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Genotoxic effects of *Azadirachta indica* and *Cynodon dactylon* on *Allium cepa* meristamatic tissue

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ABSTRACT

The genotoxic effects of aqueous extracts of two medicinal plants *Azadirachta indica* (A. Juss) and *cynodon dactylon* was evaluated using *Allium cepa*. The extracts were prepared with water. Onion bulbs were exposed to 1, 5, 10, 25 and 50%; and 1, 2.5, 5, 10 and 20% concentrations (v/v) of each of the extracts for macroscopic and microscopic analyses, respectively. There was concentration-dependent and statistically significant ($P < 0.05$) inhibition of root growth by the extracts when compared with the control. The EC₅₀ obtained for decoctions of *Azadirachta indica* and *cynodon dactylon* were 1.4 and 0.8%, respectively. It was 2.5 and 0.7 % for the squeezed extracts of *Azadirachta indica* and *cynodon dactylon* respectively. All the tested extracts were observed to have mitodepressive effects on cell division and induced mitotic spindle disturbance in *Allium cepa*. These results suggest an inhibitory, mitodepressive and turbagenic activities of the aqueous extracts of *Azadirachta indica* and *Cynodon dactylon* on *Allium cepa*.

KEY WORDS; *Azadirachta indica*; *Cynodon dactylon*; *Allium cepa*; mitodepressive; Genotoxic, Phytochemicals

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INTRODUCTION

The world of nature abounds in medicinal compounds, which constitutes a fascinating and fruitful area of scientific investigation. Nature is still the preeminent synthetic chemist and that in plants particularly; there are almost infinite reserves of fascinating chemical constituents with actual and potent effects on the human system¹. The use of medicinal plants has always been part of human culture. The world health organization estimates that up to 80% of the world's population relies on traditional medicinal system for some aspect of primary health care. Ethno-traditional use of plant-derived natural products has been a major source for discovery of potential medicinal agents. Worldwide, herbal medicine has become one of the most common forms of alternative therapy. The popularity of herbal medicines is associated with their easy access, therapeutic efficacy, relatively low cost, and assumed absence of toxic side effects. Herb that may be safe in small doses may become dangerous in higher doses. The risk of overdose is higher in herbal preparations than conventional medicines due to the product variability. *Glycyrrhiza uralensis* is a perennial herb known as the Chinese licorice. Licorice has long been valued for therapeutic use for fevers, liver ailments, dyspepsia, gastric ulcers, asthma, bronchitis, Addison's disease and rheumatoid arthritis and has been used as a laxative, antitussive and expectorant^{2,3}. *Salvia miltiorrhiza* is an annual sage plant and among the most popular medicinal herbs. It is a hardy perennial growing to 80 cm, with toothed oval leaves and clusters of purple flower^{4,5}. It is commonly used either on its own or in combination with other herbs based on the concepts of traditional medicine. Recent studies have shown that long-term exposures to herbal products might be associated with increases in the rates of morbidity and mortality. Among several medicinal plants such as, *Azadirachta indica* (A.Juss), *Morinda lucida* (Benth.), *Cymbopogon citratus* (DC Stapf.), *Mangifera indica* (Linn.) are widely used. They have been reported to have antimicrobial, pesticidal and healing properties^{6,7,8}. However, there are few reports on the toxicological properties of these plants in literature. In addition to systemic toxicity, the possible genotoxicity of herbal products has been investigated in recent years. The aim of this study was to contribute to a better understanding of the genotoxic effect of leaf extracts of *Azadirachta indica* and *Cynodon dactylon* using *Allium cepa* root tips.^{9,10,11}

MATERIALS AND METHODS

Collection of Medicinal Plants:

The medicinal plants used in the present study are *Azadirachta indica* (neem leaves) and *Cynodon dactylon* (Bermuda grass). They were selected on the basis of their ethnobotanical uses and availability. They were collected at different locations near Sholinganallur.

