Influence of decoction of seeds of Cassia tora Linn (Leguminosae) on the genotoxicity of sodium azide and acetaminophen in Allium cepa model

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Abstract
During the first experiment Allium cepa bulbs with roots grown for 24 hrs were further grown for the next 48 hr in three media, Group-I controls, tap water, Group-II six concentrations (0.31, 0.62, 1.25, 2.50, 5 & 10 mg / L) of sodium azide in tap water, Group-III all six concentrations of NaN3 as in the earlier group but each were also given a fixed (0.30 mg/ml) amount of Cassia tora seed decoction. During the second experiment 24 hr grown onion bulbs with roots were allowed to grow further for next 48 hr in three media, Group-I tap water, controls, Group-II 1000 ppm acetaminophen and Group-III 1000 ppm acetaminophen having 0.30 mg/ml Cassia tora seed decoction in it. At 72 hr following both experiments, mean root length and morphology of tips were recorded and mitotic index and aberrations were scored. In group II of first experiment sodium azide progressively lowered root growth and mitotic index, caused ‘bulb’ like swelling of tips and also disturbed the metaphase stage of mitosis. Acetaminophen exposure in group II of second experiment only declined root growth and mitotic index. All these effects of sodium azide and acetaminophen were significantly less pronounced at their lower concentrations in the presence of C.tora seed decoction (group III in both experiment I & II). Probable protective role of C.tora seed decoction is discussed and it is concluded that Cassia tora seed decoction did not enhance the genotoxicity of test chemicals.

The seeds of a medicinal plant Cassia tora Linn (Leguminosae) are used as coffee substitutes, health drinks and for curing many human ailments[1,2]. Seeds of C.tora contain anthraquinones some of which (emodin and rhein) are still debated as carcinogenic or antitumor agents[3,4]. Our recent study has shown lack of genotoxicity of C.tora seeds in Allium cepa model[5]. The present study was planned to further determine whether C.tora seed decoction (water boiled extract) enhances or reduces genotoxicity of a potent mutagen sodium azide and unprescribed analgesic-antipyretic drug acetaminophen in many part of world [6] because both are known genotoxicants in Allium cepa model[6].

MATERIALS AND METHODS

ALLIUM CEPA
Dry healthy onions of almost same size i.e. 1.5 to 2.0 cm in diameter were obtained from local market and brought to the laboratory.

CASSIA TORA
Dried seeds of the medicinal plant Cassia tora locally called 'punvad' were purchased from local herbal medicine shop. These seeds were authenticated by a professor of Botany on systemic grounds. Plant is commonly found in campus. Seeds of Cassia tora were crushed in electrical grinder to obtain a coarse powder. Each time 5 gms of C.tora seed powder was boiled in 1000 ml of tap water for five minutes to prepare decoction of seeds. After cooling, evaporated (lost) volume of solution was made up to 1000 ml with tap water. Dark brown coloration of decoction ensured consistent concentration of water soluble seed contents.

SODIUM AZIDE
Sodium azide (NaN₃) sodium azide 99% pure salt (NaN₃, MW 65.01) made by Central Drug House, New Delhi, India was used. NaN₃ was dissolved in tap water and diluted to obtain concentrations ranging from 0.31 mg/L to 10 mg/L (ppm).
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**ACETAMINOPHEN**

Acetaminophen (paracetamol) under trade name medimol dispersible 500 mg B.P. tablets is made by Synchem Laboratories, Baroda (Gujrat) were dissolved in known amount of luke warm tap water to prepare stable suspensions of desired concentrations (as clear solution can not be obtained).

**EXPERIMENT-I**

Studies of the cultivation of 24 hr grown Allium cepa bulbs with roots for 48 hrs in various concentrations of sodium azide alone or in combination with a fixed concentration of Cassia tora seed decoction were conducted to access the potential for chromosomal aberrations (Little Modified Rank And Nielson's Model) \[7\]. Descaled bulbs were grown for 24 hr in test tubes filled with tap water. Root length was recorded as per protocol \[9\] to determine initial mean root length (MRL) and onions were randomly divided into three groups.

Group-I 12 bulbs in tap water, controls, Group-II 12 bulbs in each of six concentrations of sodium azide (0.31, 0.62, 1.25, 2.5, 5 and 10 ppm) Group-III 12 bulbs in each of six different concentration of sodium azide as in earlier group but each concentration level of sodium azide also aqueous decoction of Cassia tora seeds at a concentration level of 0.30 mg/ml.

All three groups of Allium cepa were all allowed to grow for next 48 hr (equals to two cell cycles of root tip cells\[10\]. At 72 hr mean root length was recorded in each group. Also root tips were fixed in acetoalcohol for scoring chromosomal aberrations.

**EXPERIMENT-II**

Cultivation of 24 hr grown Allium cepa bulbs for next 48 hr in paracetamol alone or in combination with Cassia tora seed decoction to access chromosomal aberrations (Little Modified Rank And Nielson's Model)\[7\]. Descaled bulbs were grown in 36 test tubes filled with tap water for 24 hr Root length was recorded to get mean root length (MRL) then onions were randomly divided into three groups.

