World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Cytotoxic Studies on Selected Siddha Plants

R. Ganesan[1], Mathuram Venkatanarasimhan[2], P. Elankani[1], R. Shakila[1] and Ponniahsamy[1]

[1]Siddha Central Research Institute, Arumbakkam, Chennai.
[2]Department of Chemistry, CSMDRIA, Arumbakkam, Chennai, India

Received: 20-06-2015 / Revised: 29-07-2015 / Accepted: 22-08-2015

ABSTRACT

The methanolic extract of whole plant of *Andrographis echioides* (L.) Nees, *Ocimum tenuiflorum* L., *Barleria prionitis* L., *Coldenia procumbens* L. and chloroform extract of *Coldenia procumbens* L. were studied for the anticancer activity of the Human Ovarian Cancer Cell Line Ovkar -3 and Human Renal Cancer Cell Line 786-O in different concentrations (10,20,40 80 µg/ml) along with standard drug Adriamycin (Doxorubicin)(ADA)(Positive control compound).The results showed that all the extracts were non-cytotoxic at the dose levels studied and all of them showed activities of GI50 only at concentration > 80 µg/ml.

Keywords: Cytotoxic study, *Andrographis echioides*, *Ocimum tenuiflorum*, *Barleria prionitis*, *Coldenia procumbens* and Adriamycin (ADA).

INTRODUCTION

Andrographis (Fam. Acanthaceae) is a genus of about 40 species .In traditional systems of medicine, several *Andrographis* species have been used in the treatment of dyspepsia, influenza, malaria, respiratory infections, and as astringent and antidote for poisonous stings of some insects [1,2] . More than 20 species of *Andrographis* have been reported to occur in India.

The phytochemistry of this genus has been investigated in view of its importance and reported to contain several flavonoids [3,4] and labdane diterpenoids[5-10]. *Andrographis echioides*, an annual herb occurring in South India, is listed in the Indian Materia Medica and used as a remedy for fevers.

In recent years, phytochemicals in medicinal plants have received a great deal of attention mainly on their role in preventing diseases caused as a result of oxidative stress which releases reactive oxygen species such as tumour promotion[11-12]. Several studies suggest that antioxidants could prevent accumulation of these reactive oxygen species and be beneficial for treatment of these pathologies [12]. Tulsi, the "Queen of Herbs", is the most sacred herb of India [13]. *Ocimum tenuiflorum* (Fam.Lamiaceae) is used as a medicinal herb for headache, cough, diarrhoea, worms and kidney malfunctions. Essential oil has been utilized extensively in the food industry as a flavouring agent, and in perfumery and medical industries [14]. Previous studies reported various effects of *Ocimum* sp., such as anti-inflammatory, antioxidative, chemopreventive, blood-sugar lowering, nervous system stimulation and radiation protection [15-17].

Barleria prionitis L. is commonly called as "Porcubine flower" is a much – branched, prickly shrub, up to 3 m in height, found growing throughout the hotter parts of India. It is also commonly grown as a hedge plant in gardens [18]. It is well known for treating bleeding gums and toothache. Because of its anti dontalgic property it is known as 'Vajradanti'. *B. prionitis* has been the centre of interest to pharmacologists as it exhibits a variety of pharmacological activities viz. antibacterial activity [19] and antifungal activity [20, 21] etc. The Boraginaceae members are herbs, shrubs or trees comprising about 100 genera and 2,000 species. They have flowers in helicoid cymes and often herbage that is coarsely hairy and widely distributed in temperate and tropical regions. The genus *Coldenia* has about 24 species of prostrate, hairy herbs distributed in tropical and subtropical zones [22]. *Coldenia procumbens*, a common weed in India, is used as external application for causing suppuration of boils and for rheumatic swellings.