Preparation of the Extract:

Extractions were carried out as practiced locally. Briefly, leaves of *Azadirachta indica* (320 g) and *Cynodon dactylon* (100g) were weighed and boiled separately in 1L of tap water. The resultant decoctions were kept separately. Squeezed extracts of *Azadirachta indica* (320 g) and *Cynodon dactylon* (100g) were made by first pounding the leaves separately in a mortar before 1L of water was added to the leaves and squeezed. Both the decoctions and squeezed extracts were filtered with Whatman filter paper to remove the suspended particles and stored at 4° until use. These were considered as the stock solutions.^{13,14,15}

Allium cepa assay:

Onion bulbs (*Allium cepa*, L., 2n=16) were obtained commercially at Sholinganallur, Chennai. They were sun dried for 2 weeks and dried outer scales were carefully removed leaving the ring root primordial intact. These were used for the bioassay according to standard procedures. For the root inhibition, five concentration of each extracts (1,5,10,25 and 50%) were considered. Seven onion bulbs were utilized for each extract and the control (tap water). The base of each of the bulbs was suspended on the extracts were changed daily. At the end of the exposure period, the length of the roots of five onion bulbs with the best growth at each concentration was measured (in cm) with a ruler. From the weighted averages for the each concentration and the control, the percentage root growth inhibition in relation to the negative control and the EC50 (the effective concentration where root growth amounts to 50% of the controls) for each extract was determined. The effect of each sample on the morphology of growing roots was also examined^{16,17}

For the evaluation of induction of chromosomal aberration, 3 onion bulbs were suspended on 1, 2.5, 5, 10 and 20% concentration (v/v) of each of the extracts and the control for 48 h. At the end of 48 h, root tips from these bulbs were cut and fixed in ethanol: glacial acetic acid (3:1, v/v). These were hydrolyzed in 1N HCL at 60° for five minutes after which they were washed in distilled water. Two root tips were then squashed on each slide, stained with acetocarmine for 10min and cover slips carefully lowered on to exclude air bubble. Six slides were prepared for each concentration and the control out of which five (at 1000 cells per slide) were analyzed. The mitotic index was calculated as the number of dividing cells per 1000 observed cells

Phytochemical Analysis

The extracts were subjected to preliminary phytochemical screening to identify the presence of phytoconstituents such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides and steroids according to¹⁸.

Statistical analysis

The SPSS 10.0 statistical package was used for this analysis. The mean, with 95% confidence limits and the standard errors for each of the quantitative sets of data were calculated. Differences between the control and individual dosage group of the extract were analyzed by means of the students t-test of significance at the $p < 0.05$ level.

RESULTS

Table1: Preliminary phytochemical assay of ethanol leaf extract of *Azadirachta.indica* and *Cynodon.dactylon*

PHYTOCHEMICAL TEST	<i>Azadirachta.indica</i>	<i>Cynodon.dactylon</i>
Detection of Alkaloid		
Mayer's Test	+	+
Wagner's Test	+	+
Detection of Flavonoids		
Lead acetate Test	+	+
Detection of Steroids	+	+
Detection of Terpenoids		
Salkowski Test	+	+
<u>Detection of Phenol</u>		
Ferric chloride Test	+	+
Lead acetate Test	+	+
Detection of Carbohydrate		
Benedict's Test	+	+
Detection of Protein or Amino acid		
Ninhydrin Test	+	+

Table 2: Effects of water extracts of *Azadirachta indica* on root growth of *Allium cepa*

Conc .(%)	SAI			DAI		
	Mean root length \pm S.E	TRG(%) of control	95%CL	Mean root length \pm S.E	TRG(%) of control	95%CL
0	4.5 \pm 0.16	-	0.6	4.0 \pm 0.16	-	0.5
1	3.1 \pm 0.26	56.2*	1.0	2.0 \pm 0.21	39.5*	0.4
5	2.3 \pm 0.07	54.0*	0.2	0.8 \pm 0.08	15.5*	0.3
10	2.0 \pm 0.12	41.8*	0.4	0.4 \pm 0.11	9.1*	0.1
25	1.3 \pm 0.21	29.5*	0.3	0.3 \pm 0.08	5.1*	0.1
50	1.0 \pm 0.09	23.5*	0.2	0.2 \pm 0.03	3.1*	0.1
EC ₅₀		2.5%			0.7%	

RG(%) of control, Treated root growth expressed as % of the control. 95% CL, 95% confidence limit. SAI, squeezed *Azadirachta indica*. DAI, decoctions of *Azadirachta indica*.