Group-I consisted of 12 bulbs for further growth in tap water and served as controls. Group-II consisted of 12 bulbs for further growth in 1000 ppm suspension of paracetamol in tap water. It was found\[11\] that maximum root growth was inhibited at this concentration. Group-III consisted of 12 bulbs for further growth in 1000 ppm paracetamol suspension in tap water also containing 0.30 mg/ml Cassia tora seed decoction.

All three groups of Allium cepa were allowed to grow for the next 48 hr which equals approximately to two cell cycles\[10\]. At 72 hr mean root length was recorded in each group. Also, root tips were fixed in acetoalcohol for scoring chromosomal aberrations.

**STAINING, SQUASHING AND OBSERVATION OF SLIDES**

Root tips were squashed using N-HCl-2% acetocarmine stain. Four fields from each slide were observed to cover 50 cells i.e. total 200 cells per slide, 300 to 4000 cells were observed for each group of onion. Mitotic index was calculated as number of dividing cells per 100 observed cells.

Slides were also observed to determine incidence of mitotic arrest, chromosome fragmentation, abnormal orientation, lagging chromosomes and polyploidy etc.

**STATISTICS**

All experiments were conducted in triplicate. A Student ‘t’ test was applied at a 5% level of significance.

**RESULTS**

**EXPERIMENT-I**

Influence of NaN\(_3\) alone or in combination with C.tora seed decoction on growing roots of Allium cepa.

**MEAN ROOT LENGTH (MRL, TABLE-1)**

In controls, 24 hr grown roots grew further throughout the tenure of experimentation (Group-I). However, sodium azide exposures caused significant inhibition of root growth at all test concentrations (Group-II), which could be checked by C.tora at lower two concentrations only.(Group-III)
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Table 1: MRL (Mean Root Length as cm) of 24 hours already grown roots following further cultivation for 48 hours in sodium azide alone or in combination with seed decoction at 72 hr (Mean ± SEM)

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**MORPHOLOGY - COLOUR AND SHAPE OF ROOT TIPS (TABLE-2)**

In controls (Gr-I), no change in the morphology of tips occurred. NaN3 exposure (Gr-II) caused 'bulb' like swelling of root tips at highest three concentrations tested but did not caused a change in the colour at any concentration. NaN3 plus C.tora seed decoction (at fixed concentration) exposure (Gr-III), changed colour of roots pale at initial four concentrations and dark pale at higher two concentrations. NaN3 could not induced 'bulb' like tips at a 2.5 mg/l concentration in the presence of C.tora, however, at higher (5mg/l and 10mg/l) concentrations 'bulb' formation could not be prevented even in the presence of C.tora seed decoction.

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**MITOTIC INDEX (MI, TABLE-3)**

Sodium azide declined mitotic index at all six concentrations. In the presence of C.tora seed decoction sodium azide induced depressed mitosis could be prevented at initial two concentrations.
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**Figure 3**

Table 3: MI (Mitotic index) of 24 hours already grown roots following further cultivation for 48 hours in sodium azide alone or in combination with seed decoction.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concav (NaN₃)</th>
<th>Gr I Control</th>
<th>Gr II Sodium Azide Exposure</th>
<th>Gr III Sodium Azide + C. tora (Root conc.) exposures</th>
<th>Gr I vs Gr II</th>
<th>Gr I vs Gr III Difference Gr II vs Gr III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00 mg/l</td>
<td>44:13.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.31 mg/l</td>
<td>22:13.07</td>
<td>31:2:0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.62 mg/l</td>
<td>19:3:0.21</td>
<td>27:8:0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.25 mg/l</td>
<td>15:7:0.21</td>
<td>16:2:0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.5 mg/l</td>
<td>13:1:2.63</td>
<td>18:4:0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5 mg/l</td>
<td>7:5:0.32</td>
<td>7:6:0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10 mg/l</td>
<td>3:5:0.21</td>
<td>3:9:0:08</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant based on "t" test at 5% of significance p = (1.960) (n=200)

**CHROMOSOMAL ABERRATIONS (TABLE-4)**

No chromosomal aberrations or abnormal mitosis could be observed in controls (Gr-I) but sodium azide exposure caused a scattering of chromosomes at metaphase (disturbed metaphase arrangement) in a dose dependent manner (Gr-II). In the presence of C.tora seed decoction (Gr-III) sodium azide induced scattering of chromosomes at metaphase could be fully prevented at the initial two concentrations (0.31 mg/l and 0.62 mg/l) tested but were found significantly less pronounced at 1.25 mg/l and 2.5 mg/l and it could not be remedied at higher i.e. 5 mg/l and 10 mg/l concentration levels.

In Gr-III each concentration at NaN₃ had C. tora seed decoction at 0.30 mg/ml level.

a = Control Vs all group, b = Gr-II Vs Gr-III at each concentration level., SC = Sticky chromosome, STC = Scattered chromosome

MPA = Multipolar anaphase CB = Chromosome bridge LC = Lagging chromosome

MNC = Micronucleated cells PKC = Polykaryotytes

**EXPERIMENT-II**

Influence of paracetamol alone or in combination with C.tora seed decoction on growing root of A.capa.

