Allelopathic effect of Ashwagandha against the germination and radicle growth of *Cicer arietinum* and *Triticum aestivum*

Sangita Chandra, Priyanka Chatterjee, Protapaditya Dey, Sanjib Bhattacharya

Division of Pharmacognosy, Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly, West Bengal, India.

Submitted: 23-10-2011 Revised: 12-12-2011 Published: 27-07-2012

ABSTRACT

Background: Ashwagandha (*Withania somnifera*) is an important medicinal plant in Indian traditional system of medicine and traditionally has been used for several medicinal purposes in the Indian subcontinent. **Objective:** The present study was aimed at the evaluation of allelopathic effect of hydroalcoholic extract of ashwagandha against germination and radicle growth of *Cicer arietinum* and *Triticum aestivum* seeds. **Materials and Methods:** The extract at different concentrations was incubated in controlled conditions with the surface sterilized seeds of *C. arietinum* and *T. aestivum* and observed periodically for seed germination and radicle growth to assess the allelopathic behavior. **Results:** The extract mainly at higher concentrations demonstrated promising allelopathic potential by significantly affecting seed germination and radicle elongation of both *C. arietinum* and *T. aestivum* in a concentration dependent manner. *T. aestivum* was found to be more sensitive than *C. arietinum*. **Conclusion:** The present study demonstrated remarkable allelopathic potential of ashwagandha against the test seeds. The effect was plausibly due to the alkaloid and withanolide contents of ashwagandha.

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.99082

Quick Response Code:

Key words: Allelopathic, withanolides, ashwagandha, *Cicer arietinum, Triticum aestivum*

INTRODUCTION

The phenomenon of allelopathy, where a plant species chemically interferes with the germination, growth or development of other plant species has been known for over 2000 years. Allelopathy can be defined as any direct or indirect harmful or beneficial effect of one plant on another through the production of chemicals that it releases into the environment.[1] In 1996, the International Allelopathy Society defined allelopathy as follows: "Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects".[2] Chemicals released from plants and imposing allelopathic influences are termed as allelochemicals or allelochemics. Most allelochemicals are classified as secondary plant metabolites which are biosynthetically derived form the primary metabolites of the plant.[3] When susceptible plants are exposed to allelochemicals, germination, growth and development may be affected. Allelochemicals are present in several parts of plants that are known to interfere seed germination and growth of neighbouring or successional plants by releasing allelochemicals in their environment.[1] The search and development of new herbicides through the identification of active compounds from allelopathic plants is an interesting research and development area.[4] These compounds can be regarded as 'natural herbicides'.

Ashwagandha, also known as Indian ginseng and winter cherry, consists of the dried roots of *Withania somnifera* (L.) Dunal. (Solanaceae). It is a perennial plant indigenous to India, grown and cultivated throughout subtropical India. It has been recognized as an important herb in the Ayurveda, the traditional system of Indian medicine for more than 3000 years. Traditionally, it has been used for several important medicinal purposes in the Indian subcontinent. Recently there has been renewed interest on ashwagandha for its effectiveness in several disease conditions, adaptogenic, immunomodulator and other health benefits.[5,6] Previous researchers have reported several pharmacological properties of ashwagandha on

Address for correspondence:
Sanjib Bhattacharya, Pharmacognosy Division,
Bengal School of Technology (A College of Pharmacy),
Delhi Road, Sugandha, Hooghly - 712102, West Bengal, India.
E-mail: sakkwai@yahoo.com

animals and humans.[7-11] The present study was conducted to assess the possible allelopathic effects of ashwagandha extract on the germination and radicle growth of *Cicer arietinum* and *Triticum aestivum* seeds.

MATERIALS AND METHODS

Plant material

The dried roots of ashwagandha (*Withania somnifera*, (L.) Dunal. family: Solanaceae) were procured in the month of July, 2011 from Kangalicharan & Sons., Kolkata, West Bengal, India and identified at the Botanical Survey of India, Howrah, West Bengal, India. Just after procurement, the roots were ground mechanically into a coarse powder and kept into an air-tight container for use in the study.

