RESEARCH ARTICLE

Inhibition of DMBA induced carcinogenesis in albino mice by *Withania somnifera* extracts

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ABSTRACT

Cancer is one of the leading deaths causing disease in the world. Now a days, there are number of studies were conducted in order to eradicate cancer in the environment. From Ancient times, the medicinal plants used to treat cancer were found in different medicinal system in different parts of the world. The present study concentrates to find out the anticancer activity of medicinal plant named *Withaniasomnifera*against DMBA induced carcinogenesis in albino mice. Inorder to find the activity of the medicinal plant both invitro and invivo studies were conducted and the results confirmed that the ashwagandha has the anticancer activity and the aqueous root extract of ashwagandha had more potential when compared to the other extracts of the plant.

Keywords: Ashwagandha, DMBA, MTT assay, in-vivo studies

INTRODUCTION

Cancer is one of the major human diseases all over the world. Chemotherapy is an important method which uses chemotherapeutic drugs for the effective treatment of tumor (Prakash *et al*., 2001, Rathnasamy *et al*., 2014). Chemotherapy treats the tumor by destroying the rapidly proliferating cancer cells. Ayurveda is one of the traditional method forcuring various diseases since more than 5000 years (Balakrishnan,*et al*., 2015). It uses the *Withaniasomnifera* plant for curing more diseases especially cancer for remedy in Indian pharmacopoeia (Kataria *et al*., 2015, Christiana *et al*., 2003).

An Indian traditional plant *Withaniasomnifera* commonly known as Ashwagandhaan annual herb which belongs to the family Solanaceae has widely spread in all over the world especially in Asia (Joshi *et al*., 2014). It has been also used in ayurvedic and unani as pharmaceuticals and nutraceuticals. It has various pharmacological activities such as antioxidant, antiarthitic, anti-inflammatory, antipyretic, antimicrobial and anticancer activities due to the phytochemicals such as alkaloids, tannins, flavonoids,

and terpenes present in it (Yadav *et al*., 2010). Ashwagandha is a source of unique alkaloids and which can be act as antioxidants and steroidal hormones with favor impact on the human health (Kataria *et al*., 2015). In-vitro cytotoxicity effect has been studied against human cancer cell lines such as MCF-7, HeLa and In-vivo studies has also been performed in Swiss albino rats (Jayaprakasam *et al*., 2003, Barnes *et al*., 2016). It has also been reported that it has the ability to modulate cholinergic neurotransmission and protective in neuropsychiatric and neurodegenerative disorders. This article concentrates more on the anticancer activity of *Withaniasomnifera.*

MATERIALS AND METHODS

Chemicals & Plant collection:

The leaves and roots used were selected from the healthy, mature and disease free *Withania somnifera* plant, Collected from the Botanical Garden, Bon Secours College For Women, Thanjavur, Tamilnadu, India. All the chemicals were analytical grade from sigma and all the glass wares used were completely sterilized and every process was done at completely sterilized condition.

Extraction:

For preparing the sample in the powder form the crude methanolicand aqueous extracts of *Withaniasomnifera* roots and leaves weredried inrotary evaporator under reduced pressure and dried completely (Joshi *et al*., 2014)

Cell culture:

HeLa (Human cervical carcinoma, tumorigenic and invasive), MCF-7(human breast carcinoma, tumorigenic and non-invasive), MDA MB 231 (human breast carcinoma, ER-, tumorigenic and invasive) were purchased from Bharadhidasan University, Trichy.

Animals and treatment:

Swiss albino mice of 7–8 weeks old weighing 18–22g were used. Swiss albino mice was kept inside the 25×25×150 cm^3 cage and incubated at proper conditions like lighting, nutrition and ventilation. These mice were properly maintained to the given procedure (Sundaramoorthy *et al*., 2014). Control group animals were given (PBS) for 21 days and ASH-WEX group animals were administered 5 ml/kg ASH-WEX through oral route (equivalent to 150 mg dry weight/kg) (Prakash *et al*.,2001).

Cytotoxicity study (methyl tetrazolium-MTT assay):

The cytotoxicity effect has been studied in the above cancer cell lines using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) test. The cancer cells lines were seeded at 20,000 cells/ml in 96 well plate and were grown overnight. Then the cells were treated with increasing concentration of ASH-WEX (0.5- 1.5%) for 24 h. The culture media was replaced by fresh medium containing MTT (0.5 mg/ml) for 4 h and incubated at 37°C. The absorbance has measured at 590 nm (Kataria *et al*., 2015).

RT1, RT2, RT3, represents the aqueous root, methanolic root and acetone root extracts respectively. Similarly LT1, LT2, LT3 represents the aqueous leaves, methanolic leaves and acetone leaves extracts respectively.

In-vivo anti cancer study in DMBA induced albinomice:

In this study, six groups (6 mice for each group), control (group I), Aqueous root extract (group II), Aqueous leaves extract (group III), Methanolic root extract (group IV), Methanolic leaves extract (group V), 5 - fluorouracil (group VI) of Swiss albino mice were used. Albino mice in all the groups were injected with 7-12-Dimethyl benzanthrecene (DMBA). After the cancer had been induced,25mg/dose/animal (i.p) of the sample was given to all the six group animals for 12 weeks and the results were recorded (Davis *et al*., 2000).

