Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Special Issue [1]2022 : 1038-1043

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Antimutagenic Potential of *Murraya koenigii* on Chromosomal Aberrations Induced by Sodium Azide in *Allium cepa* Root Tip Cells

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ABSTRACT
The present study was conducted to evaluate the mutagenic/ Clastogenic potential of Sodium Azide at a concentration of 200ug/ml on the root tip cells treated with sodium Allium cepa and to determine the antimutagenic effect of Murraya koenigii at doses (5µg / ml) (10µg / ml)20µg / ml. Now a days Allium cepa has been used to asses a great number of genotoxic/Antigenotoxic agent and is used as a cytogenetic short term bioassay that has proved to be a useful tool in basic research to evaluate the genotoxic risk of known chemicals. Sodium Azide induces chromosomal breakage, Anaphase Bridge, sticky chromosomes, but when pretreated with Murraya koenigii the chromosomal aberrations were lesser, Murraya koenigii being a dietary antioxidant has free radical scavenging activity, the effective dose was found to be 20µg / ml.
Key words: *Allium cepa root meristem, sodium azide, Murraya koenigii , chromosomal aberrations, and mitotic index.*

Received 12.02.2022 Revised 27.03.2022 Accepted 12.04.2022

INTRODUCTION

Plants have been used as indicator organisms in studies on mutagenesis in higher Eukaryotes. Plant systems have a well defined genetic end point including alterations in ploidy, chromosomal aberrations and sister chromatid exchanges [1]. Among the plant systems, *Allium cepa* is the most commonly used species for the study of chromosomal aberrations, bulbs produce a large number of roots in a short period of time and chromosomes are relatively long. The Allium cepa test introduced by Levan [2], is a cytogenetic short term assay that has proved to be a useful tool in basic research to evaluate the genotoxic risk of known chemicals[3]. An effective test organism for the assessment of chromosomal aberration should have chromosomes which are easy to analyse in terms of size, morphology and number [4].The Allium cepa test allows the toxicity of aqueous samples to be evaluated through two cytological end points; root formation and growth restriction observable at the macroscopic level and root tip meristem chromosome aberration scored at the microscopic level.

The universality of genetic material and of the basis of genetics facilitates the use of non human test system to detect chemical mutagens and clastogens. As a rule, there appears to be good correlation between the chromosome breaking caused by chemicals in plants and cultured animal cells [5].

Sodium Azide NaN3 Mol wt 65.02 is colourless odourless, crystalline solid. It is a major environmental mutagen, used in medicine, agriculture and it causes cytotoxicity in several animal and plant systems, by inhibiting the protein synthesis and replicative DNA synthesis at low dosage [6] .It is used in making chemicals , as a preservative in diagnostic medicines and blood tests , as a herbicide , fungicide and soil fumigant , and is the propellent used for inflating air bags (Hazardous substances fact sheet – Right to know).

Sodium Azide fails to induce chromosomal aberrations in human lymphocytes it is most efficient mutagen in barley, Yeast, and several other higher plants ..The reason behind its non–genotoxicity in mammalian test system is the enzyme responsible for conversion of Azide into non–genotoxic azidoalanine and the lack of interaction with DNA [7] . Sodium Azide is a unique mutagen – because of the lack of induction of SCEs above background combined with previous data which demonstrates the negative clastogenic but

