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In Vitro Cytotoxic Effect of *Atropa acuminata*

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ABSTRACT

*Atropa acuminata (*AC) is a traditional medicinal plant that is commonly used for disorders like, Central nervous system abnormalities, and in ayurvedic medicine, it is used for the treatment of fever, chicken pox, cold, colitis, conjunctivitis (inflamed eyes) and diarrhea. This plant extract has also been reported for its varied biological activities such as antispasmodic, anxiety, arthritis, asthma, bedwetting and bowel disorders. It is widely distributed in South India. The present investigation evaluated the *in vitro* cytotoxic activity of the extract of the aerial part against one type of cell line, Hep2. From the data obtained, it was evident that magnitude, of the cytotoxicity was predominant on various concentration of these extract against the death rate of Hep2 cell lines. Present study thus confirms the cytotoxic property of the herb indigenous to India. These species could be considered as potential sources of anticancer compounds. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluation to demonstrate the role of *Atropa acuminata* in traditional medicine.

INTRODUCTION

In the last two centuries therapeutic agents have been extracted out of plant sources and many drugs have been found there way into doctor's prescription all over the world. Scientific development in the research field of indigenous medicinal plants is a significant aspect to have safer anti-cancer principles through their identification, isolation, characterization and biological testing. In the field of cancer chemotherapy there are many drugs available in the World [1].

Atropa acuminata is a folklore Indian medicinal plant. It is commonly known as Indian belladonna that can be cultivated in India and has established demand for their raw materials. All parts of the plant contain the alkaloids, atropine, hyoscyamine and bellodonnine, which are used as a sedative, antispasmodic, in convulsive disorders and as an antidote for poisoning. This medicinal plant also proved to be effective in homeopathic treatment for skin reactions during radiotherapy treatment for breast cancer [2].

The aerial parts are used to treat abnormal menstrual periods, acute infections, acute inflammation, anesthetic, antispasmodic, anxiety, arthritis, asthma, bedwetting, bowel disorders, chicken pox, cold, colitis, conjunctivitis (inflamed eyes), dental conditions, diarrhea [3,4] diuretic (use as a "water pill"), diverticulitis, [5,6,7] earache, encephalitis (inflammation of the brain), eye disorders (dilation of the pupils), fever, flu, glaucoma, gout, hay fever, hemorrhoids, hyper kinesis (excessive motor function), inflammation, kidney stones, measles, motion sickness, mumps, muscle and joint pain, muscle spasm (excessive unintentional muscle movements), nausea and vomiting during pregnancy, organophosphate poisoning, pain from nerve disorders, Parkinson's disease, pancreatitis, peritonitis, rash, scarlet fever, sciatica, (back and leg pain) [8]. Since the plant is reputed for its folkloric uses in various diseases [9], it draws our attention for its pharmacological screening. Plant contains flavonoids such as quarcetin, kaemferol, robinin and clitorin. It also contains starch, tannin, resin and anthocyanins [10, 11]. The phytochemical investigations revealed the presence of saponins, carbohydrates, alkaloids, proteins, anthroquinones and phytosterols. It is used as diuretics, antihelmintic, antidiabetic, antipyretic and brain tonic [12,13].

In this method, *in vitro* determination of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye by MTT Assay [14], which is

a recently developed rapid general bioassay technique for the natural products. This method indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral and pesticidal etc. [15,16]. *Atropa acuminata* is a bioactive plant and used in various diseases as folklore medicine [17]. It is also used in the treatment of chronic bronchitis, dropsy, goiter, leprosy, mucous disorders, sight weakness, skin diseases, sore throat and tumors [18].

In the regulation of development and homeostasis in multicellular organism, cell death is as important as cell proliferation. Although, physiological cell death is proliferated and inhibited apoptosis are major characteristic of cancer cells. Apoptosis is a gene regulated phenomenon that is included by many chemotherapeutic agents in cancer treatment. The induction of apoptosis in tumor cells is considered to be useful not only for the management and treatment of cancer, but also for its prevention [19,20,21]. The rich and diverse plant sources of India are likely to provide effective anticancer agents.

