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An Evaluation of the Genotoxic Effects of the Seed Decoction of *Cassia tora* Linn. (Leguminosae) in an *Allium cepa* Model

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ABSTRACT

Cytological effects of *Cassia tora* seed decoction were evaluated in *Allium cepa* root tip cells. Bulbs were grown in pure tap water (controls, Gr. I) and also in six concentrations (0.15 mg/ml, 0.31 mg/ml, 0.62 mg/ml, 1.25 mg/ml, 2.5 mg/ml and 5 mg/ml) of *C. tora* seed decoction in tap water (experimental, Grs. II). Parameters of study were 'mean root length' and morphology i.e. colour and shape of root tips at 72 hr of cultivation and 'mitotic Index', chromosomal aberrations and abnormal mitosis at 48 hr of cultivation. Physico-chemical characterization of decoction was also made. No changes in the morphology of root tips occurred at any concentration of *C. tora* seed decoction, however, change in color did occur at all concentrations. Mitotic index and mean root length remained unaffected at first two concentrations but all higher four concentrations caused progressive mitodepression hence a decline in root growth occurred. No abnormal mitosis and no chromosomal aberration occurred at all at any concentration. Results suggest that water soluble constituents of *C. tora* seeds could only lower mitosis but not caused any adverse genotoxic effects in mitotically dividing *A. cepa* root cells under laboratory condition.

Key words: *Cassia tora*, *Allium cepa*, emodin, mitosis.

INTRODUCTION

Cassia tora Linn. is a well known oriental herb used in traditional medicine[1]. Its seeds are used as coffee substitute, health drink and in curing several human ailments[2-4]. The seeds of *C. tora* contains a variety of bioactive anthraquinones including emodin, chrysophanol and rhein etc which are mainly responsible for pharmacological action ascribed to them[5-8]. Anthraquinones present in *C. tora* seeds have also been found to be mutagenic and cytotoxic too in prokaryotic and eukaryotic cells [9-14]. Present study was planned to find out the genotoxic effect of *C. tora* seed extract in *Allium cepa* test which is an internationally accept model for such studies[15].

MATERIALS AND EXPERIEMNTAL DESIGN

Allium cepa

Dry healthy common onions (2n=16) 2.0 to 2.50 cm in diameter were obtained from the local market.

Test herbal compound

Dried seeds of medicinal plant *Cassia tora* locally called 'Punvad' were purchased from local herbal medicine shop. These seeds were authenticated by Botany department of this University. Seeds were crushed in electrical grinder to get coarse powder. Each time 5 gm of *C. tora* seed powder was boiled in 1000 ml of tap water for 5 minutes to prepare decoction of seeds. After cooling evaporated (lost) volume of decoction was made up to 1000 ml with tap water.

Experimental Design

Experiments were planned as per international protocol (15) for *Allium cepa* test. A set of twelve tubes were filled with pure tap water (Group I, controls). Another series of 12 test tubes were filled with each concentration of *C. tora* seed decoction (Group II, experimental). All solutions were changed every 24 hours. After 48 hours two onions out of 12 in each series with most poorly growing roots were removed. Same day i.e. after 48 hours of cultivation 02 mm of 05 root's tips were cut off from five individual bulbs and were fixed in acetoalcohol (1:3 v/v acetic acid and absolute ethanol) for 24 hours and were stored in 70% ethanol. Every time fixation was done at a fixed time, 11:00 AM. After 72 hours of cultivation total length of 05 root bundles in each series of onions was measured to record "mean root length". Also same time i.e. at 72 hours w.m. of root tips was prepared to observe shape and colour (morphology).

Squashing of root tips and observation of slides

Root tips were squashed in N-HCl and 2% acetocarmine (BDH) stain. Four fields from each slide were observed to cover about 50 cells in each i.e. total 200 cells per slide hence 3000-4000 cells were observed for each group of onions. Mitotic index was calculated as total number of dividing cells per 100 observed cells. Slides were also observed under oil immersion lens to find out mitotic arrest, aberrations, polyploidy etc. as detailed in Table 5.

Physico-chemical Analysis of decoction

All parameters were done as per standard methods described in APHA[16].

Statistics

Experiments were repeated three times. Students 't' test was applied at 5% level of significance.

RESULTS

1. Physicochemical properties of decoction of *C. tora* is shown in (Table 1).

It shows slight deviation from pure tap water due to presence of many water soluble constituents of *C. tora* seeds.

Table 1. Physico-chemical properties of *Cassia tora* seed decoction.

