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Chemopreventive efficacy of *Wedelia calendulaceae* against 20-methylcholanthrene-induced carcinogenesis in mice

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ARTICLE INFO

Article history:
Received 22 December 2009
Received in revised form
16 August 2010
Accepted 20 August 2010
Available online 26 August 2010

Key words:
Wedelia calendulaceae
Chemoprevention
Antioxidant
Carcinogenesis
20-Methylcholanthrene

ABSTRACT

Present study reports the chemopreventive effect of methanol extract of *Wedelia calendulaceae* (MEWC) against 20-methylcholanthrene (20-MC) induced carcinogenesis in Swiss albino mice. MEWC was administered orally at 250 and 500 mg/kg body weight for 90 consecutive days after 24 h of single subcutaneous administration of 20-MC (200 μg) in mice and observed for 15 weeks to record tumor incidence (fibrosarcoma) and survival. After 15 weeks the mice were sacrificed for the estimation of hematological profiles and liver biochemical parameters viz. lipid peroxidation, reduced glutathione (GSH), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT). MEWC treatment markedly reduced tumor incidence and prolonged life span of sarcoma bearing mice as compared to 20-MC control. Hematological profiles were significantly ($p < 0.001$) restored to normal levels in MEWC treated mice. MEWC treatment significantly ($p < 0.001$) modulated the aforesaid liver biochemical parameters as compared to 20-MC control. Therefore, *W. calendulaceae* possess remarkable chemopreventive efficacy in Swiss mice.

1. Introduction

Cancer is characterized by a rapid and uncontrolled formation of abnormal cells which may mass together to form a growth or tumor, or proliferate throughout the body indicating abnormal growth at other sites. Cancer is considered as one of the most fearsome causes of morbidity and mortality in all over the world (Abdullaev et al., 2000). Although medicinal sciences have made rapid progress in the treatment of carcinogenesis till cancer remains a tragic disease (Nitha et al., 2005).

The term chemoprevention is defined as the use of specific agents (either synthetic or natural) to suppress or reverse carcinogenesis and thereby to prevent the development of cancers (Greenwald and Kellof, 1996). Carcinogenesis being a lengthy multi-step process offers unique opportunity for its prevention. An ideal anticancer agent should be both tissue and cell specific i.e. it should kill or incapacitate cancer cells without causing excessive damage to normal host cells. Unfortunately, currently available cancer chemotherapeutic agents insidiously affect the host cells especially bone marrow, epithelial tissues, reticulo-endothelial system and gonads (Mascarenhas, 1994). Therefore, the advancement of chemoprevention is important, as well as the development of cancer treatment.

It is estimated that about 80% of human cancers are due to environmental factors, principally chemicals. About 2–4% of all cancer deaths are attributed to occupational hazards (Anon., 1995). A variety of chemicals viz., polycyclic aromatic hydrocarbons, nitrosamines, alkylating agents

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doi:10.1016/j.etap.2010.08.003

and other inorganic and naturally occurring compounds are carcinogenic. (Zur Hausen, 1991; Kim et al., 1991). Generally all carcinogens are electrophiles, which attack nucleophilic groups in the DNA and RNA and proteins and thus cause genetic damage to the cell (Levin et al., 1979).

Polycyclic or polynuclear aromatic hydrocarbons (PAH) are an important class of environmental pollutant. PAHs are formed mainly as a result of pyrolytic processes, especially the incomplete combustion of organic materials (whether fossil fuel or biomass) during industrial and other human activities, such as processing of coal and crude oil, combustion of natural gas, refuse, wood, fat, incense, vehicle traffic, cooking and tobacco smoking, as well as in natural processes such as carbonization and these are one of the most widespread air pollutant. As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic. Among the different PAHs, benzo[*a*]anthracenes are known to be carcinogenic. The alkyl substituted derivatives of benzo[*a*]anthracene like 20-methylcholanthrene (20-MC) are potent carcinogens and teratogens (Anon., 1987, 1995; Menzie et al., 1992).