MATERIALS AND METHODS

Chemicals

DMSO was purchased from Merck India Ltd, Mumbai. MTT were Sigma, Media – HI MEDIA, Chloroform –CSRL Chemicals, Mumbai, FCS –Axiva sichem pvt. Ltd. Delhi, TPVG, DET, MET, and antibiotics were from Life Technologies in Chennai. All other chemicals and reagents were of pure analytical grade.

Plant materials and extraction

The Aerial part of *Atropa acuminata* was collected freshly from the Thirunelveli District, Tamilnadu, India during the month of March 2012. They were indentified and authenticated by V.Chelladurai (Research officer), Department of Botany (C.C.R.A.S), Government of India. The Voucher specimens (2012-2013) has been retained in the P.G. and Research scholar, Department of Biochemistry, Jaya College of Arts and Science, Chennai -602024, Tamilnadu, India. The aerial part of *Atropa acuminate* was dried under the shade, and leaves were coarsely powdered with a mechanical grinder, and the material was kept in the air tight container for the further study.

Cell lines and culture medium

Hep2 cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. While using the cytotoxicity test, the different concentrations of leaves extract were taken as toxic or nontoxic to the cell lines that were isolated from the laboratory. The cells were grown as Monolayer in MEM supplemented with 10% heat–inactivated fetal calf serum and 1% penicillin – streptomycin mixtures. Cultures were maintained at 370°C in 5% CO_2 and 100% relative humidity atmosphere.

Cytotoxicity assay

Cytotoxicity of sample on tumor cells was measured by micro-culture tetrazolium (MTT) assay. For the assay cells were grown in MEM at 370ºC under incubated for 6-7 hrs 5% CO_2 in a humidified incubator. Cells were harvested, counted (10 X 10, 000 cells/ml), and transferred into a 24 well plate, and incubated for 24 hrs, prior to the addition of test compound. Serial dilutions of test samples were prepared by dissolving compounds in DMSO followed by dilution with Minimal essential media (MEM) to give final concentration at 2.5, 1.25, 0.625, 0.3125, 0.516, 0.078, and 0.0.39 mg/ml.

Stock solutions of samples were prepared. Sample at 10ml and cell lines at 90ml were incubated for 72 hrs. MTT solution at 5mg/ml was dissolved in 1 ml of phosphate buffer solution (PBS), and 10ml of it was added to each of the 24 wells. The wells were wrapped with aluminum foil and incubated at 370^0C for 4 hrs. The solution in each well containing media, unbind MTT and dead cells were removed by suction and 150ml of DMSO was added to each well. Then the plant extracts were shaken and optical density was recorded using a micro-plate reader (spectrophotometer) at 595nm and DMSO as a blank. Controls and samples were assayed and replicated for each concentration and replicated three times for each cell line. After 24 hrs incubation of the mononuclear cells with plant extracts, the cytotoxicity on the cancer cell lines was evaluated using MTT assay. The cytotoxicity was obtained by comparing the absorbance between the samples and control. The values were then used to alternatively calculate the concentration of plant extracts required to cause a 50% reduction (IC50) a growth (cell number) for each cell lines.

$$\text{Cell viability (\%)} = \frac{\text{Mean OD}}{\text{Control OD}} \times 100$$

RESULTS

The standardization of ethanol extract [Table 1 and Fig. 1] of plant material has been carried out as per the standard guidelines. The anticancer activity of extract was conducted against cell lines of which ethanol extract have shown the anticancer activity against Hep 2 cell lines from the screening test. Present study implicit the observation that the ethanolic extract of *Atropa acuminata* showed a promising activity against the Hep 2 cell lines with percentage survival of less than 50% at concentration of 0.5 mg/ml. The cell viability percentage with regard to cytotoxicty was found to be 35% at 2.5mg/ml with the mean IC50 value ranging from 25.42-79.66µg/ml. The *in vitro* screening of the ethanolic extracts of *Atropa acuminate* showed potential activity against Hep2 cell lines.

