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Cytological effects of the root extracts of *Boerhaavia diffusa* on root tips of *Crinum jagus*

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Abstract
The mitotic effects of the root extracts of *Boerhaavia diffusa* on the root tips of *Crinum jagus* were investigated. The results of this study showed several chromosomal abnormalities including stickiness of chromosomes (both at metaphase and anaphase), C-metaphase, lagging chromosomes, and sticky bridges. The trend of the results showed that the higher the concentration of the extracts for treatment, the more inhibitory the effect on mitosis with more pronounced chromosomal aberrations. The root extract of *B. diffusa* was found active at concentrations of 0.1%, 0.2%, 0.4%, and 0.8%. The extract was found to accumulate metaphase and could thus be of immense help in cytological work. These results are discussed in the light of their reported use in cancer therapy, the treatment of other diseases in traditional medicinal practice and its' possible use as an alternative to colchicine in cytological work.

Keywords: *Boerhaavia diffusa*, chromosom, *Crinum jagus*, cytological effect, mitosis.

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INTRODUCTION

The general principles of the mechanisms of mitosis are best and most easily studied in the actively growing regions of plants such as a shoot or root apex. Frequently, such studies involve the use of chemicals which modify the normal course of mitosis. The best known and most commonly used extract is colchicine, an alkaloid from autumn crocus, *Colchicum autumnale* L. (Levan 1938). This compound could disturb spindle formation during cell division in *Tagetes erectus*. It is widely used in the control of mitosis with a view to studying chromosome morphology or inducing polyploidy in plants (Love and Love 1975). However, it is a rather expensive drug and hence, not easily available. Thus a common effect of medicinal plant extracts on root tip mitosis of *Allium cepa* L. is an inhibition of the spindle mechanism leading to the scattering of the chromosomes, stickiness of the chromosomes, anaphase bridge, and diverse kinds of abnormalities (Ene-Obong and Amadi 1987).

Boerhaavia diffusa L. is a common herbaceous weed with ovate, fairly long-stemmed leaves and crimson flowers in a small terminal cluster. It is a medicinal plant used in traditional medicinal practice and has been reportedly useful in the treatment of many diseases (Ayensu 1978). The swollen tap-roots when softened by boiling are applied externally as a poultice to draw abscesses and encourage the extraction of guinea worms. Apart from this, the root of *B. diffusa* is considered to have an expectorant action and thus used in the treatment of asthma, cough, stomach and intestinal colic, haemorrhage, oedema, anemia, jaundice, piles, rheumatism, eye disease, liver ailments, gonorrhea, small pox, yaws, and cancer (Bakhru 1992, Leyon et al. 2004). It has also been used as a laxative, diuretic, emetic in large doses, anti-venom, and in the treatment of heart disease.

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The root has been reported to contain about 0.04 per cent of punarvavine an alkaloid, boerhaavic acid, reducing sugars, potassium, nitrate, and tannins including phlobaphenes (Bakhru 1992). These medicinal values of *B. diffusa* and the contents of its root necessitated its use in mitotic studies.

The genus *Crinum* is represented by so many species. Two of these have been found in Nigeria and are classic ornamentals. They decorate both the landscape and private gardens when in bloom. Like most members of the family Amaryllidaceae, they flower by mid-dry season and bloom till the beginning of the rains in May. The two species are *C. ornatum* (Ail.) Bury Hexander, *C. jagus* (Thomps.) Dandy (Nwankiti 1985). Apart from *C. octabilis* (2n = 33), 14 species investigated all had 2n = 22 *C. jagus* was used for the purpose of this study.

Allium cepa (onion) is commonly used in studies of this kind. This mainly because *A. cepa* roots easily and contain few chromosomes (2n = 16) which make it very suitable for cytological studies and for any aberrations to be very clearly elucidated (Okoli and Russom 1986). However, *A. cepa* is an expensive crop since it is used as a condiment in human nutrition. On the other hand, *C. jagus*, as has been pointed out could either be found in the wild or private gardens where they decorate such places when in full bloom. No extra cost is involved in acquiring the *Crinum* species for research purposes. *C. jagus* and *A. cepa* belongs to the same family Amaryllidaceae. Using *C. jagus* therefore is an innovation aimed at finding a suitable substitute for the more expensive *A. cepa* crop. The only possible disadvantage in using C. jagus for this kind of work is that it has relatively more chromosomes (2n = 22), and its roots are quite larger than those of *A. cepa*. However, this set-back is circumvented by slicing the root into thin longitudinal sections which make them more amenable to cytological manipulations after treatment with the test substance. In this research, the cytological effects of the root extracts of *B. diffusa* on the root tips of *C. jagus* was investigated with a view to finding some possible use of these extracts as chemicals for the modification of mitosis in much the same way as colchicine. Colchicine is a very expensive drug which is not easily available for research work (Okoli and Russom 1986). The economic potentials of the observations made are discussed.

