Biomedicine & Preventive Nutrition 4 (2014) 535–541

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Protective role of *Solanum trilobatum* (Solanaeace) against benzo(*a*)pyrene-induced lung carcinogenesis in Swiss albino mice

R. Venugopal*, V. Mahesh , G. Ekambaram , A. Aadithya , D. Sakthisekaran

Department of Medical Biochemistry, Dr.ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 3 July 2014
Accepted 4 August 2014

Keywords:
Solanum trilobatum
Lung cancer
Antioxidants
Marker enzymes
Histopathology
Xenobiotic enzymes and protective effects

ABSTRACT

Objective: To investigate the protective effect of leaf extract of *Solanum trilobatum* (ELEST) against benzo(*a*)pyrene (BP) induced lung carcinogenesis.
Methods: Experiment was designed with the treatment regimen of ELEST [200 mg/kg body weight dissolved in dimethyl sulphoxide(DMSO)] for 4 weeks before (pre-initiation) and from 12th week after B(*a*)P (50 mg/kg body weight) induced lung carcinoma(post-initation).
Results: Administration of BP (50 mg/kg body weight) resulted in increased lipid peroxidation (LPO) and marker enzymes, such as arylhydrocarbon hydroxylase (AHH), gamma-glutamyl transpeptidase (γGT), 5′-nucleotidase (5′ND) and lactate dehydrogenase (LDH) along with decrease in the levels of tissue antioxidants, like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), vitamin E and vitamin C in mice. Significantly, ELEST modulated these alterations suggest the efficacy of ELEST in the chemotherapeutics of lung cancer. The histopathological studies also evidenced the protective efficiency of the extract against lung carcinogenesis. Further, significant increase in the levels of Cytochrome P_{450}, Cytochrome b_5, NADPH Cyt *c* reductase and decrease in UDP-glucuronyl transferase and quinone reductase was observed in microsomal fraction of lung and liver of BP-induced mice, whereas the treatment with ELEST resulted in reversal of modulations observed in the activities of detoxification enzymes.
Conclusions: Collectively, the present observations indicate that the treatment with ELEST exhibited protective and antioxidant effect against BP-induced lung carcinogenesis.

1. Introduction

The reactive oxygen species (ROS) are known to play a major role in either the initiation or progression of carcinogenesis by inducing oxidative stress [1]. A large number of studies support the hypothesis that oxidative damage to DNA, lipids and proteins may contribute to the development of cardiovascular disease, cancer and neurodegenerative diseases [2]. Many naturally occurring compounds with antioxidant potential are known to protect cellular components from oxidative damage and prevent diseases [3]. The genotoxic effects of the toxicants were reported to be minimized by antioxidants through modulating the physiological detoxification processes. A number of such compounds activate the detoxification enzymes, which remove the toxic elements from our system. Exposure to such phytochemicals is therefore beneficial to human health [4]. Medicinal plants have been used for therapeutic purposes since the beginning of civilization. There has been a resurgence of interest in plant compounds with beneficial pharmacological properties [5].

Traditionally, *Solanum trilobatum* Linn has been used for various ailments in different parts of India [6]. Reports have shown that the active principle sobatum isolated from *Solanum trilobatum* possess antitumour effect against chemically-induced tumor [7], antioxidant activity [8], chemotherapeutic efficacy of *solanum trilobatum* along with paclitaxel in lung cancer-bearing Swiss albino mice [9], radiation-induced toxicity [10], carbon tetrachloride-induced hepatic damage [11] and anti-inflammatory effect [12].

BP is well-recognized as complete carcinogen that induces tumors of the lung in laboratory animals when given by subcutaneous injection in the subscapular region [13]. It is well-known that BP are metabolically activated by Cytochrome P450 1A1 (CYP1A1) generates 7,8-diol-9,10-epoxide-benzo(*a*)pyrene, which is believed to be the ultimate carcinogenic metabolite of BP [14] that leads to the formation of DNA adducts.

* Corresponding author. Department of Biochemistry, DM Wayanad Institute of Medical Sciences, Naseera Nagar, Meppadi (PO), Wayanad, Kerala 673577, India. Mob: +91 815 783 0588.
E-mail address: biovenu@gmail.com (R. Venugopal).

http://dx.doi.org/10.1016/j.bionut.2014.08.001
2210-5239/

Several synthetic and naturally occurring compounds have been tested as chemopreventive agents against BP-induced lung tumorigenesis [15,16]. The present study is designed to evaluate the cancer chemopreventive activity exerted by ethanol extract of *Solanum trilobatum* and also for proper understanding of its probable mechanism of action against progression of lung carcinogenesis induced by BP with reference to detoxification systems, ROS and antioxidants status.

2. Materials and methods

2.1. Chemicals

Benzo(*a*)pyrene, 2,4,6-trinitro benzene sulfonate, reduced glutathione (GSH), oxidized glutathione (GSSG), pyrogallol, diethylenetriamine penta acetic acid (DETPA), thiobarbituric acid (TBA), pyruvic acid, reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide Phosphate (NADPH) and bovine serum albumin (BSA) were obtained from Sigma Chemical Company, St. Louis, MO, USA. Ascorbic acid was obtained from Sisco Research Laboratories (SRL), Mumbai, India. 1-Choloro-2,4-dinitrobenzene (CDNB) and 5,5′-dithionitrobenzoic acid (DTNB) were obtained from S.D. Fine Chemicals, Mumbai, India. All other chemicals used of analytical grade and obtained from SRL.