**MEAN ROOT LENGTH (MRL, TABLE-5)**

Acetaminophen exposure (Gr-II) caused significant inhibition of root growth. Even in the presence of C.tora seed decoction paracetamol inhibited root growth (Gr-III) but inhibition is significantly less pronounced in comparison to acetaminophen alone (Gr-II).
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Figure 5
Table 5: Mean Root Length (MRL, cm) of 24 hours grown root tips following further cultivation for 48 hours in acetaminophen alone or combination with seed extract (Means ± SEM)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Duration</th>
<th>Gr I - Control</th>
<th>Gr II - Acetaminophen Exposed</th>
<th>% Growth in Gr I Vs Gr II</th>
<th>% Inhibition in Gr I Vs Gr II</th>
<th>% Growth in Gr III Vs Gr II</th>
<th>% Inhibition in Gr III Vs Gr II</th>
<th>Difference between Gr II and Gr III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 hr (Initial)</td>
<td>1.80 ± 0.10</td>
<td>1.80 ± 0.30</td>
<td>1.80 ± 0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>72 hr (Final)</td>
<td>5.30 ± 0.17</td>
<td>2.52 ± 0.05</td>
<td>3.30 ± 0.11</td>
<td>25.17% Increase</td>
<td>52.40% Increase</td>
<td>131.60% Increase</td>
<td>58.30% Increase in Gr III</td>
</tr>
</tbody>
</table>

Statistically significant based on 't' test at 5% level of significance.

MORPHOLOGY - COLOUR AND SHAPE OF ROOT TIPS
No changes in the shape and colour of root tips could be noticed in any group of onions.

MITOTIC INDEX (MI, TABLE-6)
In controls (Gr-I) mean value of mitotic index did not change significantly during experimentation but acetaminophen exposure (Gr-II) declined mitotic index significantly. Presence of C.tora seed decoction could significantly check acetaminophen induced depressed mitosis (Gr-III).

Figure 6
Table 6: Mitotic Index of 24 hours grown root tips cells following further cultivation for 48 hours in acetaminophen alone or in combination with .

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Duration</th>
<th>Gr I - Control</th>
<th>Gr II - Acetaminophen Exposed</th>
<th>% Reduction in Gr I Vs Gr II</th>
<th>% Reduction in Gr III Vs Gr II</th>
<th>% Inhibition in Gr III Vs Gr II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 hr</td>
<td>44.16 ± 0.40</td>
<td>44.16 ± 0.40</td>
<td>44.16 ± 0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>72 hr</td>
<td>12.20 ± 0.30</td>
<td>12.20 ± 0.30</td>
<td>12.20 ± 0.30</td>
<td>48.42% Reduction</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant based on 't' test at 5% level of significance.

CHROMOSOMAL ABERATIONS
Abnormal mitosis or any sort of chromosomal aberrations could not be observed in any group.

DISCUSSION
Sodium azide exposure lowered mitosis, disturbed metaphase stage and induced bulb like swelling of the tips of roots but acetaminophen exposure reduced only the process of mitosis. Neither azide nor acetaminophen induced chromosomal aberrations.

Genotoxicity of sodium azide has been established in three common plant protocols namely Allium root tip test [7], Tradescantia micronuclei test and pollen mother meiosis test[12]. Sodium azide inhibited mitosis reversibly in guard mother cells of Allium cepa and stamen hairs cells of Tradescantia virginal[13]. According to a report sodium azide induced chromosomal aberrations at anaphase and telophase in Allium cepa root cells and brought mitotic index close to zero [7]. The genotoxic effects of acetaminophen in Allium cepa root tip model [14] and cytotoxicity, spindle disturbances and chromosomal effects in animal, plant and human cells are on record[15-22].

The results of the present study did not show chromosomal aberrations. It seems that differences in purity of chemical used, physicochemical properties of tap water and laboratory conditions might have been responsible for this discrepancy.

Decoction of C.tora seeds could reduce toxic effects of sodium azide and acetaminophen at lower concentrations. Very few similar earlier reports do exist in the literature. Black tea polyphenols theaflavins and thearubigins, turmeric oil, Croton lechleri latex and a herbal formula smoke shield have been found to be antimutagenic against sodium azide[25-27]. Curcumin, a constituent of Curcuma longa, seaweed sargassum polycystum and myrobalan, fruit of Terminalia chebula have been found to antagonize genotoxicity of acetaminophen in different models[21-24].

C.tora seeds have been found to exert antioxidant and free radical scavenging activities which can also be held responsible for the observed protective role in the present study[31-34]. Present findings further support our recent report on the lack of genotoxicity of C.tora seed decoction in Allium test.[5]

It is, therefore, concluded that C. tora seed decoction neither exerted genotoxicity of its own nor enhanced genotoxicity of sodium azide or acetaminophen in Allium test. This way use of C.tora seeds seems safe for human.

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