Table-1*:* Cytotoxicity assay of Ethanolic extracts of *Atropa acuminata* against in Hep 2cell line

S.no	Concentration (mg/ml)	Dilutions	Absorbance	Cell viability %
1	5	Neat	0.10	19.60
2	2.5	1:1	0.18	35.29
3	1.25	1:2	0.25	49.01
4	0.625	1:4	0.27	52.94
5	0.3125	1:8	0.30	58.82
6	0.156	1:16	0.35	68.62
7	0.078	1:32	0.48	94.11
8	Cell control	-	0.51	100

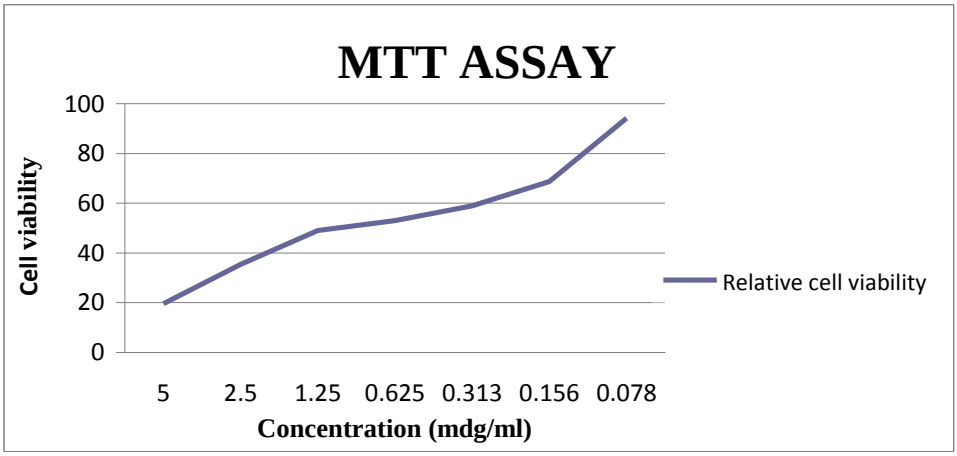
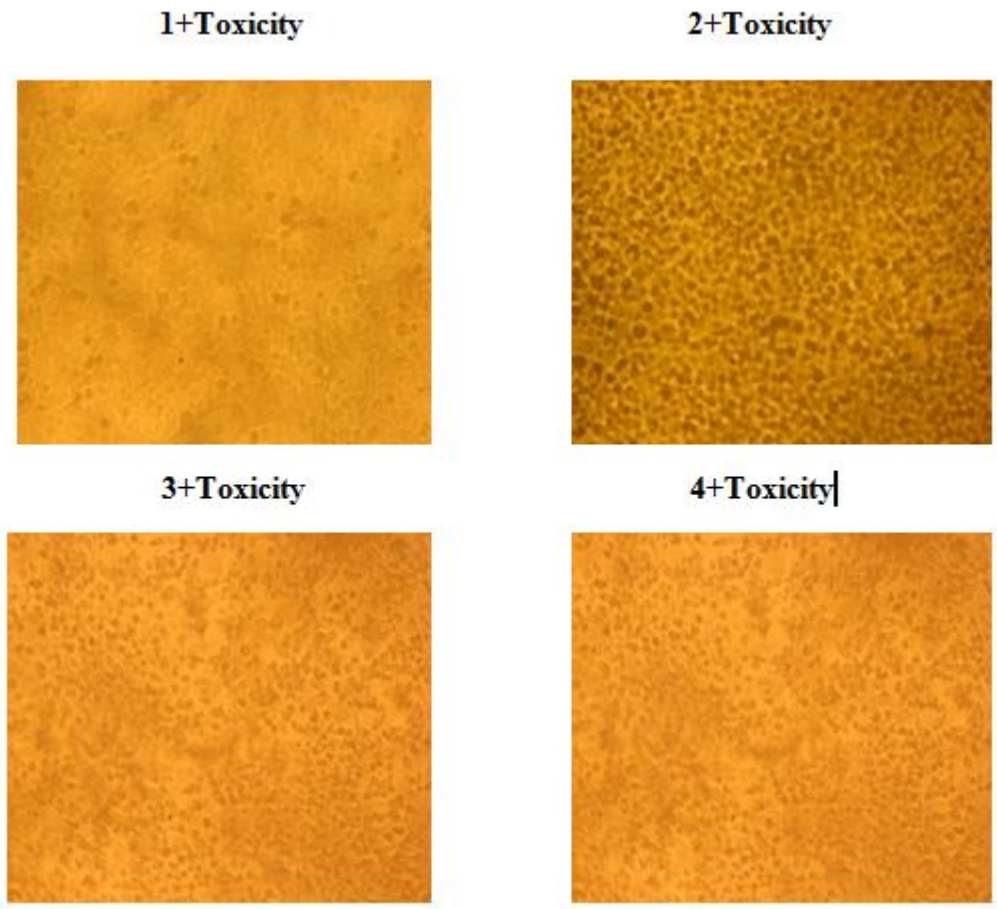
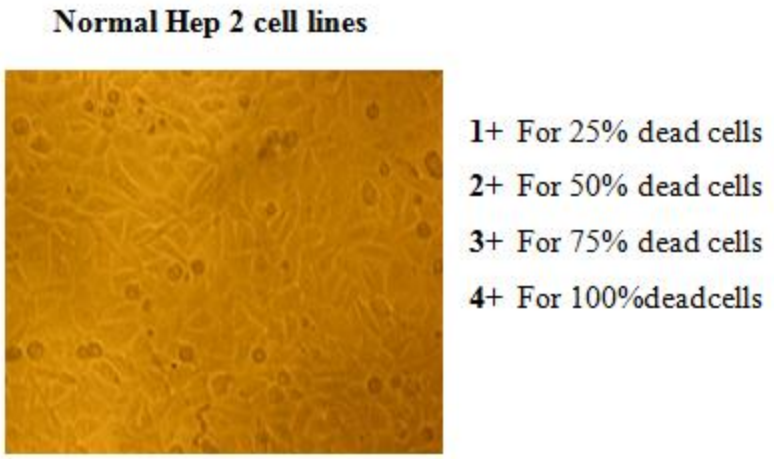


