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Analysis of Antigenotoxicity induced by Tulsi extract on *Allium cepa* Grown in Slurry Water

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ABSTRACT: The use of industrial effluents and sewage sludge for agriculture has become a common practice in the world and India too. Many harmful metals and substances get transferred and accumulated in plant tissues from the soil, causing many problems and diseases in living organisms. Management of these abnormalities/ diseases with the medicinal plant is an ancient practice that has gained momentum in recent years. Aqueous extract of *Ocimum sanctum* (Tulsi) has long been used for the traditional management of cancer due to its antigenotoxic nature. Data on genotoxicity and carcinogenicity of are rather controversial, depending on the genetic system or the assay used. The genotoxicity profile of five different concentrations of slurry water (20%, 40%, 60%, 80%, and 100%) and the antigenotoxic profile of three concentrations of aqueous extract of tulsi leaves (10%, 20% and 30%) were evaluated with genotoxicity assays by using root tip cells of *Allium cepa* plant. The parameters evaluated in the cytological assay were several cells in dividing stage, mitotic indices (MI), % mitodepressive (MD) effect, RDR and chromosomal aberrations (CA) on *A. cepa* roots. A decrease in mitotic index with increasing concentration of slurry water was observed but the reduction was not significant enough to deduce that the slurry water samples caused a decrease in the number of dividing cells. However higher concentrations of slurry water (i.e. 60%, 80% and 100%) were found to have a completely lethal effect as no root germination and growth was observed in onion bulbs treated with these three concentrations of slurry water. The antigenotoxic effect of aqueous extract of tulsi leaves (10%, 20% and 30%), on *Allium cepa* roots pretreated with 20 % and 40% concentrations of slurry water was also observed. A significant reduction in MI% was noticed with all three concentrations of aqueous extract of tulsi leaves in treated samples. A concentration-dependent increase in the antigenotoxic effect of tulsi extract was observed for an overall reduction in chromosomal abnormalities in both concentrations of slurry water-induced genotoxicity. In the present investigation response of slurry water and antigenotoxic potential of *Ocimum sanctum* has been evaluated for their cytotoxic and genotoxic effects on *Allium cepa.* On the basis of our results, we can say that aqueous extract of tulsi has a protective effect on *Allium cepa* root meristem cells against the genotoxic effects produced by slurry water.

Keywords: *Ocimum sanctum*, antigenotoxic, genotoxicity, *Allium cepa*, mitotic indices, Mito depressive effect, RDR, chromosomal aberrations.

INTRODUCTION

Population growth increased significantly as the industrial revolution gathered rapidly. The advent of the industrial era for newer materials has extremely increased the contamination of water, air, and soil by heavy metals (Patra *et al.*, 2004), considered the most common and consistent environmental pollution.

Urban-industrial and agricultural wastes can add heavy amounts of contaminants to surface water and sediments; accordingly, pollution could be a severe problem for the health of the biome and humans interacting with polluted aquatic ecosystems. The essential source of drinking and irrigation water often receive pollutants from industrial effluents, mine sewage herbicides, insecticides, and various other chemical and radioactive wastes (Ray and Barman 1987) it may influence human health such as allergy at early ages, respiratory disorders, coronary and cancer in Middle Ages.

The predominant cause of global complications and mortality is lifestyle-related chronic diseases, many of which can be addressed through Ayurveda with its focus on healthy lifestyle practices and regular consumption of adaptogenic herbs. Tulsi is also known as ''the elixir of life'' since it promotes longevity (Govind and Madhuri 2010). Different parts of plants are used in Ayurveda and Siddha Systems of Medicine for the prevention and cure of many ailments.

