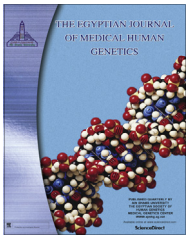
The Egyptian Journal of Medical Human Genetics (2014) **15**, 163–171

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Andrographia paniculata a Miracle Herbs for cancer treatment: *In vivo* and *in vitro* studies against Aflatoxin B1 Toxicity

Md. Sultan Ahmad [a,*], Sheeba Ahmad [b], Mohammad Arshad [c], Mohammad Afzal [d]

[a] *Department of Zoology, S.N. (PG) College, Azamgarh 276001, UP, India*
[b] *Department of Zoology, D.S. College, Aligarh 202002, UP, India*
[c] *Human Molecular Genetics Section, Department of Zoology, Lucknow University, UP, India*
[d] *Human Genetics and Toxicology Laboratory, Department of Zoology, Faculty of Life Science, Aligarh Muslim University, Aligarh 202002, UP, India*

Received 6 December 2013; accepted 29 December 2013
Available online 22 January 2014

KEYWORDS

Andrographia paniculata;
Chromosomal aberration;
Sister chromatid exchange;
Replication index;
Clastogeny

Abstract *Background:* The history of natural products used in ancient times and in folk medicine these days, around the world, is the basis for the use of many therapeutic drugs in modern day medicine. *Andrographia paniculata* belongs to the family Acanthaceae or Kalmegh and is commonly known as 'king of bitters'. It is extensively used as home remedy for various diseases in Indian traditional system as well as in tribal system in India for multiple clinical applications.

Aim: In our present work, extracts of these ayurvedic plants were tested for their anticlastogenic, antimutagenic and anticarcinogenic properties against Aflatoxin B1 induced toxicity.

Materials and methods: We used the *in vitro* method i.e. human lymphocytes culture and *in vivo* method in bone marrow cells of albino mice, while the parameters studied included chromosomal aberrations (CA), sister chromatid exchanges (SCEs) and cell growth kinetics (RI) both in the presence as well as in the absence of exogenous metabolic activation system for *in vitro* studies, whereas total aberrant cells and the frequencies of aberrations were used for *in vivo* methods.

Abbreviations: CA, chromosomal aberrations; SCE, sister chromatid exchanges; RI, replication index; AFB1, Aflatoxin B1; AE, extracts of *Andrographia paniculata*.

* Corresponding author. Mobile: +91 9335753122.
E-mail address: sultansnc@gmail.com (M.S. Ahmad).

Peer review under responsibility of Ain Shams University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.ejmhg.2013.12.006

Results: *A. paniculata* extracts significantly reduced chromosomal aberrations from 35.0%, 62.0% and 69.0% level [at 24, 48, and 72 h due to Aflatoxin B1] to 21.72%, 44.0% and 52.0%, similarly sister chromatid exchanges were reduced from 14.60 per cell to 7.50 per cell at 48 h of treatments and replication index was enhanced *in vitro* for each concentration and duration of treatment.

Conclusion: In conclusion *A. paniculata* extracts significantly reduced the number of aberrant cells and frequencies of aberration per cell at each concentration and duration of exposure *in vivo*; similarly it reduced chromosomal aberrations and sister chromatid exchanges and replication index was enhanced *in vitro* that was statistically significant at <0.05 level.

1. Introduction

The history of natural product used in ancient times and in folk medicine around the world, is the basis for the use of many therapeutic drugs in modern day medicine. Traditionally, natural plant products have been the source in the search for new drugs by pharmaceutical companies [1]. Earlier we have worked on certain bioactivators like vitamins, carotenoids, flavonoids, ellagic acid and extracts of *Caesalpinia bonducella*, *Agaricus bisporus*, *Terminalia arjuna* and their antimutagenic and anticarcinogenic potentials were noticed, using *in vitro* and *in vivo* methods [2–6].

