ± 2.09*
Hydrastis	12/15	5.04 ± 0.88*
Thuja	12/15	5.55 ± 0.68*

Data are expressed as mean ± S.D. *p<0.001, with respect to the normal, untreated group

The activity of the enzyme γ -glutamyl transpeptidase (γ -GT), a marker of cellular proliferation was found to be significantly elevated in the serum and the liver of all the NDEA treated animals (Table 2). Almost similar increases were observed in the liver tissue also. Administration of homeo drugs significantly lowered the elevated levels of γ -GT. Ruta showed the maximum decrease (55.4%) followed by Lycopodium, Hydrastis and Thuja. The level of one of the major cellular antioxidant GSH was elevated in the blood after NDEA administration (Table 2) and administration of Ruta 200c caused partial reduction.

The treatment of NDEA also elevated the activities of three major marker enzymes of hepatic function viz. ALP, GPT and GOT in the serum and the liver (Tables 3 and 4). ALP activity of the liver also showed a similar pattern (Table 4). The level of total bilirubin, which is increased in both the serum and the tissue by NDEA, was also decreased by the treatment of homeo drugs.

Histopathological analysis

Histopathological evaluation of the livers of NDEA alone (control) animals showed extensive areas of necrosis, hemorrhage and multiple Foci of carcinomatous changes. Hepatocytes were in degenerating condition. Vehicle treated group also

Table 2. Effects of Selected Homeo Drugs on the Levels of Serum and Liver γ -GT and Serum GSH

Group	γ -GT Serum (U/L)	γ -GT Liver (nmoles/mL)	GSH (nmol/min/mg protein)
Normal	22.3 \pm 1.99	0.25 \pm 0.05	16.8 \pm 1.08
Control (NDEA)	79.1 \pm 6.70 ^a	5.07 \pm 1.39 ^a	35.0 \pm 3.94 ^a
Vehicle(Ethanol)	93.6 \pm 6.13 ^a	4.24 \pm 0.71 ^a	30.0 \pm 3.10 ^a
Ruta	55.4 \pm 4.62 ^{a,b}	1.09 \pm 0.14 ^{a,b}	21.6 \pm 2.36 ^{a,b}
Lycopodium	57.5 \pm 5.00 ^{a,b}	1.58 \pm 0.25 ^{a,b}	27.0 \pm 2.87 ^{a,b}
Hydrastis	58.7 \pm 6.69 ^{a,b}	1.44 \pm 0.16 ^{a,b}	25.0 \pm 3.17 ^{a,b}
Thuja	62.0 \pm 4.02 ^{a,b}	2.03 \pm 0.34 ^{a,b}	26.1 \pm 3.09 ^{a,b}

Data are expressed as mean \pm S.D. ^ap<0.001, with respect to normal, untreated group and ^bp<0.001, with respect to NDEA alone controls

showed similar changes. In case of Ruta treated animals there was no carcinomatous change of the liver. It showed foci of hemorrhage and mild necrosis. The Hydrastis treated group showed mild degree of necrosis, hemorrhage and cellular atypia associated with fatty changes. Lycopodium treated animals also showed same architecture. Thuja treated animals showed areas of necrosis, hemorrhage, with some carcinomatous changes. The results collectively indicated that administration of homeo drugs prevented the NDEA induced hepatocarcinogenesis in rats.

Effect of selected potentized homeo drugs on the induction of 3-methyl cholanthrene induced sarcoma

The administration of Ruta 200c and Phosphorus

1M were found to significantly delayed the sarcoma induction produced by 3MC. By the end of 16th week all the animals in the control group, treated with 3MC alone, developed sarcoma. Only 4 out of 20 animals treated with Phosphorus 1M and 3 out of 20 of the animals treated with Ruta 200c developed sarcoma by the 16th week. At the end of 30th week 5 out of 20 Phosphorus 1M treated and 6 out of 20 Ruta 200c treated group developed sarcomas. The homeo drugs also elevated the survival rate of the animals harboring sarcoma. All animals in the control group died by the end of 20th week. In case of treated animals 14 out of 20 of the Phosphorus 1M treated and 9 out of 20 of the Ruta 200c groups were found to be alive at the end of 30 weeks. The results indicated a significant inhibition of 3- MC induced sarcoma by homeo drugs.

Table 3. Effects of Selected Homeo Drugs on the Serum Bilirubin, ALP, GPT and GOT

Group	Bilirubin (mg/dL)	ALP (KA units)	GOT (units/mL)	GPT (units/mL)
Normal	0.48 \pm 0.15	30.08 \pm 5.31	96.5 \pm 9.9	57.0 \pm 3.58
Control (NDEA)	0.98 \pm 0.30 ^a	64.71 \pm 6.03 ^a	421.3 \pm 11.3 ^a	156.0 \pm 19.6 ^a
Vehicle(Ethanol)	0.94 \pm 0.44 ^{a,b}	56.29 \pm 8.32 ^a	318.2 \pm 27.8 ^a	90.3 \pm 5.48 ^a
Ruta	0.35 \pm 0.12 ^{a,b}	29.92 \pm 3.84 ^{a,b}	218.2 \pm 59.8 ^{a,b}	55.8 \pm 8.05 ^{a,b}
Lycopodium	0.57 \pm 0.20 ^{a,b}	36.31 \pm 4.90 ^{a,b}	238.9 \pm 20.3 ^{a,b}	83.4 \pm 11.22 ^{a,b}
Hydrastis	0.61 \pm 0.11 ^{a,b}	35.36 \pm 4.01 ^{a,b}	209.3 \pm 61.6 ^{a,b}	79.0 \pm 9.70 ^{a,b}
Thuja	0.58 \pm 0.12 ^{a,b}	38.36 \pm 5.00 ^{a,b}	211.8 \pm 64.8 ^{a,b}	74.3 \pm 16.55 ^{a,b}