Ocimum (2n=36) could be a genus of about 35 species of aromatic annual and perennial herbs and shrubs within the family Lamiaceae. *Ocimum sanctum* (synonym – *Ocimum tenuiflorum*), commonly known as Holy Basil or Tulsi, is a small herb found throughout India from Andaman and Nicobar Islands to the Himalayas up to 1800 m above the sea level (Anonymous 1991). Within Ayurveda, Tulsi is called "The Incomparable One, "Mother Medicine of Nature" and "The Queen of Herbs," and is revered as an "elixir of life" that is without equal for both its medicinal and spiritual properties (Singh *et al.,* 2010). Tulsi has unique actions like Antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial,), mosquito repellent, anti-diarrheal, anti-oxidant, anti-inflammatory, chemo-preventive, radio protective, hepatoprotective, neuro-protective, cardio-protective, anti-diabetic, anti-hypertensive, anti-carcinogenic, anti-allergic, immunomodulatory, central nervous system depressant, memory enhancement, anti-asthmatic, diaphoretic, anti-thyroid, anti-fertility, anti-ulcer, anti-stress, anti-cataract, anti leukodermal and anti-coagulant activities (Mahajan *et al.,* 2013). Tulsi also helps to prevent cancers caused by toxic compounds by reducing DNA damage and inducing apoptosis in precancerous and cancerous cells, thereby reducing the growth of experimental tumors and enhancing survival (Jha *et al.,* 2012).

However, plant assays, such as the *Allium cepa* test, may have some advantages over microbial and mammalian cell tests for environmental monitoring. Plant assays are highly sensitive to many environmental pollutants, including heavy metals (Fiskesjo, 1988), and determination of toxicity as well as evaluation of cytotoxicity and anti-proliferative potential of medicinal plants (Bakare *et al.,* 2013; Pastori *et al.,* 2013). Furthermore, the test plants can be directly exposed to complex mixtures or environmental samples either in the laboratory or in situ (Rank, 2003). Because of the large size of their chromosomes, higher plants are suitable for cytological analysis (Sadowska *et al.,* 2001). Onion (*Allium cepa*) root-tip cells can be used to measure a variety of morphological and cytogenetic factors.

MATERIAL AND METHOD

Cytotoxicity and genotoxicity of slurry water. Slurry water sample collection: Slurry water samples were collected from the open wastewater basin of village Gesupur Baphawat, a Village in Daurala Block in Meerut District of Uttar Pradesh State, India. It belongs to Meerut Division.

Study design: The study was carried out by using an experimental study to examine the cytotoxicity, genotoxicity, and chromosomal aberrations of slurry water on *A. cepa* root tip.

Preparation of different concentrations of slurry water: Five different concentrations of slurry water i.e. 20%, 40%, 60%, 80% and 100% were used. Distilled water was taken as control.

Experimental plant material: Healthy and equal-sized bulb onions (*A. cepa* L. 2*n* = 16) were purchased from Meerut local market.

Methodology. Prior to initiating the test, the outer scales of the bulbs and the dry bottom plate were removed without destroying the root primordial and germinated at five definitive concentrations of slurry water for 5-7 days at room temperature (25 ±2 °C). Distilled water was used as a control. When the roots reached a length of 1 to 2 cm, tips were collected and fixed in Carnoy's fixative 1:3 (methanol: glacial acetic acid) for 24 hrs. Then, the root tips were placed in 70% ethanol and stored at 4°C until analysis.

Mitotic smear was prepared by as per the standard protocol of the Feulgen squash method (Sharma and Dphil 1980). The root tips were hydrolyzed with 1N HCl for 10 minutes and heat for 5 minutes to soften the root tissues. Then, the meristematic regions of root tips were cut and stained with 2% acetocarmine in 45% glacial acetic acid for 5-10 minutes and examined using bright-field microscope. A total of 3 replicas were prepared for each concentration of treatment. A total of 1000 cells were scored for mitotic index and chromosomal abnormalities in each replicate. Microscopic parameters and data analysis the mitotic activity, rate and kind of chromosomal aberrations were recorded using bright-field microscopy at total magnifications of 400X.

