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STUDY OF EFFECT OF ETHANOLIC EXTRACTS OF *Phyllanthus amarus* ON THE ROOT OF *Allium cepa* (L.) AND *Allium sativum* (L.)

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Abstract: *Cytological studies of the effects of ethanolic extract of Phyllanthus amarus is the plant has served as lead for several experimental investigators that explored its phytochemical constituents uses the roots of Allium cepa L. and Allium sativum L. treated with ethanolic extract of Phyllanthus amarus on different concentration of ethanol (20% to 80%). A degree of chromosomal aberrations and physiological disturbances during mitotic divisions were observed after the treatments. The observations showed on exponential relationship between the % of abnormalities and the concentrations of ethanol applied.*
Keyword: *Allium cepa Allium sativum, Ethanol, Leaf extract of Phyllanthus amaru, cytological effects.*

Introduction: *Phyllanthus amarus* is a plant of the family euphorbiaceae and has about approximately 800 species which are found in tropical and subtropical countries. The genus allium being a member of family liliaceae is an important crop plant. It is used an vegetables and also has medicinal values. It is rich in vitamin, minerals and trace elements. It helps in digestion, stimulates kidney function and blood purifier. *Allium* is antiseptic, ethanol extract of *Phyllanthus amarus* used in this study is shown to be a potent phytochemical mutogen in both higher and lower organisms [1]. The present work perfomed in order to investigate potential effects of *Phyllanthu amarus* leaf extacts on both the species of *Allium* and its elimination through M1 and M2 generation.

The *Phyllanthus amarus* has been found in Philippines, Cuba, Nigeria and among others. In India, *Phyllanthus amarus* is widely distributed as a weed in cultivated and waste lands. *Phyllanthus amarus* have numerous phytocompounds such as alkaloids, flavonoids, tannins, lignins polyphenolic compounds and tetracyclic triterpenoids, several phytoconstituents isolated from this plant. Antimicrobial activity of ethanol extracts of *Phyllanthus amarus* were evaluated against the test organisms *Salmonella typhi*. Ethanolic extract of *Phyllanthus amarus* were employed for antimicrobial evalution by agar cup diffusion method which are compared against standard antibiotics that were evaluated by disk diffusion method. Ethanolic extract isolated phyllanthin from *Phyllanthus amarus* leaf due to phyllathin effect of cytology of *Allium* sps.

Effect of *Phyllanthus amarus* is evident from the study in which ethanol extract of *Phyllanthus amarus* leaves caused a significant doses dependent decrease in the levels of total cholesterol, urea, total protein, uricacid and prostotic, alkaline and acid phosphatases, as partate transminase and alanine transaminase. Since increase in enzyme in these enzymes is related to hepatic and heart disorders therefore their reduction shows that the leaves *Phyllanthus amarus* have hepatic and heart disorders therefore their reduction shows that leaves have hepato protective, nephroprotective and cardioprotective proproteine. Histopathalogical study confirmed the beneficial effect of *Phyllanthus amarus* with its potential antioxidant activity [2].

Materials and Methods

Preperation of Ethanolic extracts of *Phyllanthus amarus*: *Phyllanthus amarus* leaves (100g) were cleaned with water following which the leaves were ground into solution using an electric blender and successfully extracted with 200ml of ethanol (80%).The solution was kept at room temperature for

two hours in a closed glass container. Then the contents were filtered and the clear solution (50ml) was used ·these studies.

The bulbs of *Allium cepa* L. and *Allium sativum* L. were soaked with 20%, 40%, 60% and 80% ethanol extract solution for two hours duration.Well scattered metaphase plates were obtained by pretreating the root tips with aqueous solution of paradichloro benzen (PDP) for three to four hours at 12ºc followed by overnight fixation in 1:3 aceto alcohol. As usual 2% acetocarmine staining method was followed and slides were prepared by squash technique.