***Corresponding Author Address: Dr.** R. Ganesan, Department of Biochemistry, Siddha Central Research Institute, Arumbakkam, Chennai. E.mail: ganeshbiochem@yahoo.co.in*

This current study was focused for the anticancer activity of the *A.echioides* (L.) *Nees, O. tenuiflorum L., B. prionitis L.* and *C procumbens* L. against the Human Ovarian Cancer Cell Line Ovkar -3 and Human Renal Cancer Cell Line 786-O in different concentrations (10,20,40 80 µg/ml) along with standard drug Adriamycin (Doxorubicin)(ADA)(Positive control compound). Hence it helps to know the anticancer activity or cytotoxicity nature of these plants.

MATERIAL AND METHODS

Collection of the plant material: The whole plant of *Coldenia procumbens* Linn. was collected during March 2009 from Pudukkottai district, Tamil Nadu. It was authenticated by Dr. Sasikala Ethirajulu, Department of Pharmacognosy, Siddha Central Research Institute, Chennai. The whole plant of *Andrographis echioides* L., *Ocimum tenuiflorum* L., *Barleria prionitis* L. were collected from Mettur,Salem Dt.,Tamilnadu and authenticated by Dr.Sorna subramaniyan, Research Officer (Botany),SMPG, Mettur Dam.

PREPARATION OF EXTRACTS

Methanolic Extract: Plants were shade dried and coarsely powdered separately (1 kg) and extracted with methanol in an aspirator bottle by cold percolation method at room temperature (48 hr). Each extraction was carried out twice. All extracts were filtered through Whatman No. 1 filter paper. Nearly 80% of the solvent was removed by distillation on a water bath at atmospheric pressure and the last traces were removed under reduced pressure.

Chloroform Extract: The whole plant of *Coldenia procumbens* Linn. was shade dried and coarsely powdered (1 kg) and extracted with chloroform in an aspirator bottle by cold percolation method at room temperature (48 hr.) and extraction was carried out twice. The extract was filtered through Whatman No. 1 filter paper. Nearly 80% of the solvent was removed by distillation on a water bath at atmospheric pressure and the last traces were removed under reduced pressure. Both methanolic and chloroform extracts were used for this study.

The studied cells are:

(a) Human Ovarian Cancer Cell Line Ovkar -3
(b) Human Renal Cancer Cell Line 786-O

Both Cells lines were obtained from Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai.

Sulforhodamine B (SRB) assay: The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 hrs prior to addition of experimental drugs.

After 24 hrs, one 96 well plate containing $5*10^3$cells/well was fixed *in situ* with TCA, to represent a measurement of the cell population at the time of drug addition (Tz). Experimental drugs were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100 µg/ ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

Endpoint measurement: After extracts addition, plates were incubated at standard conditions for 48 hrs and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 min. at 4°C. The supernatant was discarded, the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 min. at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells x 100. Using the six absorbance measurements [time zero (Tz), control growth (C) and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz (Ti-Tz) positive or zero

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz. (Ti-Tz) negative.

The dose response parameters were calculated for each test article. Growth inhibition of 50 % (GI50) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested [23,24].

RESULTS AND DISSCUSION

The Cytotoxic activitity on *Coldenia procumbens* (chloroform and methanol extracts), *Andrographis echioides (*methanol extract*), Ocimum tenuiflorum* (methanol extract) and *Barleria prionitis* (methanolic extract) was studied against Human Ovarian Cancer Cell Line Ovkar-3 and Human Renal Cancer Cell Line 786-O.The *in-vitro* studies were carried out at the dose level 10,20,40 and 80 µg/ml (Table 1and 3).The results are given in table 2 and 4.Tthe plant extracts are non-cytotoxic when compared with positive control Adriamycin (ADR). But the activity was observed at the dose level > 80 µg/ml.