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their therapeutic principles. *Wedelia calendulaceae* Less (synonym: *Wedelia chinensis* Merrill) (Compositae), commonly called *Bhringaraja* in Bengali and Sanskrit is a perennial herb occurring widely throughout India, particularly in humid and coastal areas. It has been traditionally used for several medicinal purposes. The leaves are used in alopecia, juice used for dyeing hair and for promoting hair growth. The leaves are considered tonic, alterative, and useful in cough, cephalalgia, and skin diseases. The whole plant is used as deobstruent; used in uterine haemorrhage, menorrhagia and abdominal swellings, as a tonic for hepatic and spleenic enlargement. An infusion of the plant is given in Indonesia for the swelling of the abdomen. The plant is very specific for viral hepatitis (Kirtikar and Basu, 2001; Khare, 2007; Prajapati et al., 2004). The whole herb contains an oil-soluble black dye, tannins, saponins and phytosterol. The leaves contain isoflavonoids, bisdesmosidic oleanolic acid saponins and wedelolactones (Govindachari and Premila, 1985; Khare, 2007). Wedelolactone, demethyl wedelolactone (Govindachari et al., 1956), norwedelolactone (Bhargava et al., 1970) and norwedelic acid were isolated from its leaves (Govindachari and Premila, 1985). Kauren diterpenes were also isolated from it (Haider et al., 2003). Wedelolactones from *W. calendulaceae* was found to possess 5-lipoxygenase and caspase inhibitory activities (Wagner et al., 1986; Kobori et al., 2004). Previous workers reported wound healing (Hegde et al., 2006), hepatoprotective (Sharma et al., 1989), sedative (Prakash et al., 2008) and post-menopausal anti-osteoporotic effects (Shirwaikar et al., 2006) of *W. calendulaceae* in animal models. In our earlier study we have reported antitumor activity of *W. calendulaceae* against Ehrlich ascites carcinoma in mice (Gupta et al., 2007). Present study was aimed to investigate the cancer chemopreventive effect of methanol extract of *W. calendulaceae* (MEWC) against chemical carcinogenesis in Swiss albino mice.

2. Materials and methods

2.1. Plant material

The mature entire plant of *W. calendulaceae* was collected during November 2008 from 24-Parganas (South), West Bengal, India. The species was identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India, and a voucher specimen (WCP-1) was deposited at Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India. Just after collection the whole plant material was washed thoroughly with water and shade dried at room temperature (24–26 °C) and ground mechanically into a coarse powder.

2.2. Drugs and chemicals

20-Methylcholanthrene, ICN Pharmaceuticals (New York, USA); 5-fluorouracil (5-FU), bovine serum albumin, Sigma Chemical Co., St. Louis, USA; trichloroacetic acid (TCA), Merck Ltd., Mumbai, India; thiobarbituric acid (TBA) and nitroblue tetrazolium chloride (NBT), Loba Chemie, Mumbai, India; 5,5′-dithio *bis*-2-nitro benzoic acid (DTNB), phenazonium methosulphate (PMS), nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH), SISCO Research Laboratory, Mumbai, India. All the other reagents used were of analytical reagent grade obtained commercially.

2.3. Preparation of extract

The powdered plant material (450 g) was macerated at room temperature (24–26 °C) with 80% aqueous methanol (750 ml) for 4 days with occasional shaking, followed by re-maceration with the same solvent for 3 days. The extracts were pooled, filtered and evaporated to dryness *in vacuo* at 40 °C temperature to yield MEWC (9.35%). The dry extract was kept in a vacuum desiccator until use. MEWC was subjected to preliminary phytochemical analysis and planar chromatographic studies (Harborne, 1998) to reveal qualitative composition of MEWC. MEWC was suspended in normal saline as per required concentrations and sonicated for 10 min immediately before administration.

2.4. Animals

Adult male Swiss albino mice weighing 20 ± 2 g were obtained from Rita Ghosh & Co., Kolkata, India. The mice were grouped and housed in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C, relative humidity 55–65%, with dark/light cycle 12/12 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Mumbai, India) and water *ad libitum*. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All experimental methods described were reviewed and approved by the University Animal Ethical Committee, Jadavpur University (367001/C/CPCSEA).