ejpmr, 2017,4(4), 349-352

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF APOPTOTIC AND GENOTOXIC ACTIVITY OF ANDROGRAPHOLIDE ISOLATED FROM *ANDROGRAPHIS PANICULATA* USING DNA FRAGMENTATION AND AMES TEST

Mohana S.J.*[1] and Umarani V.[2]

[1]Research Scholar, Gurunanak College, Chennai.
[2]Associate Professor, Department of Plant Biology and Biotechnology, Gurunanak College, Chennai.

***Corresponding Author: Mohana S.J.**
Research Scholar, Gurunanak College, Chennai.

Article Received on 24/01/2017 **Article Revised on 14/02/2017** **Article Accepted on 07/03/2017**

ABSTRACT

Mutation and Apoptosis are two key factor for converting normal cells to cancerous cells and beside developmental abnormalities, it may also lead to tumorigenesis, autoimmunity, and other serious health problems. Upregulating of apoptosis by a nonmutagenic compound may be useful as cancer therapy by inhibiting spread of abnormalities or cancer. Thus, it is essential to analyse, prove a novel phytoconstituent for anti-mutagenic and antiapoptotic property. Andrographolide, a diterpenoid lactone isolated from Andrographis paniculata, known as 'the King of Bitters', exhibits several pharmacological activities including immuno-stimulation, cytotoxicity, anti-inflammation, anticancer effect, hypotensive action cardio-protective action HIV . Though, reports on anticancer role of Andrographis sp. are rapidly increasing, there are limited reports with its derivatives. Thereby in this article a research is made to prove active phytoconstituent andrographolide as apoptosis inducer and a non-mutagen compound.

KEYWORDS: Andrographolide, mutation, apoptosis, DNA fragmentation, MTT assay.

INTRODUCTION

Apoptosis or programmed cell death is a specific form of cell death which plays a crucial role to maintain the integrity of multi cellular organisms. Alterations in the apoptotic pathways are intimately involved in the development of cancer, a leading cause of death worldwide (Fernandes et al., 2012). Apoptosis also plays a role in preventing cancer; if a cell is unable to undergo apoptosis, due to mutation or biochemical inhibition, it can continue dividing and develop into a tumor. Therefore apoptosis is required by living organisms to conserve homeostasis as well as to maintain their internal states within certain limits (Liew et al., 2010).

In genetics, genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may also lead to cancer. While genotoxicity is often confused with mutagenicity, all mutagens are genotoxic, however, not all genotoxic substances are mutagenic. The alteration can have direct or indirect effects on the DNA: the induction of mutations, mistimed event activation, and direct DNA damage leading to mutations (Kolle sussanne, 2012).

Apoptosis is characterized by a number of distinct cellular changes such as cell shrinkage, irregularities in cell shape, membrane blebbing, externalization of phosphatidyl serine in cell membrane, chromatin condensation, and inter-nucleosomal DNA fragmentation and mutations are caused by specific mutagens leading to uncontrolled cell division, apoptosis, inactivation of genes etc., Thus, cell death by apoptosis is exerted by the coordinated action of many different gene products. Mutations in some of them, acting at different levels in the apoptosis process, have been identified as cause or contributing factor for human diseases (Petra Gruber et al., 2001; Rodenhuis., 1992).

Identifying a non -mutagen and apoptosis causing phytoconstituent is an important process for screening of potential anti-cancer compound. In that case, a pharmaco-logically interesting structure is found and isolated from A*ndrographis paniculata* is Andrographolide. Andrographis paniculata (Burm. F) Nees, commonly known as the "king of bitters," is an herbaceous plant belonging to the Acanthaceae and is found throughout tropical and subtropical Asia, Southeast Asia, and India (Sajeeb et al., 2015). It is seen in abundance in the agricultural fields, roadsides and along railway tracks in Uttar Pradesh, West Bengal, Bihar and the Gangetic plains. This is a native Indian species (Thanasekaran et al., 2013).

Andrographolide is isolated from leaves of Andrographis paniculata, its chemical name of is 3α, 14, 15, 18-tetrahydroxy-5β, 9βH, 10α-labda-8, 12-dien-16-oic acid γ-lactone, and its molecular formula and weight are $C_{20}H_{30}O_5$ and 350.4 (C 68.54%, H 8.63%, and O 22.83%), respectively (Poonam et al., 2010). Although andrographolide is not very soluble in water, it is soluble in acetone, chloroform, ether, and hot ethanol. Crystalline compound was isolated and reported (Mohana et al., 2016).

MATERIALS AND METHODS

Isolation of Andrographolide

The fresh green aerial parts of the plant A*ndrographis paniculata* were collected and the leaves were dried at room temperature under shade for five days and milled into coarse powder using milling machine and subsequently subjected to the extraction by means of soxhlet apparatus.

Preparation of plant extracts

The dried blended powder (1kg) was first extracted with petroleum ether (60-80 °C) for a period of 48 h after which the powder was dried and successive extraction of the marc was repeated with chloroform and methanol for a period of 48 h respectively. All the three extracts were evaporated to dryness using Büchi Rotavapor (Switzerland) and ultimately dried in an oven. Quantitative phytochemical analysis and Structural elucidation using spectral studies are defined in Mohana et al., 2016.