positive mutagenic activity of Sodium azide confirms the uniqueness of this mutagen [8] .The Allium cepa anaphase , telophase assay was used to show genotoxicity of N- methyl –N-Nitrosourea (MNU) , maleic hydrazide, sodium Azide NaN3 and Ethyl Methyl Sulphonate (EMS) . All agents induced chromosomal aberration at statistically significant levels .*Murraya koenigii* is the most common herb used in Asia with enormous nutritive and pharmacological benefits.. It belongs to the Rutaceae, the family of flowering plants, is composed of 160 genera and a few herbaceous perennials [9]. Of the 2070 global species belonging to the family Rutaceae, only a few herbaceous varieties are available in Sri Lanka, including *M. koenigii, M. minutum*, and *C. indica*. Of the three, *M. koenigii* is a fascinating house plant grown in Asia and native to Sri Lanka, Bangladesh, and India, which had used curry leaves or "karapincha" (in Sinhala) for centuries. The dark green fresh leaflets of *M. koenigii* are widely used in Asian cooking mainly for their aroma and versatile medicinal properties [7, 8]. Furthermore, they add subtle flavors to various food preparations, from vegetables to many other dishes as a natural flavor. Kola kanda or leafy porridge is famous in Sri Lanka for its high nutritional value. Various parts of *M. koenigii* are used to treat diabetes, chronic fever, dysentery, and diarrhea [10]. *M. minutum* is also used as a flavoring agent and is reported to have medicinal value in the southern part of Thailand and many other Asian countries [10]. In particular, *M. minutum* roots are used to cure ringworms and to regulate menstruation. Other parts of *M. minutum* are used as carminatives, purgatives, and expectorants [11, 12]. The leaves of *M. minutum* are used traditionally to treat toothache and teething issues in babies, skin irritations caused by scabies, and as a remedy for stomachache and headache [13]. *C. indica* is famous as a folk medicine in many Asian countries. Leaves and roots of *C. indica* treat various health issues, such as flu, colds, joint dislocation, bone fractures, headaches, colic, and rheumatism. [14].

MATERIAL AND METHODS

Healthy onion bulbs (20- 25 gms) were grown in test tubes at room temperature When the root tips reached 2-3 mm in length, they were treated with different concentration of (5, 10, 20µg / ml of *Murraya koenigii* for 16 hrs. Following *Murraya koenigii* treatment the bulbs were washed in distilled water and then treated with 200 µg / ml of Sodiun Azide for 3 hrs. This formed group 1.

Healthy onion bulbs (20- 25 gms) were grown in test tubes at room temperature When the roots reached 2-3 mm in length, they were treated with concentration of 200 µg / ml of Sodium Azide for 3 hrs. This formed group 2.

Healthy onion bulbs (20- 25 gms) were grown in test tubes at room temperature. When the roots reached 2-3 mm in length , they were treated with different concentration 5, 10, 20µg / ml of *Murraya koenigii* for 16 hrs.This formed group 3 . Healthy onion bulbs (20- 25 gms) were grown in test tubes at room temperature 24 hrs. This formed group 4 and served as control. After the treatment schedule, the root tips were harvested and fixed in ethanol acetic acid in the ratio 3:1 and stored at 5º C.[15]

Microscopic preparation

Hydrolysed the root tips in1N HCl at 56º C for 8 mins. The root tips were washed in distilled water and were then exposed to 4% Iron Alum solution as a mordant. The root tips were washed in distilled water .A single softened root tip was then transferred to a slide and a few drops of Haematoxylin stain was added and stained for 25 mins. [16,]The excess stain was removed with 45% Acetic acid and a cover slip was placed and squashed without any air bubbles.

Analysis

The Microscopic preparations were analysed in 40x objective lens to determine the cell division intensity by calculating the Mitotic index. This is the ratio between the number of dividing cells and the total number of cells analysed in percents.

$$\text{MITOTIC INDEX} = \frac{\text{Number of dividing cells}}{\text{total number of cells}} \times 100$$

Scoring of slides – Chromosomal aberrations are scored at prophase, metaphase, anaphase and telophase as fragments, disturbed chromosomes, sticky chromatin, anaphase bridge, unequal distribution of chromosomes [18,19].

Statistical Analysis of Data - The mean values were calculated for each group of concentrations and controls for the determination of the significance among the means, Independent Samples t-Test was applied ($p<0.05$).