MATERIAL AND METHODS

Bulbs of *Crinum jagus* were used as test material for this experiment. They were dug up from the Botanical garden at the University of Port Harcourt where they had been growing or were cultivated. Different sizes were selected, the smaller bulbs having smaller roots than the bigger ones which have roots many times that of *Allium cepa*. The root tips (between 1-2 cm in length from the root apex) were cut off and sectioned to produce thinner longitudinal sections which are amenable to cytological treatments.

B. diffusa roots were obtained around the Choba campus of the University of Port Harcourt where they were mainly found to be growing near buildings and or in hard places with cement work. The roots were subjected to a very good washing with water as much as was necessary to remove all the attached soil and dirt. After this, they were sliced to expose a greater surface area and to facilitate drying. The sliced specimens were oven-dried at a temperature between 40-60°C. The dried specimen was then crushed in a mortar to a semi-powdered form. About 200 g of the crushed specimen was weighed out into a thimble of a soxhlet extractor and the reflux extraction was carried out in a fume chamber.

For the extraction, two solvents were used successively. Firstly petroleum ether was used. This was mainly for the de-fattening of the specimen. There after chloroform was used which effected the alkaloid extraction from the specimen. It took 14-16 h for the complete exhaustion of the root with each solvent. The solvent was distilled off and the extract weighed. The fatty components obtained after the extraction with petroleum ether were discarded while the extract obtained after the extraction with chloroform

was dried and weighed. It gave 0.2 g dry weight alkaloid for *Boerhaavia diffusa* and from this, various concentrations of the test solutions were made at 0.1%, 0.2%, 0.4%, and 0.8% concentrations while using tap water as control.

The roots of the *Crinum jagus* were randomly selected from the bulbs and sampled by cutting them off the bulbs with a sharp razor blade. They were sliced to desirable sizes and placed in a watch glass with water. They were then treated with the test substances in vials containing about 2 mL of extract at different concentrations for 3 h. This treatment is best from between 10.00 a.m and 1.00 p.m in the day. This is because mitotic activity has been found to be at its best within this time of the day. There after, the roots were fixed in a freshly prepared 1:3 glacial acetic acid, 95% alcohol (V/V), for at least 24 h at 4°C, then stored in 70% alcohol under refrigeration until required. For control purposes, another group of randomly-selected roots were taken and treated with tap water instead of the test solutions after they were cut and fixation was carried out as previously described.

For each period of collection, each root tip was later randomly selected for slide preparation. Hydrolysis of the roots was carried out in 8% HCl for about five min. This is to facilitate the disintegration of the middle lamella of the cell staining. This treatment preceded their stabilization before squashing was done. About 2 mm of the opaque end of the root tip was sectioned off with a sharp razor blade and used for slide preparation.

For examination of mitotic chromosomes, root tips were squashed in an FLP-orcein (2 g of Orcein dissolved in 10 mL of solvent, containing equal parts of formic acid, lactic acid, propionic acid and water), following the method of Okoli (1983). The materials were squashed directly by tapping with the blunt end of a ball point pen, to cause the cells to spread out properly. Slides were viewed at X400 magnification. The frequencies of mitotically dividing cells were scored by sampling portions of slides which showed unambiguity in the configurations of mitotic cells. The mitotic index was defined as the ratio of dividing cells to the total number of cells examined for each treatment (Balog 1982). The effect of different concentrations and duration of treatment of the extract on the frequencies of the four phases of mitosis was determined. Microphotographs of chromosomal aberrations were taken from the temporary slides following the method of Okoli and Russom (1986) (Fig. 1).

RESULTS

The extract exhibited a strong depressive effect on the mitosis of *C. jagus* roots. In the *B. diffusa* extract used, the mitotic index of the control experiment was found to be 5.27%. As for the treatment, at concentration of 0.1%, 0.2%, 0.4%, and 0.8% the mitotic indices were found to be 4.80, 3.59, 3.47, and 3.36 respectively. Thus, there was a negative correlation between the concentrations of the treatment extracts and the mitotic indices obtained from their action (Table 1). This points to an inhibition of mitosis by this extract. Inhibition of the mitotic index increased significantly with an increase in the concentration of treatment solution of *B. diffusa*. This again shows a very negative correlation between the concentration of the extract and the mitotic indices produced by the observed action. The untreated roots (controls) had high mitotic indices in all the mitotic phases. Table 2 shows the chromosome aberrations in *C. jagus* root tip cells treated with different concentrations of *B. diffusa* root extract in the experiment. Other significant observations made for the various concentrations and treatments are presented in the form of microphotographs (Fig. 1).