Preparation of extract

The powdered plant material (50 g) was extracted with 50% aqueous ethanol (400 ml) by boiling under reflux for 30 min. The extract was filtered and evaporated to dryness to yield the dry extract (HAWS, yield: 37.44%). The dry extract was kept in a refrigerator until use.

Test samples

The test samples for allelopathic bioassay were prepared freshly from the dry extract. Different concentrations of HAWS (40, 20, 10, 5, 2.5, 1.25 mg/ml) were prepared by dissolving in double-distilled water immediately prior to use.

Collection and preparation of seeds

Healthy uniform seeds of gram (*Cicer arietinum* L., family: Fabaceae) and wheat (*Triticum aestivum*, family: Poaceae) were obtained from the Agriculture Seed Store (Govt. of West Bengal) Kalyani, West Bengal, India. The seeds were soaked in distilled water for 1 h. Then the seeds were surface sterilized with 70% ethanol for 2 min, then rinsed with double-distilled water for several times for complete removal of the sterilant.

Exposure to text samples

This procedure was performed under aseptic conditions at laminar air-flow bench. The surface sterilized seeds were placed evenly in sterilized glass Petri dishes (9 mm). Each Petri dish contained 10 seeds. Then equal volume (5 ml) of varying concentrations of the test samples were introduced into each Petri dish. Similar volume of double distilled water was used as control. In case of wheat seeds, the test liquids were decanted after 30 min. Then all the Petri dishes were incubated in dark at room temperature (24-26°C). Allelopathic behavior was evaluated by recording the number of germinated seeds and radicle length using a millimeter ruler, after 48, 72 and 96 h in case of gram seeds, and after 24 and 48 h in case of wheat seeds. The indicating parameters viz., germination percentage and percentage inhibition of radicle growth were calculated by the following formulae:

Germination percentage = Number of germinated seeds/ Total number of seeds ×100

% Inhibition of radicle growth= (X–Y)/X ×100.

Where, X= Control mean radicle length and Y= Treated mean radicle length.

The extract concentration for 50% radicle length inhibition (IC_{50}) was determined by plotting percentage inhibition of radicle growth with respect to control against treatment concentration.

Statistical analysis: The data of radicle length were expressed as the mean ± standard error of mean (SEM). Same data were analyzed for statistical significance by Student's '*t*' test.

RESULTS AND DISCUSSION

Screening of plant extracts and their fractions for their effects on seed germination of various plant species are routinely used to evaluate their alleleopathic potential.[4] The present findings demonstrated negative allelopathic effects of hydroalcoholic extract of ashwagandha (*Withania somnifera*) on the germination and radicle growth of *C. arietinum* and *T. aestivum*.

The results of allelopathic effect of HAWS against *C. arietinum* are summarized in Tables 1 and 2. HAWS at all test concentrations inhibited germination of *C. arietinum* seeds in a concentration dependent way; however, all seeds were found to germinate at lower concentrations (1.25 and 2.5 mg/ml) at each time interval [Table 1]. HAWS remarkably inhibited radicle growth at the all test concentrations in a time and concentration dependent manner. The effects were found to be prominent and significant during the whole period of observation [Table 2].

The results of allelopathic effect of HAWS against

Table 1: Effect of HAWS on germination percentage of *C. arietinum*

Concentration (mg/ml)	After 48 h (%)	After 72 h (%)	After 96 h (%)
Control	100	100	100
1.25	100	100	100
2.5	80	80	80
5	70	70	70
10	70	70	70
20	60	70	70
40	60	70	70