Data was recorded by calculating the following parameters (Fiskesjo, 1985):

$$\text{Mitotic index (MI)} = \frac{\text{No. of dividing cells}}{\text{1000 total of dividing cells observed}} \times 100$$

$$\text{Mito-depressive effect (MD)} = \frac{\text{MI of control} - \text{MI of trated cells}}{\text{MI of control}} \times 100$$

$$\text{Frequency of chromosomal Abnormalities (\% CA)} = \frac{\text{Number of aberrant cells}}{\text{Total number of dividing cell}} \times 100$$

Relative division rate (RDR) calculated using the formula of Kumar and Shikha (2012)

$$\text{RDR\%} = \frac{\text{\% dividing cell in treated variant} - \text{\% of dividing cell in control}}{100 - \text{\% dividing cell in treated variant}} \times 100$$

Data analysis: The replicated data were subjected to one-way analysis of variance (ANOVA) using software OPSTAT using one way ANOVA analysis (O.P. Sheoran Programmer, Computer Section, CCS HAU, Hisar).

Antigenotoxicity of tulsi

Study design: The study was carried out by using an experimental study to examine the antigenotoxicity of aqueous leave extract of tulsi on *A. cepa* root tip.

Experimental plant material: To study the antigenotoxicity effect at the cytological level *Allium cepa* root tips were used as test material.

Preparation of *Ocimum sanctum* aqueous extract. Tulsi plants were grown in pots at College of Biotechnology, S.V.P.U.A & T., Meerut. The aqueous extract of Tulsi leaves was prepared using the protocol described by Devi and Dutta (2012). Fresh leaves were collected from the plant using the protocol; a 30% stock solution of Tulsi leaves extract was obtained. Three different dilutions i.e. 10%, 20% and 30% were prepared from this 30% stock solution using distilled water.

Methodology, Observation, and data analysis for cytological work. The pre-grown onion bulbs in slurry water were transferred to different concentrations of the tulsi leaves aqueous extract (10%, 20% and 30%) for 48 hours to analyse the possible antigenotoxic potential of the extract. The roots grown in distilled water served as the control. The squash was made and observed under the microscope as mentioned previously. The antigenotoxicity potency of the tulsi plant extract was calculated using the following formula (Sharma *et al*., 2012).

Inhibitory activity (%) = A-B/A-C × 100

Where: A= Number of aberrant cells induced by slurry water; B= Number of aberrant cells observed after treating slurry water treated roots with aqueous extract of tulsi plant; C=Number of aberrant cells observed in control

RESULT AND DISCUSSION

In this study Out of the five different concentrations (20%, 40%, 60%, 80% and 100%) of slurry water taken, germination of onion bulbs (root formation) was completely inhibited in three concentrations i.e. 60%, 80% and 100%. As compared to control, a reduction in both root length and number was observed in treated samples. The mean mitotic index for control was 12.5±2.28 and it was 10.53±1.04 and 11.2±0.91, respectively in 20% and 40% slurry water treatments (Table 3). The reduction was not significant enough to deduce that the slurry water samples caused a decrease in the number of dividing cells. However higher concentration of slurry water (i.e. 60%, 80% and 100%) were found to have a complete cytotoxic effect as no root growth was observed.

Among all the mitotic phases, prophase was showing the highest value, in control as well as in treated samples. The sequence of the number of cells in both 20% as well as in 40% Slurry Water treatment was Prophase>Anaphase>Metaphase>Telophase.

The Mito-Depressive effect (MD) (Table 3) was decreasing with an increase in concentration in the treatment. In control, RDR was zero. The maximum negative value was observed in 20% concentration of slurry water i.e., -2.22% and minimum negative value (-1.5%.) was recorded in 40% concentration of slurry water. In comparison to control, highly significant chromosomal aberrations were reported at both the tested concentrations of slurry water. Total percentage of Chromosomal abnormalities in 20% concentration of slurry water was 11.5% and in 40% concentration of slurry water was 18.4% as compared to 1.8% of control. The induction of chromosomal aberrations was concentration dependent.