Ames Test – Bacterial reverse mutation test

Bacterial strains

For mutagenicity and antimutagenicity assays, Salmonella strains TA98 and TA100 were used. These strains were kindly provided by Dr. Bruce N. Ames, Biochemistry Division, University of California, Berkeley, USA. Preparation of S9 mix: The procedure of Ames et al. (1973) and Garner et al., 1972 were used for the preparation of rat liver homogenate (S9). All the steps were performed at 0°C to 4°C with cold and sterile solutions and glassware. The S9 fractions were distributed in 2 mL aliquots in small sterile plastic tubes, quickly frozen and stored at - 80°C. The S9 mix was prepared following the method of Maron and Ames (1983). Mutagenicity and antimutagenicity assays: Standard plate incorporation tests: Plate incorporation tests were performed using S. typhimurium tester strains (TA98) with and without an exogenous metabolic system: S9 fraction in S9 mix. The andrographolide was dissolved in DMSO and different concentrations were used for the mutagenicity assays with positive mutagens. The plates were incubated for 1 hour and then inverted and placed in a dark vented incubator at 37 °C for 48 hours. Presence of background lawn on all the plates was confirmed after 48 hours of incubation. Four plates were used for each concentration. After 48 hours of incubation, the revertant colonies on the test plates were counted. A similar experiment was also carried out using liver homogenate (S9) fractions. The spontaneous reversion rates of these Salmonella strains were checked and reported. After the specific time of incubation, the numbers of revertant colonies were calculated.

DNA fragmentation studies – Anti apoptotic studies

Cell maintenance

Human hepatocellular carcinoma (Hep3B) cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM). All culture media were supplemented with 10% fetal bovine serum (FBS), 1% antibiotic and antimycotic solution (50,000 units/L of penicillin and 50 mg/L of streptomycin) and 2 mM glutamine. Cultures were held in 75 cm^2 culture flasks at 37°C, 5% CO_2 and 95% relative humidity, changing media at least twice a week.

DNA agarose gel electrophoresis

DNA extraction and agarose gel electrophoresis were performed.$1x10^6$ cells were plated in 100 mM petri dishes with MEM containing 10% FBS. The cells were incubated for 24 h under 5% CO2 and 95% air at 37^{o}C. The cells were treated with IC_{50}concentrations of Nanoparticles. After 24 h, 48 h both treated and control cells were tryspsinized and combined with the cells in the medium centrifugation at 1500 rpm for 5 min, washed twice with PBS, the resulting pellet was resuspended in 0.25 ml of lysis buffer, transferred to a microfuge tube and incubated for one hour at 37^{o}C. To this 4 µl proteinase K was added and the tubes then incubated at 50^{o}C for 3 hr. To each tube, 0.5 ml of Phenol: chloroform: isoamlyalcohol (25:24:1) was added, mixed and centrifuged at 13,000 rpm for 30 min at 4^{o}C to separate DNA containing upper aqueous phase. To the resulting aqueous phase, 2 volumes of ice cold absolute ethanol and 1/10 the volume of 3 M sodium acetate were added and kept at -20^{o}C overnight to precipitate DNA. The DNA was pelleted by centrifuging at 13,000 rpm for 10 min at 4^{o}C.

After repeating the above centrifugation step and removing the last traces of the supernatant fraction, the pellet was allowed to dry at room temperature for approximately 30 min and resuspended on 50µl of TE buffer. The DNA was quantitated by UV-visible spectroscopy and 10 µg of DNA was electrophoresed in a 1.5% agarose gel containing ethidium bromide in a mini gel tank containing TBE buffer for 2 hour under 90 V. The gel was examined under UV light and photographed.

RESULTS

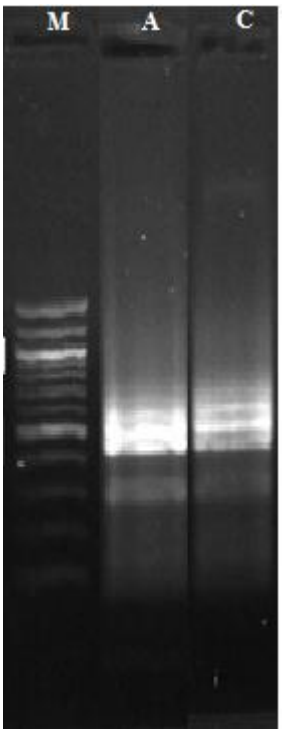
The herbal technology industry has matured and expanded at a rapid pace in the last decade, leading to the research and development of plant materials with enormous potential. The mutagenicity assay was performed with five dose levels (5000-125 µg/ml) in the absence of metabolic activation system. Inhibition of background growth of non-revertant bacteria was found at all of the five dose levels in comparison with the

mutagen (positive control) treated which showed a 3 fold increase of average revertant colonies per plate (Table no.1). Thus, exhibiting the ability to identify the samples as nonmutagen by the tester strain.

