Mitotraumatism Triggered by Alkaloids (Caffeine and Nicotine) in root meristems of *Lathyrus sativus* L. (Grass pea)

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Abstract

The alkaloids such as caffeine and nicotine, which are constantly consumed by humans, were proved to be harmful for the plants also. These alkaloids alter the biological activity of living organisms and lead to disturbances in the environmental sustainability within the ecosystem. So, the present literature explicates the comparative toxicity of both caffeine and nicotine in root meristems of *Lathyrus sativus* L. (grass pea). Moreover, genotoxicity of caffeine and nicotine was compared at different doses viz. 50, 100, 150 and 200 ppm each. This brought about various instability within chromosomes such as stickiness, scattering, precocious, c-metaphase, unorientation, etc. among which stickiness was predominant. Simultaneously, AMI (%) and TAB (%) were found to be inversely correlated. It was concluded that nicotine was more cyto-genotoxic to the root meristems as compared to caffeine.

Keywords: - Cyto-genotoxic, caffeine, nicotine, Chromosomal instability. *Lathyrus sativus* L.

1. Introduction

The Nature has created the plants with dual activity with respect to the occurrence of organic compounds. First, the organic compounds which are responsible to primary functions are known as primary metabolites, whereas those organic compounds which are not essential to normal growth of the plants but are synthesized at the time of need are termed as secondary metabolites. These secondary metabolites also play an important role in plant defense system as they protect the plants against herbivores and other pathogens. The administered material that is caffeine and nicotine are the examples of such secondary metabolites which come under the category of alkaloids. On the other hand, concerning about their effect on the chromosomes, they are termed as mutagens. Alkaloids are produced not only by higher plants but also from animals. Some alkaloids may have an ability to defend human beings from diseases like malaria and asthma.

The first alkaloid which is used in experimental work is caffeine, isolated from the plant *Coffea arabica* L. family, Rubiaceae and belongs to purine alkaloids. Caffeine was discovered in 1820 by a German Scientist named Friedrich Ferdinand Runge. It is synthesized in green tissues of the plant. It is founds in most commonly consumed beverages. The molecular formula of caffeine is C_{10}H_{10}N_{2}O_{2} and IUPAC name is 1, 3, 7-trimethylpurine-2, 6-dione [1]. The molecular structure was determined by Fischer, 1897 [2]. The radiomimetic effect of caffeine on plant chromosomes has been reported by Sax & Sax in 1966 [3]. High doses of caffeine may lead to cancer in human beings. The physiological effects of caffeine are thought to be mediated through adenosine receptors [4].

Nicotine, the active ingredient in tobacco is second only to caffeine as the most widely used central nervous system stimulant [5]. It was discovered in 1828 by Poselt and Reimann. It is a pyridine alkaloid. The structure of nicotine [1-methyl-2-(3-pyrildyl-pyrrolidine), C_{10}H_{14}N_{2}] was confirmed in 1895 by synthesis [6]. The molecular formula of nicotine shows that it is an oxygen free compound. The main source of nicotine is *Nicotiana tabacum* L. family Solanaceae. It is synthesized in roots and accumulates in leaves. It is also found in tobacco smoke. Nicotine is a nicotinic acetylcholine receptor agonist [7, 8].

The biological material used for the experimental work is *Lathyrus sativus* L. commonly known as Grass pea or Khesari Dal. It belongs to the economical family Leguminosae. This plant is proved to be easy for experiment due to its prolific seed production and less number of chromosomes (2n = 14). The plant has nutritional value and is rich in protein. But, recent studies evidence that this plant contains neurotoxic
compound ODAP (β-n-oxalyl-amino-l-alanine) inside seeds which causes lathyrism in human beings.

The objective of present study was to determine toxicity and mutagenecity of these two alkaloids on the chromosomes in the root meristems of *Lathyrus sativus* L.

2. Materials and Methods
2.1 Biological material-
The pure inbred seeds of the plant *Lathyrus sativus* L. were achieved from SHIATS, Allahabad.

2.2 Tested Compounds-
The organic compounds caffeine and nicotine were used in the experiment of this study.

2.3 Method-
The seeds were kept inside Petri dish for germination on moist filter paper. After germination the seeds were subjected to both caffeine and nicotine treatment by dilution method for 3 hours at different doses (50 ppm, 100 ppm, 150 ppm and 200 ppm). Then, the roots were washed in running tap water. The control sets were also prepared (by maintaining the roots in distilled water for three hours) only with water soaked seeds. The fixation of root tips was done for about 24 hours in Carnoy’s fixative [3(Alcohol):1 (Acetic Acid)] at room temperature followed by preservation in 70% alcohol. After this, the hydrolysis was done in 1 N HCl. The root tips were stained in aceticarmine. Then, the slides were prepared and photographed under 40 X Resolution using PCTV software.