1	Colour	Yellowish
2	Odour	Odorless
3	Turbidity	98 NTU
4	pH value	7.2
5	Total alkalinity	90 mg/l
6	Carbonate	8 mg/l
7	Bicarbonate	140 mg/l
8	Hardness	162 mg/l
9	Chlorides	52 mg/l
10	BOD	5.2 mg/l
11	COD	9.3 mg/l
12	Fluoride	0.43 mg/l
13	Dissolved oxygen	4.2 mg/l

2. Morphology : color and shape of root tips (Table 2)

Among controls color of root tips was white and tips were straight and pointed in shape which are usual features of growing roots of *Allium cepa* bulbs. Bulbs grown in *Cassia tora* seed decoction at four initial concentrations (0.15 mg/ml to 1.25 mg/ml) revealed no chance in the shape of tips but they appeared pale yellow in color while at last two concentrations (2.5 mg/ml and

5 mg/ml) they appeared dark yellow, however, no changes in their morphology could be noticed.

Table 2. *Morphology of Allium cepa root tips following 72 hrs. cultivation in Cassia tora seed decoction. (n=75).*

S.N.	Concentration of *Cassia tora* mg/ml	Morpohology i.e. Shape of Root Tips				Colour of Root Tip		
		Abnormal			Normal	Normal	Abnormal	
		Crochet Hooks	Bulbs	Broken Tip	Straight	White	Pale Yellow	Dark Yellow
1	Control 0.00	NO	NO	NO	YES	YES	NO	NO
2	0.15 mg/ml	NO	NO	NO	YES	NO	YES	NO
3	0.31 mg/ml	NO	NO	NO	YES	NO	YES	NO
4	0.62 mg/ml	NO	NO	NO	YES	NO	YES	NO
5	1.25 mg/ml	NO	NO	NO	YES	NO	YES	NO
6	2.5 mg/ml	NO	NO	NO	YES	NO	NO	YES
7	5 mg/ml	NO	NO	NO	YES	NO	NO	YES

3. Mean Root length (Table 3).

Cassia tora seed decoction at concentrations 0.15 mg/ml and 0.31 mg/ml could not affect root growth but at all higher concentrations from 0.62 mg/ml to 5 mg/ml caused significant progressive inhibition in root growth.

Table 3. Mean root length of *Allium cepa* after 72 hours of cultivation in different concentration of *Cassia tora* seed decoction (Mean ± SEM).

S.N.	Concentration (mg/ml)	Mean Root Length (cm)	% Change in Comparison to Controls
1	0.00 mg/ml	6.52 ± 0.21	Mean root length of control is taken as 100%
2	0.15 mg/ml	6.48 ± 0.10	0.61% Inhibition
3	0.31 mg/ml	6.41 ± 0.04	1.68% Inhibition
4	0.62 mg/ml	4.78 ± 0.07^{ac}	26.68% Inhibition
5	1.25 mg/ml	3.67 ± 0.13^{ad}	43.71% Inhibition
6	2.5 mg/ml	2.36 ± 0.12^{ae}	63.80% Inhibition
7	5 mg/ml	1.12 ± 0.10^{af}	82.82% Inhibition

Statistically significant base on 't' test at 5% level of significances
(p = 1.98) (n=100)
a = control vs all experimental groups (1 vs 2, 3, 4, 5, 6 & 7), b =group 2 vs 3 , c = group 3 vs 4 , d = group 4 vs 5, e = group 5 vs 6, f = group 6 vs 7

4. Mitotic Index (Table 4)

No significant change in the value of mitotic index could be found at 0.15 mg/ml and at 0.3 mg/ml concentrations of *C. tora* seed decoction, however, progressive significant decline in mitosis in root cells could be observed at all concentrations i.e. from 0.62 mg/ml onward upto 5 mg/ml.

Table 4. Mitotic Index of *Allium cepa* root tip cells after 48 hours of cultivation in different concentrations of *Cassia tora* seed decoction (Mean ± SEM).