Fig. 1: Cytotoxicity Effect of Ethanol extracts of *Atropa acuminate*





DISCUSSION

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use [22]. Agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction are currently used for the treatment of cancer [23]. The use of multiple chemo preventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer treatment [24]. Medicinal plants are playing an important role in the healthcare immemorial. Activities of medicinal plants were due to the safe, compared with costly synthetic drugs that have adverse effects. Flavonoids also are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity [25, 26, 27]. Further more, flavonoids have a chemo preventive role in cancer through their effects on signal 1+ For 25% dead cells 2+ For 50% dead cells 3+ For 75% dead cells 4+ For 100% dead cells transduction in cell proliferation [28] and angiogenesis [29]. The cytotoxicity and anticancer properties are due to the presence of flavonoids. Phenolic compounds, including flavonoids are especially promising candidates for cancer prevention [30]. Much information is available on the reported inhibitory effects of specific plant phenolic compounds and extracts on mutagenesis and carcinogenesis [10]. The potential ability of polyphenol combinations to prevent cancer progression has not been adequately studied. Scientists have suggested that it appears extremely unlikely that any one substance is responsible for all of the associations seen between plant foods and cancer prevention because of the great variety of dietary phenolics, including flavonoids and the many types of potential mechanisms reported [31, 8]. Plant extracts containing catechin, epicatechin,

quercetin, kaempferol, rutin etc, have shown to decrease proliferation of breast, pancreatic, prostate and other cancer cell lines [32]. Multi-component prescription is a common feature in cancer treatment. Our observations are in agreement with that made by [33]. Usually in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [34, 35].

CONCLUSION

The ethanolic extract of *Atropa acuminata* plant showed potent cytotoxic activity against Hep2 cells. The phytochemical agents in the ethanolic extracts of the plant could be responsible for theses noteworthy activites. Further studies are in progress in our laboratory in synthesis of novel derivatives and investigation of molecular mechanisms, responsible for the cytotoxic activity of this plant. This study may contribute to the improvement of scientific understanding of chemical constituents and functionality of the tested traditional medicinal plants which are needed to provide insights into *in vivo* cytotoxicity of the plant with a view to obtain a useful chemotherapeutic agent.

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