Table 1: Abnormal mitotic stages profile induced by slurry water in root tip cells of *Allium cepa.*

Treatment	Prophase		Metaphase		Anaphase		Telophase		Total abnor-mal cells	Total dividing cells	CA%
	N	AB	N	AB	N	AB	N	AB			
C(DW)	219	0	54	9	34	9	50	0	18	375	4.8
SW 20%	110	47	28	24	20	37	43	7	115	316	36.39**
SW 40%	74	46	18	60	18	69	42	9	184	336	54.76**

N=Normal cells, AB= Abnormal cells, CA= Chromosomal abnormalities

Several types of chromosome abnormalities were reported in different stages of cell division (Fig. 1) which included vacuolated cells at prophase (V), shrinked cells at prophase (SC), sticky metaphase (SM), metamorphic plate failure (PF), irregular chromosome at metaphase (IC), fragmented chromosome (F) at metaphase, anaphase and telophase, sticky anaphase (SA), spindle failure at anaphase (SF), bridge at anaphase (B) and telophase etc. as shown in Table 2.

Table 2: Mitotic chromosomal abnormities profile (mean±SE) induced by slurry water in root tip cells of *Allium cepa*.

Treatment	Types of Chromosomal Abnormalities												CA%
	P Ab.		M Ab.			A ab.				T Ab.			
	V	SC	SM	PF	IC	SA	SF	F	B	F	B	CC	
Control	0	0	3±1	2.67±0.67	0	1±0.58	0.67±0.33	2±1	1± 0.58	0	0	0	4.99±0.65
SW 20%	8.67± 2.19	7± 0.58	2± 1.15	3.33±1.67	2.67± 1.45	4.67±1.45	1.67±1.2	6± 2.08	0	0.33 ±0.33	0.33 ±0.33	1.67±0.88	37.36±4.92*
SW40%	18±9.54	4.67± 2.19	13± 5	5.33±2.33	15±6.11	9±3.61	5.33±2.33	17.67±7.22	6± 2.89	0	0	5± 2.08	55.12±6.25*

V=vacuolated cells at prophase, SC=shrinked cells at prophase, SM=sticky metaphase, PF=metamorphic plate failure, IC=irregular chromosome at metaphase, F=fragmented chromosome at metaphase, anaphase and telophase, SA=sticky anaphase, SF=spindle failure at anaphase, B=bridge at anaphase and telophase, CC=chromosome clumping.

Table 3: Antigenotoxic effects of the combination of aqueous extracts of tulsi leaves extract on Mitotic Index (MI), MD, RDR and CA on root meristem of *Allium cepa*.

Treatments	Mitotic Phase				MI%	MD%	RDR	CA%
	P (%)	M(%)	A(%)	T(%)				
Control	7.3± 2.01	2.1± 0.153	1.43± 0.145	1.667± 0.088	12.5± 2.28	—	—	4.99± 0.65
SW 20%	5.233± 0.801	1.733± 0.133	1.9± 0.2	1.667± 0.470	10.53± 1.04	12.9± 9.48	-2.22± 1.84	37.36± 4.92*
SW 20% +T1	3.067± 0.41	1.5± 0.231	1.067± 1.33	0.767± 0.120	6.4± 0.78*	45.67± 10.74	-6.5± 2.63	63.92± 3.75*
SW 20% +T2	2.9± 1.021	1.167± 0.033	1.333± 6.01	1.367± 0.088	6.77± 1.63*	46.91± 2.87	-6.18± 0.82	44.33± 8.7*
SW 20% +T3	1.766± 0.328	1.933± 0.433	1.767± 0.333	1.6± 0.321	7.07± 1.2*	41.89± 10.42	-5.85± 2.15	20.9± 4.39
SW40%	4.0± 0.557	2.6± 0.2646	2.9± 0.252	1.7± 0.1	11.2± 0.91	7.33± 8.5	-1.5± 1.58	55.12± 6.25*
SW 40% +T1	1.9± 0.1732	1.3± 0.058	1.367± 0.088	1.433± 0.684	6± 0.71*	50.8± 4.15	-6.94± 1.79*	43.52± 8.54*
SW 40% +T2	3.7± 0.781	1.767± 0.348	0.833± 0.088	1.233± 0.088	7.53± 0.33*	36.9± 7.87	-5.39± 2.14	29.91± 7.28*
SW 40% +T3	4.333± 0.841	1.567± 0.481	1.633± 0.233	1.767± 0.145	9.3± 0.87	19.19± 17.76	-3.47± 3.31	14.26± 4.96
CD	2.76	N/A	0.90	N/A	3.623	30.024	-	17.947
SE(M)	0.92	0.28	0.30	0.25	1.21	9.929	2.145	5.994
SE(d)	1.30	0.4	0.42	0.36	1.711	14.042	3.033	8.477
CV	42.05	27.9	32.94	29.9	24.399	52.595	-78.129	29.727