CONCLUSION

The Cytotoxic studies on Human Ovarian Cancer Cell Line Ovkar-3 and Human Renal Cancer Cell Line 786-O using chloroform extract of whole plant of *Coldenia procumbens* Linn. showed non-cytotoxic at the dose levels studied. The methanolic extract of whole plant of *Coldenia procumbens* Linn. *Andrographis echioides* L., *Ocimum tenuiflorum* L. *and Barleria prionitis* L. also showed that non-cytotoxic at the dose levels studied. All of them showed activity of GI50 only at concentrations > 80 µg/ml. Hence, it can be concluded that these medicinal plants studied, can be used in the treatment of various ailments as mentioned in the traditional systems of medicine.

GI50	Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase
TGI	Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
LC50	Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.
GI50 value of ≤ 10^-6 (i.e. 1 µmole) or ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤ 20µg/ml is considered to demonstrate activity.	
Yellow highlighted test values under GI50 column indicate activity.	

Table:1 HUMAN OVARIAN CANCER CELL LINE OVKAR-3:

	Human Ovarian Cancer Cell Line Ovkar-3															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
CPC	89.7	87.9	86.8	75.4	100.0	100.0	87.7	68.0	100.0	100.0	83.4	67.3	96.6	96.0	85.9	70.2
CPA	100.0	97.3	96.6	92.4	100.0	100.0	99.8	76.6	100.0	100.0	92.8	80.7	100.0	99.1	96.4	83.3
GT	100.0	100.0	100.0	100.0	100.0	100.0	100.0	88.1	100.0	100.0	100.0	92.0	100.0	100.0	100.0	93.4
KT	100.0	88.3	80.7	76.5	100.0	98.9	72.0	57.9	100.0	95.1	79.3	59.9	100.0	94.1	77.4	64.8
SM	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.0	100.0	100.0	100.0	93.6	100.0	100.0	100.0	96.5
ADR	5.5	4.4	-4.6	-11.4	2.5	0.4	-4.3	-14.8	6.6	4.4	-5.9	-14.1	4.8	3.1	-4.9	-13.4

Table:2

Drug concentrations (µg/ml) calculated from graph

Ovkar-3	LC50	TGI	GI50
CPC	>80	>80	>80
CPA	>80	>80	>80
GT	>80	>80	>80
KT	>80	>80	>80
SM	>80	>80	>80
ADR	>80	49.6	<10

Table: 3: HUMAN RENAL CANCER CELL LINE 786-O:

	Human Renal Cancer Cell Line 786-O															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
CPC	87.4	82.8	71.1	59.6	86.1	83.0	74.6	64.1	89.1	85.0	76.3	60.1	87.5	83.6	74.0	61.3
CPA	89.4	85.3	82.0	80.9	87.6	87.6	85.7	79.4	85.4	85.4	83.5	72.1	87.5	86.1	83.7	77.5
GT	78.0	77.7	75.2	75.2	85.1	79.3	79.1	76.9	80.3	78.0	75.3	74.8	81.1	78.3	76.5	75.6
KT	80.4	78.5	76.4	66.9	85.2	81.1	73.5	72.5	76.9	76.5	68.4	68.4	80.8	78.7	72.8	69.3
SM	83.7	83.5	78.6	77.8	82.6	81.9	80.4	79.2	76.9	76.7	74.3	73.6	81.1	80.7	77.8	76.9
ADR	-19.4	-37.7	-56.0	-61.3	8.1	-27.9	-39.7	-53.2	-36.3	-38.1	-53.0	-55.0	-15.9	-34.6	-49.5	-56.5

Table: 4

Drug concentrations (µg/ml) calculated from graph

786-O	LC50	TGI	GI50
CPC	>80	>80	>80
CPA	>80	>80	>80
GT	>80	>80	>80
KT	>80	>80	>80
SM	>80	>80	>80
ADR	58.4	23.0	<10

CPC -Chloroform extract of *Coldenia procumbens* Linn.
CPA- Methanolic extract of *Coldenia procumbens* Linn.
GT -Methanolic extract of *Andrographis echioides* L.,
KT -Methanolic extract of *Ocimum tenuiflorum* L.
SM -Methanolic extract of *Barleria prionitis* L.
ADR- Adriamycin (Doxorubicin), Positive control compound.