Calculations
The Active Mitotic Index (%) and Total Abnormality percentage are estimated by following formulas-

\[
\text{Active mitotic index} = \frac{\text{Total Number of Dividing Cells}}{\text{Total Number of Cell Observed}} \times 100
\]

\[
\text{Total Abnormality Percentage} = \frac{\text{No. of abnormal cell}}{\text{Total Number of Cell Observed}} \times 100
\]

Statistical assessment-
The data obtained from the study were analyzed by the use of SPSS 16.0 software. There were three replicates for each treatment and one independent variable was used. A one way analysis of variance (ANOVA) and Duncan’s multiple range test (DMRT, \( P < 0.05 \)) were performed for mean separation and the graph was plotted by using sigma plot 10.0 software. Actual mean and standard error were calculated. The data were subjected to analysis of variance.

3. Result
Chromosomal organization is an important aspect for studying variations in living organism. In normal metaphase stage (Fig.1A), they are organized at equatorial plate whereas reached at the pole in normal anaphase stage (Fig.1B), as reported in control sets. After giving treatment of alkaloids, normal behavior of chromosomal organization was found to be altered.

The present cytological experiment also resulted in decreased AMI (Active Mitotic Index) on increased doses (50 ppm to 200 ppm) whereas TAB % (Total Abnormality Percentage) was found to be inversely proportional to that of AMI (Table-1). The AMI of control set was found 12.34%, which was considerably decreased in treated set (50 ppm to 200 ppm) ranging from 11.89% to 8.08% and from 11.43% to 6.46% in caffeine and nicotine treated sets respectively. In contrast to AMI, TAB % was increased from 1.27% to 4.32% in caffeine treated sets and from 1.92% to 5.94% in nicotine treated sets. These data show that AMI was reduced in both treated set but it was more declined in nicotine treated set as compared to caffeine. Simultaneously, it was also confirmed that TAB% was increased in both treated set. But, this increased in TAB% in caffeine treated set was found to be less than nicotine treated set.

The result of the given experiment was also concerned about presence of various chromosomal abnormalities in both treated sets. Chromosomal stickiness was found to be dominant at both metaphase (Fig.1 c) and anaphase stages (Fig.1 h). Other abnormalities observed were viz. scattering of chromosomes (Fig.1 d), precocious movement of chromosome (Fig.1 e), unorientation of chromosome (Fig.1 f), C-mitosis at metaphase (Fig.1 g), scattering with 2 laggards chromosomes at anaphase (Fig.1 i). The Figure 2 and 3 shows comparative studies of AMI % and TAB% in both treated sets, respectively.

4. Discussion
It has been found that mutagens have potential to cause cyto-genotoxic effects within the plants. These mutagens might be responsible for copy error at gene level by forming dimmers of nitrogen bases. Genetic toxicological studies have given rise to a number of testing
procedures, both in vitro and in vivo. They have been designed to assess the effects of chemicals on the genetic material and, consequently, to assess the risk to living organisms including humans. To provide a broad coverage of the mutagenic and presumably carcinogenic potential of different levels, e.g. the gene, the chromosomes and the cellular apparatus necessary for chromosome segregation [9]. Cytogenetical analysis with respect to both mitotic and meiotic behavior is the most dependable indices to estimate the potency of mutagens [10].

The present study reveals about various types of chromosomal abnormalities found in treated sets whereas control set was found to be normal. These abnormalities have significant value as it results in genetic damage that is handed over to the next generation [11]. The augmentation in percentage of chromosomal anomalies was observed in a dose dependent manner. Higher doses show more toxicity than lower ones.

The AMI % is an important parameter while determining the rate of root growth [12]. Hence, decreased in AMI % was also related with the increased in Total Abnormality Percentage (TAB %) on adding mutagens. According to Badr & Ibrahim (1987) [13], decrease of mitotic index level shows that experimental material had mitodepressive effect resulting into the inhibition of cells access to mitosis. These results inferred about more toxicity of nicotine as compare to caffeine in root meristems of the plant. The metabolism of nicotine is known to produce reactive intermediates capable of binding to proteins and DNA [14]. Regarding the caffeine mechanism action, it is possible that this compound acts as solubilizing factor and forms molecular complexes, thus favoring the generation of chromosomal aberrations. It can interact with DNA and forms molecular complexes, thus favoring the generation of chromosomal aberrations.