PI= Prophase index, MI= Metaphase index; AI= Anaphase index; TI= Telophase index; MI= Mitotic index; MD= Mito- Depressive Effect; RDR= Relative Division Rate; CA=Chromosomal Abnormalities; †= significantly toxic if MI tests≤ ½ of control ; T1= 10% extract of tulsi leaves, T2= 20% extract of tulsi leaves, T3= 30% extract of tulsi leaves.

Table 4: Inhibitory activity of different concentration of tulsi extract on *Allium cepa* grown in slurry water.

Treatments	Inhibitory activity%
T1+ SW20%	-6.35±8.67
T2+SW20%	16.57±40.82
T3+SW20%	69.19±15.56
T1+SW40%	60.06±13.32
T2+SW40%	69.77±10.35
T3+SW40%	89.02±5.73
C.D	61.014
SE(M)	19.584
SE(d)	27.697
CV	68.237

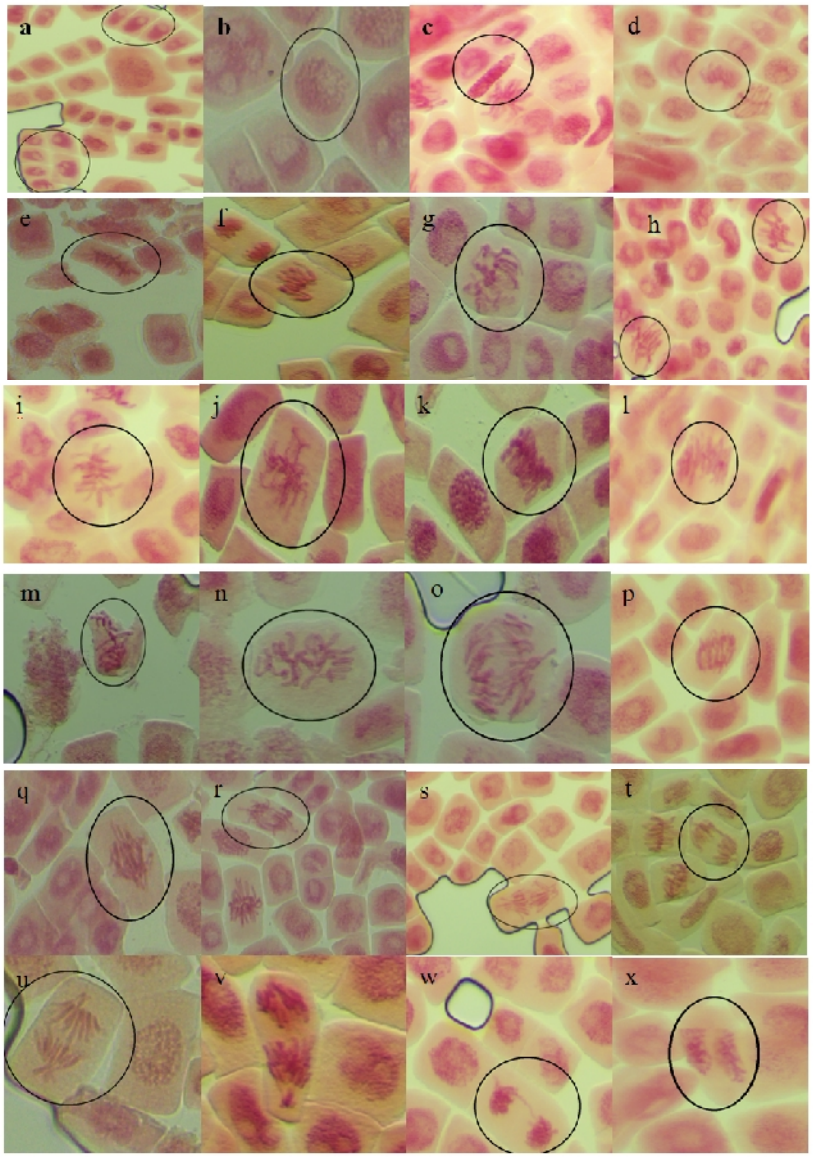

Fig. 1. A microphoto graphs of onion root tip types of aberrant cells induced by different concentration of slurry water and by combination of slurry water(20% and 40%)+ different concentration of tulsi extract(10%.20%,30%): a,b=vacuumed cells, c=sticky chromosome, d-f=chromosome arranged on one side in metaphase, e,g,i=metamorphic plate failure, j=irregular chromosome, k-l=sticky metaphase, m-n=fragmented chromosome, 0=spindal failure, p-r=sticky anaphase, t=bridge at anaphase, u=distorted poles at anaphase, v=late anaphase with clumpy chromosome, w=telophase bridge, x=early telophase with clumping.

Antigenotoxicity. For the antigenotoxic a significant reduction in MI% was noticed with all the three concentration of aqueous extract of tulsi leaves in treated samples. The value of prophase index (PI) was the highest in negative control (DW) and a decreasing trend was noticed with each treatment dose reflecting the antigenotoxic potential of tulsi leaves extract. In comparison to control, values of MI, AI and TI with lower doses of tulsi leaves extract (10% & 20%) exhibit reduction, but at higher concentration (30%) of tulsi extract values of MI, AI and TI were at par with control. No relationship was noticed for concentration of tulsi extract and its antigenotoxic effect of slurry water-inducedity. However, a