REFERENCES

1. Kirtikar KR, Basu BD. *Indian Medicinal Plants*,Periodical Experts Book Agency, New Delhi, India, 3;1884–1886. 1975.
2. Chopra RN et al. *Glossary of Indian Medicinal Plants*. Council of Scientific and Industrial Research; New Delhi, India,18. 1980.
3. Harborne JB. The Flavonoids: Advances in Research Since 1986. Chapman and Hall; London UK 1994; 280–290.
4. Iinuma M, Mizuno M. Natural occurrence and synthesis of 2′-oxygenated flavones, flavonols, flavanones and chalcones. Phytochemistry. 1989; 28:681–694.
5. Kleipool RJC. Constituents of Andrographis paniculata Nees. Nature 1952;169:33–34.
6. Chan WR et al. The structure and stereochemistry of neoandrographolide, a diterpene glucoside from *Andrographis paniculata* Nees. Tetrahedron 1971; 27:5081–5091.
7. Balmain A,Connolly JD. Minor diterpenoid constituents of *Andrographis paniculata* Nees. J. Chem. Soc., Perkin Trans 1. 1973; 1:1247–1251.
8. Fujita T et al. On the diterpenoids of *Andrographis paniculata*: X-ray crystallographic analysis of andrographolide and structure determination of new minor diterpenoids. Chem. Pharm. Bull 1984; 32:2117–2125.
9. Matsuda T et al. Cell differentiation-inducing diterpenes from *Andrographis paniculata* Nees. Chem. Pharm. Bull. 1994; 42:1216–1225.
10. Reddy MK et al. A flavone and an unusual 23-carbon terpenoid from *Andrographis paniculata* Phytochemistry, 2003;62: 1271–1275.
11. Ames N B. Dietary carcinogens and anticarcinogens-oxygen radicals and degenerative diseases. Sci 1983; 221: 1256-1264.
12. Horton J W. Free radicals and lipid peroxidation mediated injury in burn trauma: the role of antioxidant therapy. Toxicol 2003; 189: 75-88.
13. Mabberley D J. The plant Book: A portable Dictionary of the Higher plants of genera and families of angiosperms. J. California Native Plant Soc 1997; 30(2): 1-36.
14. Simon J E et al. Basil: a source of essential oils. In: Janick J, Simon J E. (Eds). Advanced in New Crops. Timber Press, Portland, OR 1999; 484-489.
15. Chattopadhyay R R. A comparative evaluation of some blood sugar lowering agents of plant origin, J. Ethnopharmacol 1999; 67: 367-372.
16. Prakash J, K S Gupta. Chemopreventive activity of Ocimum sanctum seed oil. J. Ethanopharmacol 2000; 72: 29-34.
17. Umadevi P. Radioprotective, anticarcinogenic and antioxidant properties of the Indian holy basil, *Ocimum Sanctum* (Tulasi). Indian J. Exp. biol 2001; 39: 185-190.
18. Banerjee AK et al. Barleria prionitis Linn.: A review of its traditional uses, phytochemistry, pharmacology and toxicity. Research Journal of phytochemistry 2012; 6: 31-41.
19. Aneja KR et al. Potency of Barleria prionitis L. bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin. New York Sci. J 2010; 3.
20. Amoo SO et al. Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three Barleria species. S. Afr. J. Bot 2011; 77: 435-445.
21. Anonymous, The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, Council of Scientific & Industrial Research (CSIR), New Delhi, India, 1950; 2: 46-47.
22. Anonymous, The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, Council of Scientific & Industrial Research (CSIR), New Delhi, India, 1950; 2: 307.
23. Vanicha V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening, Nature Protocols, 2006; 1: 1112 – 1116.
24. Skehn P et al .New colorimetric cytotoxicity assay for anticancer drug screening Journal of National Cancer Institute, 1990; 82(13):1107-12.