2.2. Preparation of plant extract

Solanum trilobatum was collected from Indian Medical Practitioners Co-operative Pharmacy & Stores Ltd., Chennai, India. To prepare the extract, 500 g of dried leaves of *Solanum trilobatum* were chopped into small pieces and soaked overnight in 1.5 L of 95% ethanol. This suspension was filtered, and the residue was resuspended in an equal volume of 95% ethanol for 4 days and filtered. The extract was then filtered and evaporated in a rotary evaporator under reduced pressure at 60° C and stored in vacuum desiccators.

2.3. Animal experiments

Healthy male Swiss albino mice (*Mus muscullus*) with 20–25 g of body weight selected for the present study. The animals were procured from King Institute of Preventive Medicine, Chennai, India and maintained in a controlled environmental condition of temperature and humidity on alternatively 12 h light/dark cycles. All animals were fed standard pellet diet (Gold Mohor rat feed, M/s Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The Experimental Ethical Committee for Animal Research approved the protocols used in this study.

A total of 30 animals (18 BP-treated animals and 12 normal animals) were divided into 5 groups of 6 animals each. The treatment groups were summarized as follows:

- group 1: normal untreated mice;
- group 2: mice treated with ELEST alone (200 mg/kg b.w.) for 4 weeks;
- group 3: BP-treated animals (50 mg/kg body weight dissolved in corn oil, orally) twice weekly for 4 successive weeks to induce lung cancer;
- group 4: ELEST (200 mg/kg body weight) treated with orally for 4 weeks prior to BP administration;
- group 5: BP-treated animals treated with ELEST after the induction of lung cancer i.e. from the 12th week of BP treatment up to 16th week.

At the end of the experimental period, the animals were fasted overnight and sacrificed by cervical decapitation. The lung and liver tissues were removed and perfused immediately with cold 0.9% NaCl and thereafter carefully removed, trimmed free of extraneous tissue and rinsed in ice-cold 0.01 M Tris–HCl buffer.

The lung and liver homogenate were used for the biochemical analysis, such as lipid peroxidation [17], superoxide dismutase [18], catalase [19], glutathione peroxidase [20], glutathione reductase [21], glutathione [22], vitamin C [23] and vitamin E [24] levels.

The aryl hydrocarbon hydroxylase (AHH) assay was carried out according to the method modified from the Buening et al. [25]. The activity of LDH [26], γ-glutamyl transpeptidase [27], adenosine deaminase [28], 5′-nucleotidase [29], and the levels of polyamines [30] were assayed in the liver and lung homogenate.

The microsomal fraction were prepared by ultracentrifugation at 105,000 × *g* for 60 min (Beckman ultracentrifuge, Model-L870 M) and used for assaying Cytochrome P_{450} and Cytochrome b_5 [31], NADPH Cyt *c* reductase [32], UDP-glucuronyl transferase [33], glutathione *S*-transferase [34] and quinone reductase [35].

The samples for the estimation of histamine, putrescine, spermidine and spermine were prepared using CM-cellulose Column Chromatography. In brief, 100 mg of lung tissues were homogenized in ice-cold 0.4 M $HClO_4$ containing 2 mM EDTA and centrifuged at 3000 rpm for 5 minutes. The neutralized supernatant was applied to a CM-cellulose column. Each sample solution (0.5–3 Ml) was applied to a CM-cellulose column (0.6 × 10 cm) equilibrated with phosphate buffer 0.01 M, pH 6.2. After that the column was washed with 15 mL of 0.01 M phosphate buffer pH 6.2 and 15 mL of phosphate buffer 0.03 M, pH 6.2, histamine, putrescine, spermidine and spermine were eluted out from the column with borate buffer without NaCl (30 mL), borate buffer containing 0.03 M NaCl (20 mL), borate buffer containing 0.075 NaCl (20 mL), and borate buffer containing 0.15 M NaCl (20 mL), respectively. An amount of 3 mL fractions were collected at a flow rate of about 3 mL/min. Then, 1 mL of TNBS reagent was added to the elute (3 mL) from the CM-cellulose column. The reaction was carried out at 50 °C for 10 minutes and terminated by cooling the reaction mixture in water. Then, the absorbance at 420 nm was measured within 20 min

2.4. Histopathological studies

Histopathological analyses were carried out to confirm the induction of tumor in BP-treated mice and to assess the impact of ELEST on tumorigenesis. After sacrificing the mice, all five lobes of lung were collected, washed repeatedly in phosphate buffered saline (PBS) and soaked in blotting paper to remove blood. Tissues were then fixed in 10% neutral buffered formalin for 24 h. The tissue samples were dehydrated in ascending concentration of ethanol, cleared in xylene and embedded in paraffin to prepare the block. Serial sections of five lobes of lung were cut and stained with hematoxylene and eosin for microscopical observation and photomicrographs were taken [36].

2.5. Statistical analysis

All the data were expressed as mean ± SD ($n = 6$). The data for various parameters were tested using ANOVA using SPSS version 7.5 (SPSS Inc., Cary, NC, USA) and the group means were compared by Duncan's multiple range test. Values were considered statistically significant at $P < 0.05$.

3. Results

The antitumour effect of the *Solanum trilobatum* extract on BP-induced lung tumor incidence in mice were represented in Table 1. The animals treated with *Solanum trilobatum* extract