Table 2: Effect of HAWS on radicle growth of *C. arietinum*

Concentration (mg/ml)	After 48 h		After 72 h		After 96 h	
	Radicle length (mm)[§]	% Inhibition of radicle growth	Radicle length (mm)[§]	% Inhibition of radicle growth	Radicle length (mm)[§]	% Inhibition of radicle growth
Control	19.20±2.50	-	39.50±5.43	-	60.0±5.45	-
1.25	18.88±2.72	1.67	38.0±6.60	3.80	55.0±9.90	8.33
2.5	16.40±3.48[¶]	14.58	36.43±8.07	7.77	40.70±11.80[¤]	32.17
5	16.43±3.20[¶]	14.43	28.10±5.19[¤]	28.86	33.60±6.83*	44.0
10	10.50±2.30[¤]	45.31	23.0±5.28[¤]	41.77	30.43±4.76*	49.28
20	10.14±2.14[¤]	47.19	22.43±5.68[¤]	43.22	29.43±6.33*	50.95
40	6.17±2.06*	67.86	15.70±3.53*	60.25	24.06±5.44*	60.0

[§]Data are expressed as mean ± SEM. [¶]$P < 0.5$, [¤]$P < 0.01$, *$P < 0.001$ compared with control

Table 3: Effect of HAWS on germination percentage of *T. aestivum*

Concentration (mg/ml)	After 24 h (%)	After 48 h (%)
Control	60	90
1.25	40	80
2.5	20	50
5	20	30
10	20	30
20	20	20
40	0	0

Table 4: Effect of HAWS on radicle growth of *T. aestivum*

Concentration (mg/ml)	After 24 h		After 48 h	
	Radicle length (mm)[§]	% Inhibition of radicle growth	Radicle length (mm)[§]	% Inhibition of radicle growth
Control	2.17±0.30	-	2.67±0.47	-
1.25	2.0±0.65	17.05	2.50±0.50	6.36
2.5	1.5±0.50[¶]	30.87	2.0±0.23[¶]	25.09
5	1.5±0.29[¶]	30.87	2.0±0.19[¶]	25.09
10	1.0±0.36*	53.91	1.8±0.20[¶]	32.58
20	1.0±0.43*	53.91	1.5±0.29*	43.82
40	0	100	0	100

[§]Data are expressed as mean ± SEM. [¶]$P < 0.5$, *$P < 0.001$ compared with control

Table 5: IC_{50} values of HAWS on radicle growth of *C. arietinum* and *T. aestivum*

Treatment time	IC_{50} (mg/ml)	
	C. arietinum	*T. aestivum*
After 24 h	-	9.25
After 48 h	23.0	22.75
After 72 h	28.0	-
After 96 h	15.5	-

T. aestivum are presented in Tables 3 and 4. HAWS at all test concentrations inhibited germination of *T. aestivum* seeds in a concentration dependent fashion; however, no germination was observed in case of highest concentration of HAWS (40 mg/ml) during 48 h [Table 3]. HAWS significantly and concentration dependently inhibited radicle growth at all the test concentrations during 48 h of observation. No detectable radicle growth was observed at the highest concentration of HAWS (40 mg/ml) even up to 48 h [Table 4]. The IC_{50} values of HAWS for both *C. arietinum* and *T. aestivum* are summarized in Table 5.

The most frequently reported allelochemical-induced gross morphological effects on plants include inhibited or retarded seed germination, effects on coleoptile elongation and on radicle, shoot and root development.[12] Here, germination percentage and radicle growth were recorded to monitor the allelopathic behavior. However, in the present study, radicle growth appeared to be the most sensitive parameter and IC_{50} values based on this parameter, very clearly indicated the differential allelopathic effect of HAWS on both the test seeds [Table 5]. From these values, it becomes evident that *T. aestivum* was more sensitive to HAWS, being effective in lower concentrations; than *C. arietinum*.

Plants exhibit allelopathic activity due to release of allelochemicals of different chemical classes mainly polyphenolic compounds (flavonoids, tannins), cyanogenic glycosides and alkaloids.[3,13] The inhibitory effect of the test extracts on seed germination and radicle length may be due to the presence of putative allelochemicals. The main constituents of ashwagandha are several alkaloids and steroidal lactones commonly called as withanolides which are responsible for its wide ranging biological effects.[9,14,15] In the present study, allelopathic effect of ashwagandha can be attributed to its alkaloid and withanolide contents. The effect may be due to synergistic effect rather than single constituent.