**Table I: Abnormalities induced by Caffeine & Nicotine in the root meristems of *Lathyrus sativus* L.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses (ppm)</th>
<th>AMI % (Mean ± SE)</th>
<th>Metaphasic abnormalities (%) (Mean ± SE)</th>
<th>Anaphasic abnormalities (%) (Mean ± SE)</th>
<th>Others</th>
<th>TAB % (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>St</td>
<td>Sc</td>
<td>Pr</td>
<td>Un</td>
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<tr>
<td><strong>Caffeine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>12.34±0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50 ppm</td>
<td>11.89±0.06</td>
<td>0.30±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.31±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>100 ppm</td>
<td>10.46±0.07</td>
<td>0.41±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.29±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
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<tr>
<td>150 ppm</td>
<td>9.38±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.61±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.41±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>200 ppm</td>
<td>8.08±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.32±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.20±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td><strong>Nicotine</strong></td>
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<tr>
<td>Control</td>
<td>12.34±0.06</td>
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<tr>
<td>50 ppm</td>
<td>11.43±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>0.16±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.32±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 ppm</td>
<td>9.85±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.36±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>150 ppm</td>
<td>8.28±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.46±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.18±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.64±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>200 ppm</td>
<td>6.46±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.98±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.66±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.73±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Where: St - Stickiness, Sc - Scattering, Pr - Precocious movement, Un - Unorientation, Cm - C-Mitosis, Lg - Laggard, Oth - Others

Means followed by lowercase letter are statistically significant at p < 0.05 in Duncan’s Multiple Range Test.
chromosomes. Many authors suggest own view regarding to the cause of sticky chromosomes. Stickiness may results from the entanglement of chromatin fibres [16]. Chromosome stickiness is caused probably through immediate reactions with DNA during its inhibition periods, causing DNA-DNA or DNA-protein cross linking [17] or it might also be the result of incomplete replication of chromosomes by defective enzymes [18]. Gaulden (1987) [19] considered that stickiness may result from defective functioning of one or two types of specific non histone proteins involved in chromosomal separation and segregation. According to Patil and Bhat (1992) [20], chromosomal stickiness is a type of physical adhesion involving mainly the proteinacious matrix of chromatin material. Stickiness is indicative of highly toxic usually irreversible effect leading to cell death [21]. Klasterska et al., (1976) [22] suggested that stickiness arose due to improper folding of chromosome fibers into single chromatid. Stickiness has been reported to be a result of partial dissociation of nucleoproteins and alteration in the pattern of organization of chromosomes [23] or due to disturbances in cytochemically balanced reactions [24]. The scattering of chromosomes appeared due to loss of microtubules of spindle fibres [25, 26]. Kumar and Rai, (2007) [27] reported that unorientation at metaphase and scattering of chromosomes may be due to inhibition of spindle formation or destruction of spindle formed.

Precocious movement of chromosomes was observed when doses of mutagens increased. It might have been caused by the early terminalization, stickiness of chromosomes or because of the rest during anaphase [28]. A precocious chromosome is the result of unequal spindle movement in which some chromosome arms are pulled towards the extremity of the pole [29].

In occurrence of abnormalities, c-mitosis was also observed. Levan, (1938) [30] described colchicine mitosis as an inactivation of the spindle followed by a random scattering of the chromosomes over the cell. In the above experiment, the action of nicotine and caffeine was found to be similar to that of colchicine. In this case chromosomes become thick, appear in c-shape and remain scattered in cytoplasm. In C-metaphase, the chromosomes lose their ability to continue to anaphase and are arrested at metaphase [31, 32, 33, 34, and 35]. It was also shown that disturbances in microtubules results in c-mitotic aneuploidy [36].

The induction of Laggard chromosomes could be attributed to irregular orientation of chromosomes [37]. These Laggards may be distributed randomly to either poles both at anaphase and telophase (I and II) which result ultimately in aneuploidy [34] or may give rise to micronuclei at telophase [35]. The occurrence of lagging chromosomes may be due to abnormal spindle formation and as a result spindle fibres failed to carry the respective chromosomes to the polar region and resultantgently lagging chromosome appeared [38]. Lagging chromosomes occur because of improper movement of chromosomes during anaphase separation [39]. Laggard at metaphase could be attributed to failure of the spindle apparatus to organize and function in a normal way [40].

Conclusion

The above mutagenic analysis deduced the deleterious effect of both caffeine and nicotine, which are responsible for decreased in Active Mitotic Index and lead to various chromosomal anomalies at genomic level in the plants. On comparing to each other nicotine had more toxicity than caffeine. The amplitude of chromosomal abnormalities was directly proportional to the doses. This aberration leads to variations in biological evolution. Sometimes these variations are benefitted to the environment and selected by nature. However, the result of given experiment suggested about limited use of these alkaloids, as they causes genetic toxicity to plants which leads to disturbance both phenotypically and genotypically.

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