concentration-dependent in antigenotoxic effect of tulsi extract was observed for overall reduction in chromosomal abnormalities in both concentration of slurry water induced genotoxicity (Table 3) and the inhibitory activity of tulsi against slurry water was also higher in higher concentration of tulsi leaf extract i.e., 30%(T3) as shown in Table 4.

Population growth increased significantly as the industrial revolution gathered rapidly. With the advent of industrial era for newer materials has extremely increased the contamination of water, air, soil by heavy metals (Patra *et al.*, 2004) which are considered as most common and consistent environmental pollution. The

pollution of water resources could be a worldwide problem (Ohe *et al.*, 2003; Monte Egito *et al.,* 2007) further the direct health effects, pollutants also wait quite dangers in this they will be mutagenic or toxic and cause human calamities like cancer, atherosclerosis, cardiovascular diseases and premature aging.

Root meristematic tissue is widely used as an effective indicator of cyto and genotoxic potency of environmental pollutants. Concerning the cytological analysis of treated *A. cepa* roots with slurry water and aqueous extract of tulsi leaves the mitotic activity of meristematic cells markedly changed across tested concentrations: the detected inhibition came progressively increasing with increasing concentration of slurry water. The reduction of mitotic activity seems to be a common effect of industrial effluents, waste water etc. on mitosis (Renata-Kontek *et al*., 2007). In present investigation root growth was found to be completely inhibited by a higher concentration, of slurry water taken.

MI reduction of 50% is considered a threshold value for sub lethal and lethal effects on test organisms (Panda and Sahu, 2004; Antonise-Wiez, 1990). Additional data from the literature have shown that the decreased mitotic activity in various test organisms might be due to the environmental presence of pesticides and trace metals (Amin, 2002).