Andrographia paniculata belongs to the family Acanthaceae or Kalmegh and is commonly known as 'king of bitters'. It is widely distributed throughout tropical Asian countries often in isolated patches. Native populations of plants are spread throughout the south India and Sri Lanka which perhaps represent the center of origin and diversity of the species. It is extensively used in Ayurveda, Unani and Siddha medicines as home remedy for various diseases in India. The therapeutic value of kalmegh is due to its mechanism of action by enzyme induction. It is used to treat gastro intestinal tract, upper respiratory infections, fever, herpes, sore throat, hepatitis and a variety of other chronic and infectious diseases [7]. Therapeutically important active principal of kalmegh found in aerial part is Andrographolide ($C_{20}H_{30}O_5$, mp 230–239 °C). It is colorless, crystalline bitter in taste and known as diterpene lactone [8].

Sheeja et al. [9] reported that administration of methanolic extract of kalmegh produced complete inhibition of carrageenan induced inflammation compared with the control. Verma and Vinayak [10] studied the effect of the aqueous extract of *A. paniculata* on antioxidant defense system in lymphoma bearing AKR (an ecotropic N-tropic murine leukemia virus) mice in liver. Oral administration of the aqueous extract of plant in different doses caused a significant elevation of catalase, superoxide dismutase and glutathione-s-transferase activities. Sheeja et al. [9] explored the antioxidant and anti-inflammatory properties in methanolic extract of the plant and found it to inhibit the formation of oxygen derived free radicals such as superoxide (32%), hydroxyl radicals (80%), lipid peroxidation (80%) and nitricoxide (42.8%) *in vitro* system. *In vivo* studies using BALB/c mice models showed significant inhibition in phorbol-12-myristate-13-acetate (PMA) induced superoxide (32.4%) and nitric oxide (65.3%) formation. Tripathi and Kamat [11] examined aqueous extract for antioxidant activity using rat liver subcellular organelles as model systems and found that the extract shows potent antiradical agent against various pathophysiological oxidants. The isolated fractions effectively inhibited the toxic effect of snake venoms *in vitro* than *in vivo* [12].

Andrographolide inhibited LPS (lipopolysaccharide)-induced increase in tumor necrosis factor-alpha (TNF-α) and granulocyte–macrophage colony stimulating factor [13]. Neoandrographolide also inhibits PGE2 (Prostaglandin E2) synthesis and TNF-α in LPS-stimulated macrophages and its oral administration to mice significantly suppresses dimethyl benzene-induced ear edema and acetic acid-induced vascular permeability [14]. A refined extract of *A. paniculata*, also significantly reduces activities of lipid peroxide, while the activities of nitric oxide, cyclic guanosine monophosphate (cGMP) and superoxide dismutase are significantly enhanced in experimental atherosclerotic rabbits [15]. The aim of present investigation is to highlight the anticarcinogenic, antimutagenic, and anticlastogenic potential of extracts of *A. paniculata* in the *in vitro* and *in vivo* model.

2. Materials and methods

2.1. Materials

The whole plant was powdered into a mixture and the hydro-alcoholic extract was prepared by refluxing with the double distilled water (DDH_2O) and alcohol (3:1) in a round bottom flask for 48 h at 60 °C. The liquid extract was filtered, cooled and concentrated by evaporating its liquid contents in oven and collected. The powdered extract, termed *Andrographia* extract (AE), was re-dissolved in DDH_2O and the required doses for treatment were prepared and five concentrations of 50, 75, 100, 150 and 200 mg/kg body weight were selected for *in vivo* experiments and four concentrations of 50, 100, 200 and 250 mg/l of culture for *in vitro* experiments.

2.2. Aflatoxins B_1

Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus* at any time during growth and post harvest storage of a number of foodstuffs and the levels of contamination are enhanced under poor food harvesting and storage practices [6,16] that lead to aflatoxin B1 exposure to human. The major concern with respect to human health derives from the high potency of aflatoxins to produce cancer in laboratory animals