Results and Discussions

The result of this experiment releaved that administration of *Phyllanthus amarus* caused significant in functional activity of Mitosis of *Allium* sps. The control sections of the mitosis showed normal features. The data obtained for the percentage of chromosomal aberrations i.e. stickiness, endomitosis, fragments, tropokinesis bridges and micronuclei in the root cells of *Allium cepa* and *Allium sativum* have been set out in the Table A and B. The perusal of the table indicates that there was an exponential relationship between the percentage of aberrations and concentration of phytochemical mutagen. The two species showed decrease in mitotic index as the close increased .It was higher in M2 generation

The metaphase abnormalities gradually increased by the increase in concentrations of mutagen in *Allium cepa* is 0.50 to 1.50 at 20% to 80% doses of ethanol leaf extract of *Phyllanthus amarus*. Where as in *Allium sativum* formation of satellites occurred at higher concentration 2e at 80% concentration of mutagen which was absent in M2 generation. The aberrations recorded 2e bridges, fragments micronuclei, stickiness tro-poliness were in exponential relationship with the concentrations of mutagen applied. Aberrations also showed decreasing trends at M2generations with the interval of time.

These trends of chromosomal aberrations have been earlier studied [3] in *Vigna*, [4] in *Allium*, [5] in *Aloe* and *Asparagus*. The decrease in mitotic index may be due to arrest of cells in Go phase or retardation in the pace of events during S or G2 phase. This indicates that ethanol extract of *Phyllanthus amarus* interferes with normal sequence of cell cycle to reduce the number of cells starting to divide at interphase, stickinees resulted by denaturation of histone proteins or due to delay in chromose separation caused by disturbances at cytochemical level. Torpolinesis was due to functioning of the spindle. C-mitosis, endomitosis was caused by doubling of chromosomes failure of cell plate formation and abnormal spindle behavior. Bridges were formed by nonseparation of kinetochoricgenes, centric fragments appeared by failure anaphasic movement. The difference in magnitude of these aberrations in both the species showed that *Allium. Sativum* was more sensitive to ethanol extracts than *Allium cepa*.

Conclusion

Phyllanthus amarus herb is widely used tropical countries including India. It has significant traditional uses, some of them have been experimentally established and an attempt has been made to isolate potential chemical constituents and their mechanism of action. Present view had compiled the traditional uses, Pharmacological properties and chemical constituent present, which can be useful information for further study on this plant

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Table-A: Mitotic abnormalities induced by ethanol extract of Phyllanthus amarus in Allium cepa (M1 and M2 generation) M1 GENEzRATION

concentration	Total number of cell observed	Total number dividing cells	Mitotic index	Prophase clumping	Metaphase % Abnormality stickiness	fragment	Endomitosis	trypokinesis	Anaphase %Abnormality Single bridge	Doule bridge	multiple	laging	Fragment arrangement	Telophase Diagonal seperation	unequal	micronuclei
Control	1000	900	90	-	-	-	-	-	-	-	-	-	-	-	-	-
20%	1030	930	90.2	0.50 ±0.0028	0.50± 0.007	0.50 ±0.0033	0.1±0.002	-	0.50± 0.0055	-	-	1.96± 0.0036	1.96 ± 0.0035	-	-	2.94 ± 0.0017
40%	985	875	89.2	0.713± 0.0052	0.71± 0.004	0.62± 0.0021	1.52± 0.0031	-	0.71± 0.0031	0.50 ± 0.002	0.30 ± 0.0033	2.03± 0.0024	2.03 ± 0.0018	0.51 ± 0.0022	-	0.51 ±0.0021
60%	995	845	85.3	0.82± 0.0022	0.81± 0.0047	0.82± 0.0021	1.02± 0.0034	1.02+- 0.0018	0.61± 0.0021	0.61 ± 0.0026	1.39± 0.0221	1.02± 0.0028	2.02 ± 0.0056	-	0.51 ±0.034	0.51 ± 0.0021
80%	1020	820	81.4	1.50± 0.670	1.50± 0.6707	0.99± 0.0021	1.99±0.0018	0.99+- 0.0018	0.99± 0.0021	1.99± 0.0031	1.99± 0.0031	1.50 ± 0.0017	2.50 ± 0.0027	0.99± 0.0033	1.50± 0.0034	1.50 ± 0.0082
M2 Generation																
Control	970	975	98.99	-	-	-	-	-	-	-	-	-	-	-		-
20%	1010	960	94.51	0.26± 0.0036	0.026 ± 0.0098	0.28 ± 0.0035	0.79 ± 0.0033	-	0.28 ± 0.01	-	-	-	0.99 ± 0.0033	0.99 ± 0.0045	-	1.50 ±0.0033
40%	980	810	82.4	0.40 ± 0.0034	0.41 ± 0.0028	0.41 ± 0.0035	1.03 ± 0.0036	-	0.51 ± 0.0035	0.31 ± 0.0046	-	-	1.03 ± 0.0035	1.03 ± 0.0021	-	2.07 ± 0.0021
60%	1000	900	90.0	0.61 ± 0.0050	0.70 ± 0.0034	0.60 ± 0.0034	1.57 ± 0.0049	-	0.80 ± 0.0035	0.50 ± 0.0067	0.50 ± 0.0066	0.40 ± 0.0039	1.51 ± 0.0037	1.51 ± 0.0036	0.52 ± 0.0021	0.52 ± 0.0036
80%	1030	898	89.35	0.78 ± 0.003	0.78 ± 0.0021	0.88 ± 0.01	1.96 ± 0.0033	-	0.88 ± 0.0043	0.76 ± 0.0033	0.70 ± 0.0020	0.50 ± 0.0051	1.96 ± 0.0035	1.95 ± 0.0036	0.99 ± 0.0027	0.50 ± 0.0033