In the present study, a significantly high number of chromosomal abrasions were observed in the *Allium cepa* root meristematic cells exposed to water collected from the effluent discharge point during both seasons. The occurrence of different types of chromosomal abnormalities indicates the presence of genotoxic agents in the industrial effluents (Rahman *et al.,* 2017) Chromosomal bridges and breaks are categorized as indicators of clastogenic effects which lead to alteration in DNA structure. According to Pathiratne *et al.* (2015) the most frequent and easily recognizable chromosomal abnormality in the *Allium cepa* root meristematic cells was vagrant chromosomes. Chromosomal adherence may occur due to increased chromosomal contraction and condensation (Haq *et al.,* 2016). According to the Fiskesjö (1997), the presence of chromosomal adherence is considered as a common sign of toxic effects on chromosomes.

Our results indicating the antigenotoxic potential of Tulsi are in good agreement with those of Rani *et al.* (2005), who observed the antigenotoxic potential of the ether extract of *Withania somnifera* in root tip cells of *Allium cepa*.

CONCLUSION

The cytotoxic and genotoxic effects of wastewater discharges on receiving water bodies were confirmed in *A. cepa* root tip cells. The discharge of industrial effluent and municipal wastewater into local water bodies, which flow into the local river, may cause DNA damage and affect downstream organisms in general, including humans, due to accumulation in the food chain. Tulsi leaf aqueous extract contains a significant number of phytochemicals with high anticancer activity relevant to DNA protection. These findings suggest that this plant contains a significant amount of natural anticancer compounds, which may be useful in preventing various chromosomal activities and reverting abnormalities to a normal state. However, more isolation of bioactive compounds would help to determine their potency and safety as a lead candidate of antigenotoxic substances for pharmaceutical applications.

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Conflict of Interest: None.

REFERENCES

Amin, A. W. (2002). Cytotoxicity testing of sewage water treatment using *Allium cepa L.* using chromosome aberrations assay. *Pakistan Journal of Biological Sciences*, *5*(2), 184–188.

Antonise-Wiez, D. (1990). Analysis of the cell cycle in root meristem of *Allium cepa* under the influence of Ledakrin. *Folia Histochemical Cytobiologia*, *26*, 79-96.

Bakare, A. A., Alabi, O. A., Gbadebo, A. M., Ogunsuyi, O. I. and Alimba, C. G. (2013). In vivo cytogenotoxicity and oxidative stress induced by electronic waste leachate and contaminated well water. *Challenges*, *4*(2), 169–187.

Devi, O. I. and Dutta, B. K. (2012). Allelopathic effect of the aqueous extract of *Parthenium hysterophorus* and *Chromolaena odorata* on the seed germination and seedling vigour of *Zea mays L. Academic Journal of Plant Sciences*, *5*(4), 110-113.

Fiskesjo, G. (1985). The Allium test as a standard in environmental monitoring, *Hereditas, 102*, 99-112.

Fiskesjo, G. (1988). The Allium test—an alternative in environmental studies: the relative toxicity of metal ions. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *197*(2), 243-260.

Fiskesjö, G. (1997). Allium test for screening chemicals; evaluation of cytological parameters. *Plants for environmental studies*, 11, 307-333.

Govind, P. and Madhuri, S. (2010). Pharmacological activities of *Ocimum sanctum* (tulsi): a review. *Int. J. Pharma Sci. Rev. Res.*, *5*(1), 61–66

Haq, I., Kumari, V., Kumar, S., Raj, A., Lohani, M. and Bhargava, R. N. (2016). Evaluation of the phytotoxic and genotoxic potential of pulp and paper mill effluent using *Vigna radiata* and *Allium cepa*. *Advances in Biology*.

Jha, A. K., Jha, M. and Kaur, J. (2012). Ethanolic extracts of *Ocimum sanctum*, *Azadirachta indica* and *withania somnifera* cause apoptosis in SiHa cells. *Res. J. Pharm Biol Chem*., *3*, 557-562.

Kontek, R., Osiecka, R. and Kontek, B. (2007). Clastogenic and mitodepressive effects of the insecticide

dichlorvos on root meristems of *Vicia faba*. *Journal of applied genetics*, *48*(4), 359-361.

Kumar, D. N., Shikha, S. W. A. T. I., George, V. C., Suresh, P. K. and Kumar, R. A. (2012). Anticancer and anti-metastatic activities of Rheum emodi rhizome chloroform extracts. *Asian J Pharm Clin Res*, *5*(3), 189-194.

Mahajan, N., Rawal, S., Verma, M., Poddar, M. and Alok, S. (2013). A phytopharmacological overview on Ocimum species with special emphasis on *Ocimum sanctum*. *Biomed Prev Nutr*, *3*,185-192.

Monte, Egito, L. M., das Gracas, Medeiros, M., Batistuzzo de, Medeiros, S. R. and Agnez-Lima, L. F. (2007). Cytotoxic and genotoxic potential of surface water from the Pitimbu river, northeastern/RN Brazil. *Gen Mol Biol*, *30*, 435-431.

O. P. Sheoran Programmer, Computer Section, CCS HAU, Hisar. http://14.139.232.166/opstat/.

Ohe, T., White, P. A. and DeMarini, D. M. (2003). Mutagenic characteristics of river waters flowing through large metropolitan areas in North America. *Mutat Res Genet Toxicol Environ Mutagen*, *534*, 101–112.

Panda, S. and Sahu, S. K. (2004). Recovery of acetylcholine esterase activity of Drawida willsi (*Oligochaeta*) following application of three pesticides to the soil. *Chemosphere*, *55*(2), 283-290.

Pastori, T., Flores, F. C., Boligon, A. A., Athayde, M. L., da Silva, Cde B., do Canto-Dorow T. S. and Tedesco, S. B. (2013). Genotoxic effects of *Campomanesia xanthocarpa* extracts on *Allium cepa* vegetal system. *Pharmac Biol.*, *51*(10), 1249–1255.

Pathiratne, A., Hemachandra, C. K. and De Silva, N. (2015). The efficacy of *Allium cepa* test system for screening cytotoxicity and genotoxicity of industrial effluents originated from different industrial activities. *Environmental Monitoring and Assessment*, *187*(12), 1-12.

Patra, M., Bhowmik, N., Bandopadhyay, B. and Sharma, A. (2004). 'Comparison of Hg, Pb and as genotoxic effects on plants and the development of genetic tolerance.' *Environmental and Experimental Botany*, *52*: 199-223.

Rahman, M. M., Rahman, M. F. and Nasirujjaman, K. (2017). A study on genotoxicity of textile dyeing industry effluents from Rajshahi, Bangladesh, by the *Allium cepa* test. *Chemistry and Ecology*, *33*(5), 434-446.

Rani, G., Kaur, K., Wadhwa, R., Kaul, S. C. and Nagpal, A. (2005). Evaluation of the anti-genotoxicity of leaf extract of Ashwagandha. *Food and chemical toxicology*, *43*(1), 95-98.

Rank, J. (2003). The method of Allium anaphase- telophase chromosome aberration assay. *Ekologia.*, *1*, 38–42.

Ray, M. and Barman, S. (1987). Effects of industrial waste water on root meristem cells of *Allium sativum L. Phytomorphology*, *37*, 359-364.

Sadowska, A., Pluygers, E., Niklinska, W., Maria, M. R. and Obidoska, G. (2001) Use of higher plants in the biomonitoring of environmental genotoxic pollution. *Folia Histochem Cytobiol*, *39*, 52-53.

Sharma, A. K. and Dphil, A. S. (1980). Chromosomes techniques, Fakenham press ltd, Norfolk. 95-100.

Sharma, S., Nagpal, A. and Vig, A. P. (2012). Geno protective potential of *Brassica juncea* (L.) *Czern.* against mercury-induced genotoxicity in *Allium cepa L. Turkish Journal of Biology*, *36*(6), 622-629.

Singh, N., Hoette, Y. and Miller, R. (2010). Tulsi: The Mother Medicine of Nature. 2nd ed. Lucknow: *International Institute of Herbal Medicine*, 28-47.

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