DISCUSSION

The worldwide use of *B. diffusa r*oots to treat liver disorders was validated when researchers demonstrated, in 1980 and 1991 that its root extract had antihepatotoxic properties (Chandan et al. 1991, Rawt et al. 1997). The plant has also been shown to

Table 1. Mitotic effects of root extract of *B. diffusa* on the *C. jagus* root tip cells.

Concentration	Mitotic stages					
	Prophase	Metaphase	Anaphase	Telophase	Total cell number	Mitotic index %
Control	11	13	1	2	512	5.27
0.1%	7	6	1	1	311	4.80
0.2%	4	3	3	3	362	3.59
0.4%	7	1	1	1	288	3.47
0.8%	7	2	1	2	357	3.36

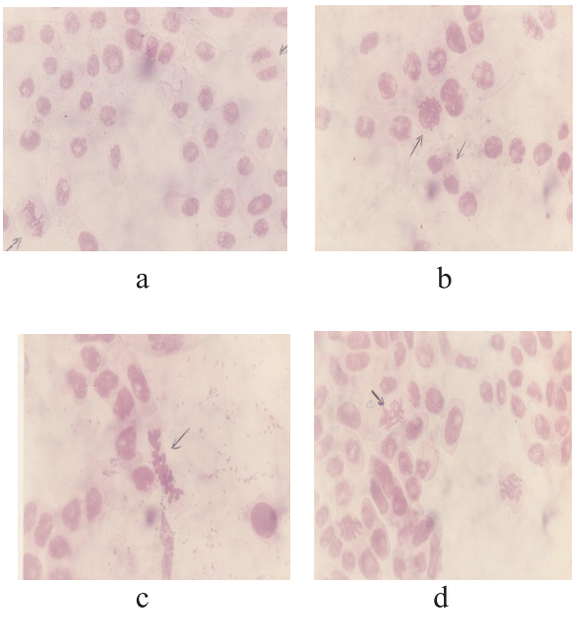

Fig. 1. Microscopic effects of aqueous extracts of *B. diffusa* on *C. jagus* root tips.
(a) Regular metaphase and telophase from control X400.
(b) Sticky chromosome and seriously disturbed anaphase caused by *B. diffusa* extract at 0.8% concentration, X400.
(c) Sticky metaphase caused by *B. diffusa* extract at 0.8% concentration, X400.
(d) Metaphase caused by *B. diffusa* extract at 0.2% concentration, X400.

possess strong anti-cancer properties (Rupyoti et al. 2003, Leyon et al. 2004), antimicrobial properties (Olukoya et al. 1993), anticonvulsant and antispasmodic effects (Adesina et al. 1979, Borelli et al. 2006) as well as being used in the control of viral diseases of vegetable crops (Awasthi et al. 1985). In Nigeria, the roots of B. diffusa are used to manage dropsy and infertility problems (Aiyeloja and Bello 2006). Onyenwe (1983) had reported that the root extract of *B. diffusa* is used in traditional medicinal practice while Ene-Obong and Amadi (1987) had reported that the water extracts of *B. diffusa* exerted mitodepressive effects on *A. cepa* root tip mitosis. This result agrees with the results obtained in this present work. In the Indo-Nepal Himalayan terai region, the tribals harvest this plant for medicinal purposes, mainly for flushing out the renal system and to treat seminal weakness and blood pressure (Mitra and Gupta 1997). In the light of the results obtained in the present study, these observations above may be due to the nucleotoxic action of the extracts or the disturbance of the formation of spindle fibres during cell division which leads to chromosomal aberrations. Stickiness and clumping of the chromosomes were some of the most common effects of these extracts on the treated root tips. These abnormalities have also been reported for several extracts and chemicals already investigated (Badr and Elkington 1982, Misra 1982, Nwakanma et al. 2009). Stickiness usually leads to the formation of anaphase and telophase bridges and this end up inhibiting metaphase and cytokinesis respectively and thus hampering cell division. Stickiness might be due to the ability of the extracts to cause DNA depolymerization and partial dissolution of nucleoproteins, breakage and exchanges of the basic folded units of chromatids and the stripling of the protein covering of DNA in chromosomes as also observed by Onyenwe (1983). The consistently high frequency of interphase observed in all the concentrations was expected since that stage lasts much longer than the other stages of mitosis. Even though many aberrations were observed at metaphase in high concentration of the extract, the frequency of prophase was still high enough to indicate that even the treated cells to some degree, go through prophase of mitosis normally. This observation further suggests that these extracts are potent spindle fibre inactivators and thus can supplement the use of colchicines or hydroxyquinoline in pretreating materials for mitotic studies. This is in agreement with the findings of some earlier workers (Ilevbare 1983, