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ANTIGENOTOXIC EFFECT OF MANGROVE BARK

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ABSTRACT

Mangroves are plants that are growing in intertidal regions of the world. They comprise of herbs, shrubs, trees, grasses, climbers etc. Mangrove forests have importance as they defend towards Tsunami, high winds, high waves, soil erosion, binds soil, useful to produce ecosystem in saline water. They provide income generating sources to people live in this area. Mangroves plants are rich sources of secondary metabolites along with primary metabolites.These plants defend extremely adverse conditions. From ancient times, a variety of plants used to cure various diseases. In this study an attempt was made, to find out whether mangrove plant *Avicennia officinalis*possess antigenotoxic potential in them. Antigenotoxicity was studied by *Allium cepa* test. *Allium cepa* root tip meristem cells are treated with 0.75ppm mercury chloride were used for producing anti-genotoxicity. For this purpose, experiments were performed with *A. cepa* onion bulbs treated for 48 h with water and then with different concentrations (1, 1.25 and 2.50%) of aqueous extract with pre, post and simultaneous-treatments with 0.75 ppm mercury chloride. Significant induction in mitotic index was recorded in treatment groups over positive control. The bark of *A. officinalis* shows potential of antigenotoxic activity at higher concentration.

Keywords: Chromosomal aberrations, mangrove, antigenotoxicity, *Allium cepa* test.

1. INTRODUCTION

Avicennia officinalis L. (family Avicenniaceae) is a small sized tree occurringcommonly in coastal areas of the world. This plant is rich in several medicinally active components that exhibit the therapeutic effects such as antibacterial, antiviral and antifungal activities. This plant has prime importance from the wide broad spectrum of uses including medicinal purposes, a source of tannins, timber and a dye plant.

This plant was mainly used to help prevent coastal erosion and in restoration of mangrove habitats (Duke *et al.* 2010). The bark is used in tanning and a dye can be extracted from plantparts such as bark and leaves. Various parts of the plant are used in folk medicines and also useful as antidiabetic and antihyperglycemic activities (Das*et al*. 2016, Aljaghthmi*et al.* 2017 &Satyavani*et al.* 2019). Mangroves are rich in various bio active compounds (Patra *et al.* 2015). The bark extract is used in controlling diarrhoea, nausea and vomiting (Bandaranayake, 2002). The *Allium cepa* assay is an efficient test for chemical screening and *In situ* monitoring forgenotoxicity of environmental contaminants. This test has been used widely to study genotoxicity of many plant extract revealing that these compounds can induce chromosomal aberrations in root meristems of *Allium cepa*.

The use of antimutagens and anticarcinogens in everyday life is the most effective procedure for preventing human cancer and genetic diseases. There are several ways in which the action of mutagens can be reduced or prevented. Chemicals which act to interfere with DNA repair or with mutagen metabolism can be effective antimutagens (Ferguson, 1994).

STATEMENT OF RESEARCH PROBLEM

The antimutagenic effect of certain naturally occurring compounds extracted from plants has been well established in bacteria and mammalian cells (Kuroda, 1990 &Bootman*et al.* 1988). An attempt was made to study antigenotoxic potential of white mangrove, *Avicennia officinalis.*

OBJECTIVES OF THE STUDY

To study antigenotoxic potential of mangrove bark of A. *officinalis by using Allium cepa test.*

SIGNIFICANCE OF THE STUDY

Genotoxicity is the capacity of chemical major representative that damages the chromosomal material within a cell causing changes in structure commonly known as mutations, which may cause Carcinoma.All mutagens are genetically toxic, in naturewhile, not all genotoxic chemicals are mutagenic in nature. The alteration can have direct or indirect effects on the genetic materials.The induction of mutations, mistimed event activation, and direct DNA damage leading to mutations. Among desmutagenic agents, antioxidants are of special interest because they are implicated in inhibition of tumour initiation, promotion and progression. The mechanisms of inhibition of mutagenesis and the initiation step of carcinogenesis by antioxidants include scavenging of reactive oxygen species, inhibition of certain enzymes involved in metabolism of xenobiotics and inhibition of mutagen/carcinogen binding to DNA (De Flora,1998).The permanent, heritable changes can affect either body cells of the organism or gamete cells to be accepted on to future generations (Kolle, 2012). Cells prevent expression of the genotoxic mutation by either chromosomal structures; however, the damage may not always be static leading to genotoxic. This study will enrich the knowledge of use of phytochemicals as a natural productto for antigenotoxic potential which are having no side effects than chemicals used in cancer treatmentsand they are also useful in deduction of genetic disorders.

There are many phytochemicals are found in the bark tissue. Outer bark is the living tissue and have more amounts of secondary metabolites.This study will enrichthe knowledge about use of mangrove extracts to decrease the genetic variability and antimutation activity from white mangrove,*A. Officinalis*. This plant is found abundantly along coastline of Indiaand use of this plant parts for therapeutic use.

RESEARCH METHODOLOGY

Allium cepa test was carried out as described by Fiskesjo(1985) further it modified by Nielsen and Rank (1994). 20 g of oven dried mangrove bark powders of *Avicennia officinalis* stem bark was soaked in 200 ml distilled water for 12 hours at room temperature (27-30° C). The extract was filtered through a filter paper and stored in a refrigeration and used this as a stock.

To study antigenotoxicity of barks by using *Allium* test the method given by Sharma and Vig (2012) was followed by giving 3 different treatments under the same conditions to the onion roots. Equal sized bulbs of an onion (*Allium cepa*) were purchased from the local market. Before use, loose the outer scales and dry bottom roots were removed carefully without destroying the root primordia. A series of 10 cleaned onion bulbs were placed on coupling jars, each filled with tap water for 48 hours. Select out only healthy root growth onions for treatments.

Treatment 1:

The selected onions were transferred to treatment jars filled with 0.75 ppm concentration of mercury for 3 hours then roots were washed and treated with different concentration 1, 1.25 and 2.5% of mangrove bark extracts for 3 hours. After the treatment the root tips we kept in fixative (Ethanol: glacial acetic acid 3:1, V/V) and then processed for the preparation of microscopic slides. The tap water used as negative control and 0.75 ppm mercury was used as positive control.

Treatment 2:

The selected onions were transferred on the treatment jars filled with different concentrations (1, 1.25, 2.5%) of aqueous extracts mangrove bark for 3 hours. After extract treatment onion bulbs were washed and treated with 0.75 ppm mercury for 3 hours and again roots were washed and fixed in a fixative.

Treatment 3:

The effect of both aqueous extract and mercury treatment were investigated. A series of 8 cleaned onion bulbs were transformed to coupling jars filled with concentrations 1, 1.25 and 2.5% of extract and 0.75 ppm of mercury simultaneously. Further procedures are same as that of tratment1.

At the end of treatment period the length of roots of each onion bulbs with the best growth of each concentration were measured in cm using thread and scale. After the completion of 6 hours treatment fixed in fixative were hydrolysed in 1-part 1N HCl and 9 part of Aceto-orcin were squashed and heated for 3-5 minutes. (2% Orcein dissolved in 45% acetic acid). The well stained root tips were squashed in 45% acetic acid on a slide with coverslip and sealed with DPX and examined for microscopically.The slides were examined per onion and six onions in each treatment. The mitotic index (MI) was determined by counting the number of dividing cells among the total number of cells scored per slide.

$$\text{Mitotic index (MI)} = \frac{\text{Number of cells in Mitosis}}{\text{Total number of cells analysed}} \times 100$$

Different chromosomal aberrations were characterized and percent chromosomal aberrations frequency with and without extract in cells were calculated.

DATA ANALYSIS AND DISCUSSION

Table 1. Effect of pre, post and simultaneous treatments of different concentrations of aqueous extract of stem bark of *A. officinalis*, on average root length of *Allium cepa*

1	Negative control		1.30 + 0.50	
2	Positive control		0.90 + 0.65	
Sr.No.	Concentrations	I	II	III
1	1 %	1.99 + 0.95**	2.17 + 0.65**	1.39 +0.05
2	1.25 %	1.48 + 0.60*	1.75 + 0.25**	1.86 + 0.70**
3	2.50 %	1.59 + 0.05	1.51 + 1.45	1.49 + 0.05*

Negative control – Distilled water Mean of Three replicates in cm + S. D.
Positive control – 0.75 ppm Mercuric chloride I -pre, II – post and III- simultaneous treatments.
* Shows significant difference from control at 5% level
* * Shows significant difference from control at 5% and 1% level significance for Mercuric chloride (0.75 ppm)

Effect of pre, post and simultaneous treatments of mercuric chloride and aqueous extracts of *A. officinalis* on average root length of *Allium cepa* is shown in Table 1**.**From the table it is observed that pre and post treatment at 1% concentration and pre, post and simultaneous treatments at 1.25 % shows significant at 5% and 1% level significance for Mercuric chloride. These tests show negative impacts on treatments and shows significant growth of *Allium* root. treatments of bark extracts induce root length.

Table 2. Effect of pre, post and simultaneous treatments of Mercuric chloride and aqueous extracts of bark of *A. officinalis*on Mitotic index in *Allium cepa*.

1	Negative control		30.43	
2	Positive control		20.56	
Sr.No.	Concentrations	I	II	III
1	1 %	41.41	43.54	42.10
2	1.25 %	44.08	46.73	43.58
3	2.50 %	45.65	47.82	46.42

*All values are mean of triplicates
Negative control – Distilled waterPositive control – 0.75 ppm Mercuric chloride
I -pre, II – post and III- simultaneous treatments.

Effect of pre, post and simultaneous treatments of Mercuric chloride and aqueous extracts of mangrove on mitotic index of *A. cepa* is shown in Table 2**.**From the table, it was clear that the pre, post and simultaneous treatments of Mercuric chloride and bark extracts results in the induction of mitotic index as compared to mercuric chloride as positive
control.

Table 3.Effect of pre, post and simultaneous treatments of Mercuric chloride and aqueous extracts of prop root and stem bark of *A. officinalis*, onphysiological and clastogenic aberrations in root tip cells of *Allium cepa*.

	Negative Control	Positive Control	1%			1.25%			2.50%		
Physiological aberrations (P A)											
			I	II	III	I	II	III	I	II	III
C-Mito	3	5	6	5	3	4	3	2	3	1	1
Delayed anaphase	3	6	7	11	3	4	5	2	2	1	1
Laggards		3	3	2	3	2	2	1	1	-	-
Stickiness		3	3	2	2	2	1	2	1	-	-
Vagrants	1	4	2	4	3	2	4	3	1	3	2
Total PA	7	21	21	24	14	14	15	10	8	5	4
Clastogenic aberrations (C A)											
Bridges	2	3	3	3	2	2	3	1	1	1	1
Rings	-	2	-	-	-	-	-	-	-	-	-
Breaks	1	5	3	4	3	2	3	3	1	2	1
Total CA	3	10	6	7	5	4	6	4	2	3	2
Total aberrations	10	31	27	31	19	18	21	14	10	8	6

*All values are mean of triplicates
Negative control – Distilled water Positive control – 0.75 ppm Mercuric chloride
I -pre, II – post and III- simultaneous treatments.

Effect of pre, post and simultaneous treatments of mercuric chloride and aqueous extracts of *A. officinalis*on physiological and clastogenic aberrations is shown in Table 3. and it is reported that physiological and clastogenic aberrations are increased in response to 1% aqueous extract of bark while 1.25 and 2.5% bark extracts reduce physiological and clastogenic aberrations. This inhibition is more significant in response to 2.5% bark extracts. It is observed that the post treatment and simultaneous treatments of *A. officinalis* exhibits significant reduction in these aberrations while the formation of laggards and stickiness are totally inhibited showing antigenotoxic nature of these bark extracts.

Chromosomal aberrations such as C-mitosis, delayed anaphase, laggards, stickiness, vagrants, bridges, rings and breaks were observed in positive control and some aberrations in treatment groups. The highest percentage of chromosomal aberrations was noticed in positive control. A significant reduction in chromosomal aberrations was recorded in root tips exposed to 0.75ppm mercury chloride treated by 2.5% extract. 75ppm mercury chloride induced chromosomal aberrations were reduced due to bark extract treatment indicates anti-genotoxic potential of the *Avicennia officinalis*.

INTERPRETATION

The tremendous use of chemical pesticides in modern agriculture isincreasing day by day this results in the accumulation of the residual effects ofpesticidal compounds are found in the living organisms. This will increase the chances of carcinoma orgenetic disorders (Ozmen &Sumer, 2004 &Metin, & Burun 2010). The use of herbicides to controlweeds inthe field (Cork& Krueger, 1992) has day to day taking into considerationof their adverse effects on living organisms includes plants,

animals, including human beings (Ajay& Sarbhoy, 1988). Many researchers have confirmed that these herbicidescause carcinogenic/mutagenic effects on non-target organisms (Pavlica*et al*. 1998 &Sharma*et al.* 2012). In reportsof Fluoride action network pesticide (2001) indicate that the herbicideCiluron with cyhalofop-butyl as dynamic constituent; used to control grassin rice, causes low oral, dermal and inhalation toxicity includingabnormalities in liver and kidney. Heavy metals have been directly orindirectly accumulated through food chain in the body of aquatic andterrestrial organisms (Memon*et al*. 2001; Akinola& Ekiyoyo, 2006 &Obasohan*et al*. 2006). Among heavy metals, mercury (Hg) is a known asmutagen and causes various types of problems in plants and animals(Florea, & Busselberg, 2006)**.** It leads to nerve, brain,kidney damage, lung, eye irritation, vomiting, diarrhoea, can damage DNAand chromosomes. The genotoxin interfere with routine progress of mitosis.Thus, prevent number of cells from entering in the prophase and blocksthe cycle of mitosis, during interphase. Mutagen causes various types ofproblems in plants and animals (Badr & Ibrahim, 1987).

LIMITATIONS AND DIRECTION FOR FURTHER RESEARCH

Further this study requires animal trials to give positive response and corelated to use of the bark sample as a source for pharmaceutical industries.

FINDINGS, SUGGESTIONS AND CONCLUSION

The post treatment of bark as well as simultaneous treatments of bark extracts induces root length. The pre, post and simultaneous treatments of mercuric chloride and bark extracts resulted in the induction of mitotic index as compared to mercuric chloride as positive treatment. Physiological and clastogenic aberrations were increased in response to 1% aqueous extract of bark while 1.25 and 2.5% bark extract reduce physiological and clastogenic aberrations. This inhibition was more significant in response to 2.5% bark extracts. The post treatment and simultaneous treatments of *A. officinalis*, exhibited significant reduction in these aberrations while the formation of laggards and stickiness were totally inhibited showing antigenotoxic nature of this bark extract.

The presence of high phenolic content and tannic acid were reported in bark tissues of *A. officinalis* which may be responsible for the repair of these of chromosomal aberrations. Thus, it can be concluded that these bark extracts of mangroves have antigenotoxic potential against mercuric chloride induced chromosomal aberrations in *A. cepa*. The bark extract of mangrove can be utilized in various natural products as an agent of neutralizing the genotoxic effects of various chemicals, chemical pesticides and hazardous pollutants in the environment.

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