From the present preliminary investigation, it can be concluded that ashwagandha root exhibited remarkable negative allelopathic potential by significantly affecting the germination and radicle growth of both *C. arietinum* and *T. aestivum*. *T. aestivum* was found to be more sensitive than *C. arietinum*. The observed allelopathic effect was plausibly due to its alkaloid and withanolide contents. To the best of our knowledge, this is the first report of allelopathic effect of ashwagandha. Further studies are necessary to

determine the exact chemical constituents of ashwagandha accounting for its allelopathic activity. Allelopathic effects of ashwagandha under field conditions also need further research in pursuit of a new effective natural herbicide.

ACKNOWLEDGEMENT

The authors are thankful to the authority of the Bengal School of Technology (A College of Pharmacy), Sugandha, Hooghly, 712102, West Bengal, India, for providing necessary facilities for the present study.

REFERENCES

1. Rice EL. Allelopathy. New York: Academic Press; 1984.
2. Torres A, Oliva RM, Castellano D, Cross P. First world congress on allelopathy, a science of the future. Cadiz, Spain: SAI (University of Cadiz); 1996.
3. Einhellig FA. Mechanisms of action of allelochemicals in allelopathy. In: Inderjit, Dakshini KM, Einhellig FA, editors. Allelopathy. Organisms, processes, and applications (ACS Symposium Series 582). Washington, DC: American Chemical Society; 1995. p. 96-116.
4. Macias FA. Allelopathy in the search for natural herbicides models. In: Inderjit, Dakshini KM, Einhellig FA, editors. Allelopathy. Organisms, processes, and applications (ACS Symposium Series 582). Washington, DC: American Chemical Society; 1995. p. 310-29.
5. DerMarderosian A. The reviews of natural products. St. Louis, Missouri: Facts and Comparisons; 2001.
6. Duke JA, Bogenschuz-Godwin MJ, duCellier J, Duke PK. Handbook of medicinal plants. 2nd ed. Boca-Raton: CRC Press; 2002.
7. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. Altern Med Rev 2000;5:334-46.
8. Khanam S, Devi K. Antimutagenic activity of Ashwagandha against lead nitrate: A genetic and non-genetic biomarkers' evaluation. Pharmacogn Mag 2005;1:97-101.
9. Gupta GL, Rana AC. *Withania somnifera* (Ashwagandha): A review. Pharmacogn Rev 2007;1:129-36.
10. Gupta GL, Rana AC. Synergistic effect of *Withania somnifera* Dunal. and L-dopa in the inhibition of haloperidol-induced catalepsy in mice. Pharmacogn Mag 2009;5:46-50.
11. Singh G, Sharma PK, Dudhe R, Singh S. Biological activities of *Withania somnifera*. Ann Biol Res 2010;1:56-63.
12. Kruse M, Strandberg M, Strandberg B. Ecological effects of allelopathic plants - A review (NERI technical report no. 315). Silkeborg, Denmark: National Environmental Research Institute; 2000.
13. Einhellig FA. Allelopathy - current status and future goals. In: Inderjit, Dakshini KM, Einhellig FA, editors. Allelopathy. Organisms, processes, and applications (ACS Symposium Series 582). Washington, DC: American Chemical Society; 1995. p. 1-24.
14. Evans WC. Trease and Evans Pharmacognosy. 15th ed. New Delhi: Reed Elsevier India Pvt. Ltd.; 2002.
15. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 39th ed. Pune (India): Nirali Prakashan; 2007.

Cite this article as: Chandra S, Chatterjee P, Dey P, Bhattacharya S. Allelopathic effect of Ashwagandha against the germination and radicle growth of *Cicer arietinum* and *Triticum aestivum*. Phcog Res 2012;4:166-9.

Source of Support: Nil, **Conflict of Interest:** None declared.