Table-B: Mitotic abnormalities induced by ethanol extract of Phyllanthus amarus in Allium cepa (M1 and M2 generation) M2 GENERATION

Total number of cell observed	Total number dividing cells	Mitotic index	Prophase clumping	Nacl-eoli	Metaphase percentage stickiness	fragment	Endomitosis	trypokinesis	Sarelike chromosome	Anaphase percentage Single bridge	Mobility bridge	Double bridge	Logging	Fragment	Telophase Diagonal-arras	Spen	Micro-nuclear
970	945	97.42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
985	910	92.39	0.20 ± 0.0017	-	0.20 ± 0.0017	-	1.10 ± 0.0167	-	-	0.20 ± 0.0036	-	-	0.53 ± 0.0046	-	-	-	6.59 ± 0.0033
1020	800	78.43	1.96 ± 0.0021	-	1.96 ± 0.0021	0.49 ± 0.0017	0.49 ± 0.0034	2.45 ± 0.0052	-	0.49 ± 0.0034	-	0.50 ± 0.0018	0.98 ± 0.0028	1.95 ± 0.0018	2.45 ± 0.0036	-	6.86 ± 0.0036
990	770	77.78	2.32 ± 0.0022	-	2.32 ± 0.0022	1.01 ± 0.0033	1.01 ± 0.0036	3.03 ± 0.0036	-	0.81 ± 0.0045	0.51 ± 0.0035	0.71 ± 0.0033	0.51 ± 0.0036	2.52 ± 0.0036	2.52 ± 0.0036	-	6.06 ± 0.0033
101	785	77.72	2.47 ± 0.0036	-	2.47 ± 0.0036	0.49 ± 0.0034	0.49 ± 0.0035	0.99 ±0.0045	1.48 ± 0.0028	0.99 ± 0.0045	0.99 ± 0.0035	0.99 ± 0.0021	0.99 ± 0.0021	2.97 ± 0.0036	2.97 ± 0.0074	0.99 ± 0.0035	4.95 ± 0.0060
M2																	
978	950	97.14	-	-	-	-	-	-	-	-	-	-	-				
1025	975	95.12	-	0.98 ± 0.0032	-	-	-	-	-	-	-	-	-	0.29 ± 0.0033	0.78 ± 0.0028	0.98 ± 0.0028	1.49 ± 0.0045
195	900	90.45	-	1.51 ± 0.0045	0.30 ± 0.0028	0.30 ± 0.0028	18.01 ±0.0033	0.30 ±0.0034	0.19 ±0.0021	-	-	-	-	0.50 ± 0.0034	1.01 ± 0.0018	1.31 ± 0.0036	1.81 ± 0.0034
110	890	89.84	1.01 ± 0.0026	2.02 ± 0.0072	0.81 ± 0.0045	0.51 ± 0.0045	1.52 ±0.0033	0.20 ± 0.0052	0.10 ± 0.00219	0.30 ± 0.0028	0.30 ± 0.0014	-	-	0.30 ± 0.0021	0.51 ± 0.0036	1.01 ± 0.0015	1.52 ± 0.0021
105	920	91.54	1.49 ± 0.0029	1.79 ± 0.0033	0.99 ± 0.0033	0.19 ± 0.0034	0.99 ±0.0028	0.09 ± 0.0001	0.09 ± 0.0053	0.19 ± 0.0013	0.19 ± 0.0013	-	-	0.29 ± 0.0034	0.29 ± 0.0032	0.79 ± 0.0036	0.99 ± 0.0346