Table 1: **Comparison of Mitotic Index between different treated groups**

Treated group	Mitotic index (%)
Control	40.58
Sodium azide (200µg / ml)	20.22
Murraya koenigii (5µg / ml)	35.05
Murraya koenigii (10µg / ml)	35.72
Murraya koenigii (20µg / ml)	35.00
Murraya koenigii (5µg /ml) +Sodium Azide (200µg / ml	24.98
Murraya koenigi (10µg /ml)+Sodium Azide (200µg)	24.98

Table 2: ANOVA for the effect of Sodium Azide Treated With *Murraya koenigii* at 5µg on Chromosomal Aberrations in *Allium cepa*

Mitotic stages	Different concentration	Mean and standard deviation	P value
Prophase	Sodium Azide	42.70 ±11.23	0.000***
	Murraya 5µg	11.32 ±3.44	
	Sodium Azide & *Murraya* 5µg	50.72±4.49	
Metaphase	Sodium Azide	60.60 ±7.69	0.000***
	Murraya 5µg	11.21 ±1.94	
	Sodium Azide & *Murraya* 5µg	51.38±5.28	
Anaphase	Sodium Azide	54.55±12.87	0.203ns
	Murraya 5µg	13.28±14.37	
	Sodium Azide & *Murraya* 5µg	65.66±57.36	
Telophase	Sodium Azide	55.02±15.95	0.017*
	Murraya 5µg	11.65±06.32	
	Sodium Azide & *Murraya* 5µg	32.78±43.58	

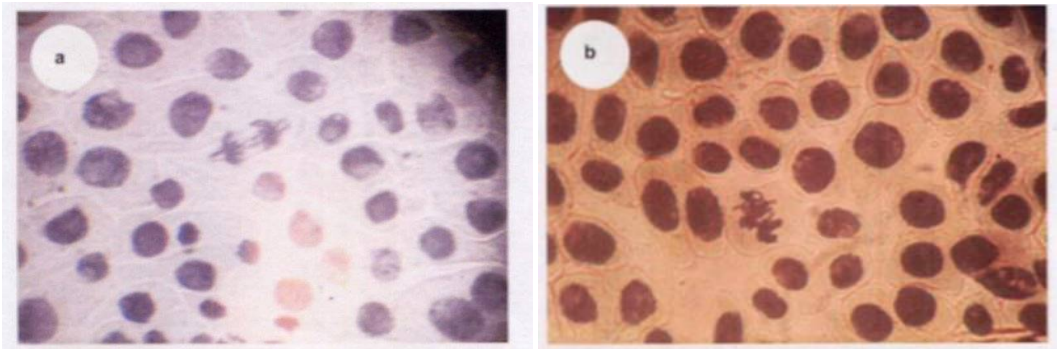
Table 3: ANOVA for the Effect of Sodium Azide Treated With *Murraya koenigii* at 10µg On Chromasomal Aberrations *Allium cepa*

Mitotic stages	Different concentration	Mean and standard deviation	P value
Prophase	Sodium Azide	42.70 ±11.23	0.002***
	Murraya 10µg	10.18 ±3.594	
	Sodium Azide & *Murraya* 10µg	44.90±7.07	
Metaphase	Sodium Azide	60.61 ±7.69	0.530***
	Murraya 10µg	10.34 ±0.73	
	Sodium Azide & *Murraya* 10µg	32.33±55.73	
Anaphase	Sodium Azide	53.55±13.67	0.329
	Murraya 10µg	12.39±11.47	
	Sodium Azide & *Murraya* 10µg	31.56±26.53	
Telophase	Sodium Azide	56.02±14.98	0.005*
	Murraya 10µg	9.56±7.23	
	Sodium Azide & *Murraya* 10µg	32.05±1.33	

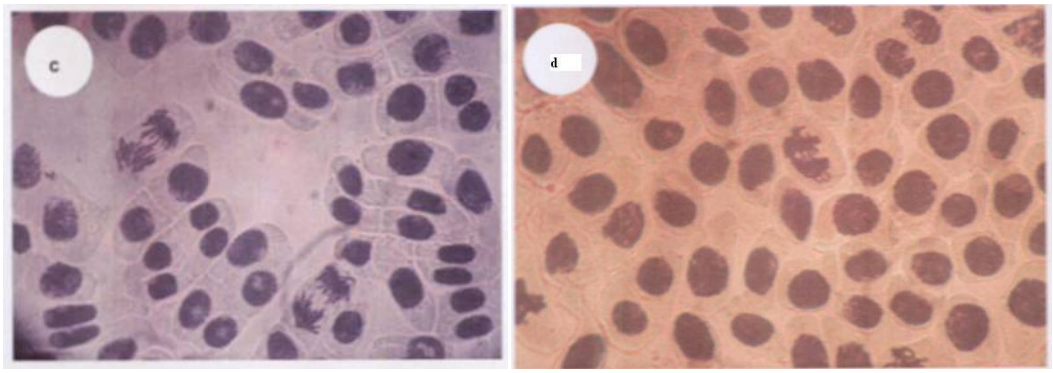
Table 4: ANOVA for the Effect of Sodium Azide Treated With *Murraya koenigii* at 20µg On Chromasomal Aberrations *Allium cepa*