Characterization of *invitro* herbal particles uptake and localization is intrinsically linked to cytotoxicological studies because uptake provides evidence of plant material's cell interaction, wherein the delicate intracellular machinery is exposed to nanoparticles. Invitro cytotoxicity analysis of Andrographolide., were evaluated on HepG2 cell line for proliferation and survival of HepG2 cells by exposing to 5-0.31mg/ml of test samples for 24 h. Further the cell lysates were collected and DNA agarose gel electrophoresis were run. Figure 2Aand 2B showed that Andrographolide induces cell death in a dose- dependent manner, as determined. Thus, the results shows that the Andrographolide inhibits the proliferation of the cell and shows apoptotic activity against hepatocellular carcinoma cells (HepG2 cell line).

Table: 1 Mean Colony Count - Strain TA 98 - spontaneous mutation

Samples	Test Concentration (µg/plate) / (mg/ml)	Histidine Revertant Colonies			
		CFU/Plate		Mean	S.D
Positive Control	Sodium azide (5 mg/plate)	1110	998	1054	2.12
Andrographolide	5000	25	32	28.5	4.9
	2500	22	27	24.5	3.5
	1250	16	20	18.0	2.8
	500	15	11	13.0	2.8
	250	9	5	7.0	2.8
	125	6	3	4.5	2.1



Lane M- Marker, Lane A – Andrographolide after 24 hrs of treatment; Lane C - control
Figure no. 1 DNA fragmentation studies

DISCUSSION

Kerr et al., (1972) were the first to report on the concept of apoptosis. Apoptosis is ubiquitous in most of the tumor cells and plays an important role in the genesis and progression of tumors (Chiarugi and Giannoni, 2008). Previous studies have demonstrated that antitumor drugs generally inhibit tumors through the induction of apoptosis of sensitive tumor cells, and their antitumor effects relate to the interior activation of the apoptosis of the tumor cells induced by the drugs. Therefore, the induction of apoptosis to treat tumors has become a new target for the development of antitumor drugs and constitutes a new direction in the current research in tumor pharmacology.

DNA fragmentation occurs in cells that produce intrinsic apoptosis activity when induced by a variety of agents. This cleavage produces ladders of DNA fragments that are the size of integer multiples of a nucleosome length (180–200 bp). As a biochemical hallmark of intrinsic

apoptotic cell death, DNA fragmentation was used to determine whether the antiproliferative effect of Andrographolide on cells acts through apoptosis pathway. The treatment of Hep G2 cells treated at 24 hrs (Lane A; Figure 1; Lane M is marker) showed typical features of DNA laddering on an agarose gel, whereas untreated cells produced intact genomes (Lane C; Figure 1). Therefore, the Andrographolide synthesized from *Andrographis paniculata* can induce nucleosomal DNA fragmentation of HepG2 cells. ROS plays an important role in triggering many cellular pathways which can lead to cellular death, including cytokine activation (Brown et al., 2004) and caspase activation (Kim et al., 2009). They can also cause damage to the nuclear DNA by altering the chemical structure of the nucleotide bases and the deoxyribosyl backbone.

Andrographolide is a labdane diterpenoid isolated from leaves of Andrographis paniculata, is an extremely bitter substance. Andrographolide has gained much worldwide attention for its antitumor effects. As shown in this preliminary study, Andrographolide inhibits the proliferation of various tumor cells, with particularly significant effects on hepatocellular cancer.

REFERENCES

1. Rodenhuis S. rasand (1992). Human tumors. Semin Cancer Biol., 3: 241-7.
2. Fernandes J, Guedes PG, Lage CL, Rodrigues JC, Lage Cde A (2012) Tumor malignancy is engaged to prokaryotic homolog toolbox. Med Hypotheses, 78(4): 435–441. 20.
3. Liew JC, Tan WS, Alitheen NB, Chan ES, Tey BT (2010) Over-expression of the X-linked inhibitor of apoptosis protein (XIAP) delays serum deprivationinduced apoptosis in CHO-K1 cells. J Biosci Bioeng 110(3): 338–344.
4. Kolle, Susanne (2012). "Genotoxicity and Carcinogenicity". BASF The Chemical Company. Pg 1-6.
5. Chiarugi P and Giannoni E: Anoikis: a necessary death program for anchorage-dependent cells. Biochem Pharmacol, 2008; 76: 1352-1364.
6. Yang C, Jo SH, Csernus B, Hyjek E, Liu Y, Chadburn A and Wang YL: Activation of peroxisome proliferator-activated receptor gamma contributes to the survival of T lymphoma cells by affecting cellular metabolism. Am J Pathol, 2007; 170: 722-732.
7. Brown DM, Donaldson K, Borm PJ, Schins RP, Dehnhardt M, Gilmour P, *et al*. Calcium and ROS-mediated activation of transcription factors and TNF-alpha cytokine gene expression in macrophages exposed to ultrafine particles. Physiol Lung Cell Mol Physiol 2004; 2(2): 344-53.
8. Kim S, Cho JE, Choi J, Chung K, Park K Yi J, Ryu. Oxidative stress dependent toxicity of silver naaoparticles in human hepatoma cell. J Toxicol *In Vitro* 2009; 23(6): 1076-84.