Data are expressed as mean \pm S.D. ^ap<0.001, with respect to normal, untreated group and ^bp<0.001, with respect to NDEA alone controls

Table 4. Effects of Selected Homeo Drugs on the Liver Bilirubin, ALP, GPT and GOT

Group	Bilirubin (mg/dL)	ALP (KA units)	SGOT (units/mL)	SGPT (units/mL)
Normal	0.38 ± 0.04	7.48 ± 0.86	247.5 ± 37.3	26.5 ± 3.81
Control (NDEA)	1.12 ± 0.17 ^a	28.8 ± 8.24 ^a	683.8 ± 88.3 ^a	196.0 ± 31.7 ^a
Vehicle(Ethanol)	0.85 ± 0.22	28.6 ± 3.46	554.6 ± 61.8	126.3 ± 16.5
Ruta	0.48 ± 0.09 ^{a,b}	13.6 ± 2.33 ^{a,b}	364.6 ± 53.0 ^{a,b}	53.5 ± 3.59 ^a
Lycopodium	0.60 ± 0.11 ^{a,b}	21.0 ± 3.72 ^{a,b}	406.2 ± 65.7 ^{a,b}	69.8 ± 2.95 ^{a,b}
Hydrastis	0.51 ± 0.07 ^{a,b}	17.7 ± 3.70 ^{a,b}	405.3 ± 46.2 ^{a,b}	63.7 ± 3.86 ^{a,b}
Thuja	0.59 ± 0.06 ^{a,b}	22.4 ± 4.27 ^{a,b}	423.2 ± 75.1 ^{a,b}	77.6 ± 7.20 ^{a,b}

Data are expressed as mean ± S.D. ^ap<0.001, with respect to normal, untreated group and ^bp<0.001, with respect to NDEA alone controls

Discussion

Homeopathy is a holistic medicine and it differs from the conventional medicine. Homeopathy administers micro doses of a substance that can produce symptoms of illness in a healthy person when administered in large doses. The conventional treatment modalities are mostly targeting the symptoms of a particular illness, while homeopathic medicines are targeted towards the individual. So homeo drugs, instead of merely removing the disease symptoms in the body, it removes the central disturbance in the individual's energetic balance. Since homeopathy uses micro doses or higher dilutions of the substances most of the time it is difficult to measure its quantity by conventional methods. The dilutions are often goes beyond the Avagadro's concept (Jonas *et al.*, 2003).

In the present study we explored the possible inhibitory role of selected potentized homeopathic preparations on two cancer models. Both Ruta 200c and Phosphorus 1M delayed the sarcoma formation and increased the life span of sarcoma bearing animals. Ruta 200c also showed the maximum inhibition in NDEA induced HCC. Other 3 drugs tested were also found to be effective in decreasing the liver tumor burden. Though the drugs failed to control the liver weight they were able to decrease the elevated marker enzymes levels. Thus the conversion of the normal hepatic cells into neoplastic cells by the treatment of NDEA is prevented by the homeo drugs as seen by the morphological, histopathological and enzymatic evaluation of the liver tissue.

Alkaline phosphatase is a direct marker liver damage and neoplasia. It increases as a result of liver dysfunction and hepatic toxicity. Elevated the activity

of ALP observed in the hepatic cancer model is due to the NDEA induced transformation of normal liver cells into neoplastic cells as well as associated hepatotoxicity. Similarly the elevated activities of GOT and GPT have also been linked to hepatic injury and neoplastic changes. In the present study the elevated levels of these marker enzymes were decreased considerably by the administration of homeopathic drugs thus ameliorating the toxic effects of NDEA.

3-methyl cholanthrene, which comes under the category of polyaromatic hydrocarbons, is oxidized in the body into 1-hydroxy-, 2-hydroxy-, 9,10-dihydrodiol-, and 3-methoxymethyl-methyl cholanthrene (Lu *et al.*, 1990). Both these products form adduct with the DNA that leads to the formation of irregular reading frame, wrong base pair insertion during replication and finally leads to a mutagenic chain of events responsible for tumor initiation. The NDEA is metabolized in the liver and one of the products namely ethyl radical is responsible for the induction of carcinogenesis. Ethyl radical so formed attack the DNA and produce genetic changes which in turn results in carcinogenic changes. It also produces the conversion of certain proto-oncogenes to oncogenes. The action of homeo drugs result in the activation or deactivation of certain signal transduction pathways that could possibly help to elicit further signals which can activate or repress certain transcriptional activities thereby controlling the expression of certain key genes.

How the homeopathic "similimum" administered in micro doses bring such changes to over come the action of carcinogens is still not clear. One possibility is that homeo drugs act through certain high affinity receptors that regulate the expression of specific genes (Khudha-Bukhsh, 1997). Homeo drugs have been

shown to be capable of repairing DNA damage and chromosome damage induced by X-ray and chemical mutation. Since DNA repair is linked to the expression of certain genes there can be a link between the treatment of homeo drugs and gene expression (Khudha-Bukhsh, 1991).

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