Mitotic stages	Different concentration	Mean and standard deviation	P value
Prophase	Sodium Azide	43.60 ±12.23	0.017*
	Murraya 20µg	8.07 ±3.51	
	Sodium Azide & *Murraya* 20µg	29.49±8.72	
Metaphase	Sodium Azide	60.61 ±7.69	0.001**
	Murraya 20µg	7.65 ±1.78	
	Sodium Azide & *Murraya* 20µg	25.47±3.19	
Anaphase	Sodium Azide	55.55±13.87	0.090ns
	Murraya 20µg	10.17±9.30	
	Sodium Azide & *Murraya* 20µg	22.67±2.83	
Telophase	Sodium Azide	55.02±15.98	0.008**
	Murraya 20µg	8.07±6.72	
	Sodium Azide & *Murraya* 20µg	27.0±0.36	

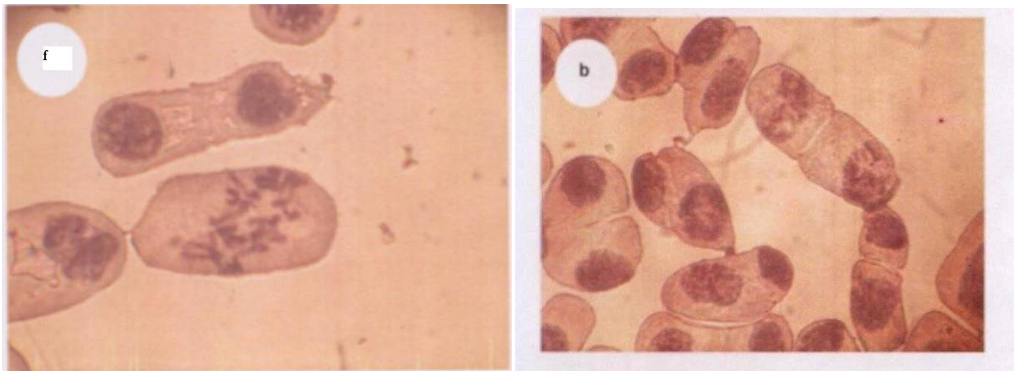
* Significant difference at p value < 0.05at 5%; ** Significant difference at p value < 0.01at %
*** Significant difference at p value < 0.001at 0.001%; ns Not significant difference at p > o.o5.



A) Anaphase with two bridges **b)** Sticky metaphase



C) Anaphase with single bridge d) Sticky anaphase



e)Gaps and breaks at metaphase breaks at metaphase f)Pulverized chromosome

Fig 1: Chromosome aberrations in Different stages

RESULTS AND DISCUSSION

The effect of treated groups on Mitotic Index

When the root tips were exposed to Sodium Azide, the Mitotic Index was 20.22%, when compared to control 40.58%. This clearly shows the genotoxicity of Sodium Azide. When root tips were exposed to *Murraya koenigii* at 5µg, 10 µg and 20 µg, the Mitotic Index was, 35.05%, 35.72%, 35.00 % .This shows that *Murraya koenigii* does not reduce the Mitotic Index at this concentration, and it was only a very negligible difference when compared to control. The Mitotic Index reduced as the concentration of *Murraya koenigii* increased. When root tips were previously grown in *Murraya koenigii* at 5µg, 10 µg and 20 µg for 16 hrs and then exposed to Sodium Azide (200µg) for 3hrs , the was a significant increase in Mitotic Index at all concentrations , *Murraya koenigii* at 20 µg was found to be the effective dose. [20, 21]

The effect of Treated groups on Chromosomal aberrations.