RESULTS AND DISCUSSION

Cytotoxicity effect (MTT assay):

The samples were evaluated against the Hela, MCF-7 (breast), MDA MB -231, and HCT-15 (colon)cancer cell lines. When compare with the standard our samples also has the activity against the tested cancer cell line this is due to the presence of phytochemical in the *Withania somnifera* leaves and roots. After the depth analysisit is observed in our studies that the root extract had the more efficient activity when compare with others. In addition to that the aquaeous root extract had the significant activity in the group.

Anticancer activity of the extracts against DMBA induced mice:

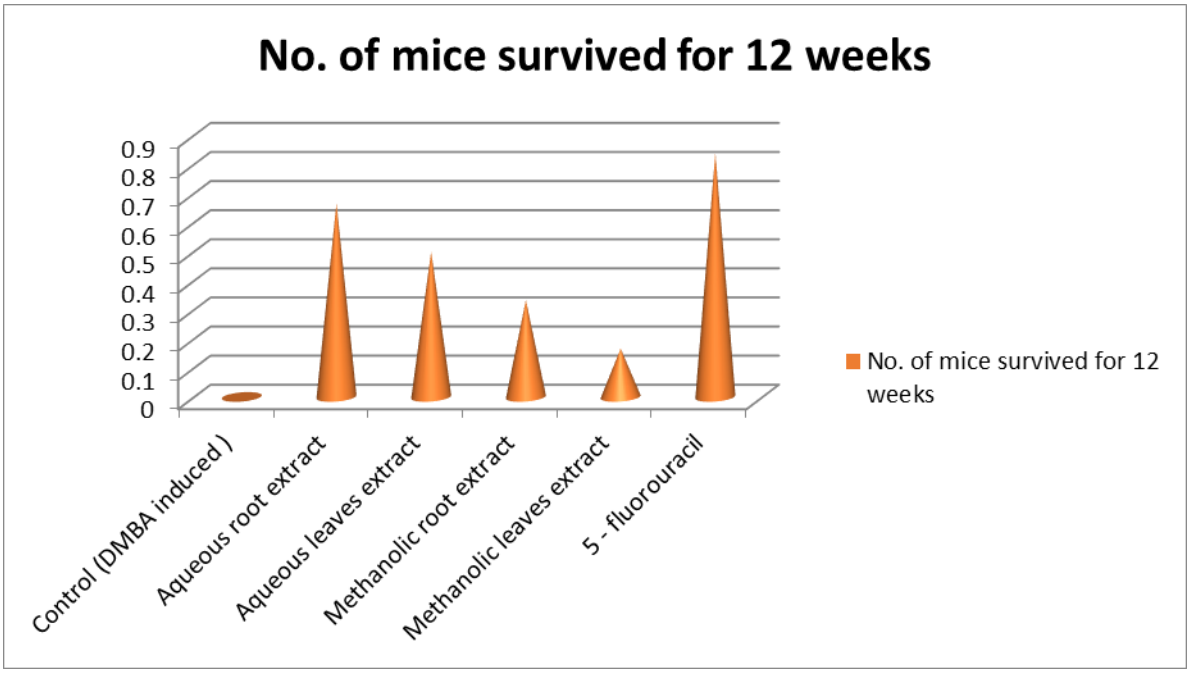
The *in vivo* anticancer activity against DMBA induced mice was studied in six groups. Thus, the result indicates that aqueous root extract of *withania somnifera* showed more potent activity (80%) among the others.

Table 1: In vitro cytotoxicity effects of *Withaniasomnifera* roots and leaves extracts against various human cancer cell lines

Samples	Concentration (µg/ml)	IC_{50} values			
		Hela	MCF-7	MDA MB -231	HCT-15
RT1	100	20	22	26	30
RT2	100	60	26	30	38
RT3	100	56	32	46	50
LT1	100	40	46	50	40
LT2	100	44	58	56	48
LT3	100	56	60	68	66
5-fluorouracil	2×10^{-5}	-	14	-	55

Table 2: Effect of *Withaniasomnifera*root and leaf extracts against DMBA induced cancer in mice

Group	No. of mice survived for 12 weeks
Control (DMBA induced) (GroupI)	0/6
Aqueous root extract(GroupII)	4/6
Aqueous leaves extract(GroupIII)	3/6
Methanolic root extract(GroupIV)	2/6
Methanolic leaves extract(GroupV)	1/6
5 – fluorouracil(GroupVI)	5/6



The fig.1 indicates the percentage mice survival for 12 weeks

CONCLUSION

There are number of studies were available related to different types of cancers. But till now, the complete eradication of cancer is difficult to achieve around the world. So the current study is mainly concentrated to give advancement in the cancer disease and the medicinal plant (ashwagandha) extracts were used to reduce the number of cancer cells (MTT assay) and the size of cancer tumor (Albino mice). Finally our results revealed that the ashwagandha is the most potential natural substance to eradicate cancer from our environment. In addition to that the aqueous root extract of ashwagandha had more potential activity against the cancer studied in our research.

Conflicts of interest: The authors stated that no conflicts of interest.

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