S.N.	Concentration mg/ml	Group.I Control	Group.II Experimental	% Change Gr. I Vs. Gr. III

			C. tora exposed	
1	0.00 mg/ml	44.61±1.07		
2	0.15 mg/ml		44.24 ± 0.49	0.82%
3	0.31 mg/ml		44.60 ± 0.13	0.02%
4	0.62 mg/ml		26.40 ± 0.16^{ac}	40.82%
5	1.25 mg/ml		20.10 ± 0.66^{ad}	54.94%
6	2.5 mg/ml		13.80 ± 0.37^{ae}	69.00%
7	5 mg/ml		5.50 ± 0.57^{af}	87.60%

Statistically significant based on 't' test at 5% of significances

(p = 1.96) (n=2000)

a = control vs all experimental groups (1 vs 2, 3, 4, 5, 6 & 7), b =group 2 vs 3 , c = group 3 vs 4 , d = group 4 vs 5, e = group 5 vs 6, f = group 6 vs 7

5. Abnormal mitosis and chromosomal aberration (Table 5)

The analysis of large number of metaphases and anaphases did not reveal any type of abnormal mitosis or aberrations in controls and in any experimental groups.

Table 5. Cytological effects in *Allium cepa* root tips cells after 48 hr. of cultivation in different concentration of *Cassia tora* seed decoction.

S. No.	Treatments	Number of Counted M*etaphase* Anaphase	Microscopic effects in percent									
			Normal Metaphase	Normal Anaphase	Sticky Chromosome	C. Mitosis	Vagrant (lagging) chromosome	Multipolar Anaphase	Bridges	Fragments	MNC micronucleated cells	Polykaryocytes
1	Control (Tap water) 0.00 mg/ml	1000	+	+	-	-	-	-	-	-	-	-
2	0.15 mg/ml	1000	+	+	-	-	-	-	-	-	-	-
3	0.31 mg/ml	1000	+	+	-	-	-	-	-	-	-	-
4	0.62 mg/ml	1000	+	+	-	-	-	-	-	-	-	-
5	1.25 mg/ml	1000	+	+	-	-	-	-	-	-	-	-
6	2.5 mg/ml	1000	+	+	-	-	-	-	-	-	-	-
7	5 mg/ml	1000	+	+	-	-	-	-	-	-	-	-

+ Present , - Absent

DISCUSSION

Results of the present study have shown that seeds of *Cassia tora* did contain water soluble constituents because deco of dark brown colour and tips of roots of onions grown in decoction were also coloured (Table 2). The dark brown col decoction might have been due to presence of a yellow pigment - torachrysone like substance.[17] It can be safely argue

results of present experiments are only due to presence of water soluble constituents of *C.tora* seeds.

Careful perusal of results indicated that when *Allium cepa* bulbs were grown in *C.tora* seed decoction, only at higher concentrations mean root length and mitotic index declined, however, mitotic disturbances and chromosomal aberrations were totally absent. Mitodepression i.e. low mitosis hence low mean root length in root cells of *A.cepa* growing in the presence of *C.tora* seed decoction can be explained and understood on the basis of existing related reports.

The cytotoxicity of several medicinal and edible plants including seeds of *Cassia tora* were screened in K 562 human leukemia cells and found very very low i.e. only 4.6% inhibitory activity[12]. Bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay[13]. They tested alcoholic extracts of seeds of *C.tora* and found very poor LC_{50} value (725 mg/ml; 24 hr). Present results are in very good confirmity with these two reports that only at higher concentrations of *C.tora* seed decoction only growth of onion root was inhibited hence low mean root length and low mitotic index could be recorded and no other adverse effect like disturbances in mitotic or any chromosomal aberrations could be seen.

It is suggested that because of quinone structure, emodin may interfere with electron transport process and in altering cellular redox status may account for its cytotoxic properties in different systems.[14]

Infact substances which can lower cell division without causing genotoxicity can be used as anticancer agents. Of late, molecular mechanism of emodin action was studied in mammalian cells and emodin a laxative component was found to be a future candidate as antitumor agent. According to authors, inhibitory effect of emodin on mammalian cell cycle modulation in specific oncogene overexpressed cells formed the basis of using this compound as anticancer agents. During apoptosis cells treated with cytotoxic agents are eliminated and emodin also induced apoptosis hence role of emodin in combination chemotherapy with standard drugs to reduce toxicity and to enhance efficacy is pursued vigrously. Its additional inhibitory effect on angionic and metastasis regulatory processes make emodin a sensible candidate as a specific blocker of tumor associated events[14].

Recently re-evaluation of an old chinese remedy rhubarb (*Rheum plamatuness* or *R. Officinalis*) was made[18] and it was concluded that emodin could be a promosing candidate for the research and development of new anticancer drugs.

On the basis of present study it does not seem proper to argue either in favour or against controversial carcinogenic potential of anthraquinones laxatives, however, total absence of abnormal mitosis and chromosomal aberrations show lack of genotoxicity of water soluble constituents of *C. tora* seeds at least in *Allium cepa* model.

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