The data for Chromosomal aberration associated with exposure to Sodium Azide are presented in (Table 2,3 & 4) . The results showed that the number of aberrations induced by Sodium Azide is increased when compared to *Murraya koenigii* at different concentrations, *Murraya koenigii* was non clastogenic in plant systems. The most frequent aberrations were sticky chromosomes (Fig b &d), pulverized chromosomes,(Fig e) fragments,(Fig f) Anaphase Bridge. (Fig a &c), But when the root tips were pretreated with

Murraya koenigii , the number of aberrations were significantly reduced, which indicated its antimutagenic potential. [22]

DISCUSSION

Sodium Azide is a colourless odourless, crystalline solid. It is a major environmental mutagen , used in medicine , agriculture and it causes cytotoxicity in several animal and plant systems, by inhibiting the protein synthesis and replicative DNA synthesis at low dosage .It is used in making chemicals , as a preservative in diagnostic medicines and blood tests , as a herbicide , fungicide and soil fumigant , and is the propellent used for inflating air bags Hazardous substances fact sheet – Right to know. When Sodium Azide is dissolved in water it forms a toxic hydrogen azide gas, with the generation of azide ions being the possible reason for its genotoxicity and cytotoxicity in plant systems. Sodium Azide induces chromosomal aberrations in *Allium cepa* root tip cells at statistically significant levels [23]. It is delivered into human heteroploid HEp-2 cells via Liposomes and it produces chromosome aberrations and other major genetic damages . The use of anti – mutagens in everyday life is the most effective way for preventing human cancer and genetic disease. Chemicals which act to interfere with DNA repair or with mutagen metabolism can be effective anti-mutagen

Murraya koenigii the active ingredient of curry leaf plant is anti-mutagenic and it has protective effect. The Phytochemicals present in *Murraya koenigii* includes saponins, Tannins, steroids, alkaloids , terpenoids and its derivatives are due to their diversified role in combinations of cell signaling pathways at multiple levels in various diseases. Furthermore it has antimutagenic potential against cyclophosphamide and BAP induced genotoxicity in microbial and mammalian tests in a dose dependent manner. *Murraya koenigii* exhibits antimutagenic potential against Sodium azide induced damage in dose dependent manner Araujo et. al reported that curcumin was clastogenic in mammalian cell culture . Extensive gaps, chromosome fragments and exchanges appeared at doses above (20µg / ml) at all treatment hours. The percentage of clastogenicity was higher at doses above 30µg / ml for *Murraya koenigii* . Furthermore *Murraya koenigii* acts as a potent anti carcinogenic compound through induction of Apoptosis and also inhibits cancer at initiation, promotion and progression stages of development against benzo(a) pyrene induced skin tumors in female Swiss mice . In our present study *Murraya koenigii* has proved to be an effective antimutagen at a particular dosage. The molecular mechanisms of its action and its interaction with xenobiotics remain to be elucidated.

CONCLUSION

Sodium Azide has wide application in various fields for human welfare. But it is mutagenic / clastogenic in plant test system. The universality of genetic code has facilitated the use of non- human assay system. *Murraya koenigii* is antimutagenic and has the capacity to reduce the mutagenic potential of Sodium Azide .

ACKNOWLEDGEMENTS

We are grateful to The Principal , Vice Principal, Head of the Department for their help rendered in carrying out the experiments. We acknowledge the support extended by the Department of Biotechnology, Mohamed Sathak College of Arts and Science, Chennai.

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CITATION OF THIS ARTICLE

N Najeeb, G.Priya and P.Priyadarshini. Antimutagenic Potential of *Murraya koenigii* on Chromosomal Aberrations Induced by Sodium Azide in *Allium cepa* Root Tip Cells. Bull. Env.Pharmacol. Life Sci., Spl Issue [1] 2022 : 1038-1043