Table3: Effects of water extracts of *Cynodon dactylon* on root growth of *Allium cepa* .

Conc.(%)	SCD			DCD		
	Mean root length \pm S.E	TRG(% of control)	95%CL	Mean root length \pm S.E	TRG(%) of control	95%CL
0	4.9 \pm 0.16	-	0.6	4.9 \pm 0.17	-	0.5
1	2.0 \pm 0.26	40.8*	0.8	1.9 \pm 0.19	39.5*	0.4
5	1.9 \pm 0.19	38.8*	0.4	0.8 \pm 0.08	18.4*	0.4
10	1.1 \pm 0.06	22.25*	0.4	0.7 \pm 0.10	15.3*	0.4
25	0.6 \pm 0.03	12.3*	0.1	0.4 \pm 0.07	9.2*	0.3
50	0.2 \pm 0.03	4.1*	0.1	0.3 \pm 0.04	8.1*	0.2
EC50		1.4%			0.8%	

RG(%) of control, Treated root growth expressed as % of the control. 95% CL, 95% confidence limit. SCD, squeezed *Cynodon dactylon* .DCD, decoctions of *Cynodon dactylon*

Table 4: Cytological effect of water extract of *Azadirachta indica* and *Cynodon dactylon*

Conc.%*	DAI			SAI			DCD			SCD		
	No of Dividing cells	MI	% of aberrant cells	No of Dividing cells	MI	% of aberrant cells	No of Dividing cells	MI	% of aberrant cells	No of Dividing cells	MI	% of aberrant cells
0	220	40	-	226	43	-	229	45	-	228	46	-
1	105	22	0.2	175	35	0.06	190	35	0.02	185	36	-
2.5	75	16	-	172	45	0.11	145	25	-	164	33	0.08
5	32	6	-	108	22	0.13	100	21	0.02	144	29	0.04
10	42	7	0.04	144	29	0.15	20	4	-	77	15	0.04
20	50	9	0.1	74	16	0.01	-	-	-	65	12	0.02

MI, mitotic index, SAI, squeezed *Azadirachta indica*. DAI, decoctions of *Azadirachta indica*. SCD, squeezed *Cynodon dactylon* .DCD, decoctions of *Cynodon dactylon*

*1000 cells/per concentration of each extract and the control.

DISCUSSION

The phytochemical test was done to show the alkaloids, flavonoids, terpenoids, steroids, saponins, carbohydrates, proteins or amino acids present in both the extract were also presented in Table 1 Table 2 shows the results of root growth, at tested concentration of root growth was highest in 1% concentration of two extract when compared with 50% concentration. Inhibition of root growth was concentration dependent and statistically significant ($P < 0.05$) at tested concentration. The EC50 value of squeezed extract of *Azadirachta indica* and *Cynodactylon* were 2.5% and 0.7% respectively. Mean while the boiled decoction extract of *Azadirachta indica* and *Cynodactylon* were 1.4% and 0.8% respectively.^{19,20} Data on the effects of the boiled and squeezed extracts on root growth on *Allium cepa* showed that there was concentration dependent decrease in root growth. Comparatively, the boiled decoction of *Azadirachta indica* and *Cynodactylon* has more inhibitory and mitodepressive effects than the Squeezed extracts. In this study, spindle disturbances were observed and might have been due to the presence of secondary metabolites. The induction of spindle disturbances in cell *Allium cepa* by these extracts may lead to aneuploidy and or micronucleus formation at the next stage of cell division(Table 4). This usually arises from irregular

separation of chromosomes at anaphase there by making some chromosomes to reach the poles before the other. The complete arrest of cell division by the boiled extract of *Azadirachta indica* and *Cynodactylon*. Alkaloid such as vincristins were reported to be responsible for chromosome aberration observed with the water extract of a *Borreria filiformis*. In this study spindle formation was observed might be due the presence of secondary metabolite alkaloid.

CONCLUSION

The results herein suggests that the tested extracts possess inhibitory, mitodepressive and genotoxic effects on root growth cell division and chromosomes behaviour of *Allium cepa*. The genotoxicity effect on these medicinal plants in albino rats are in progress.

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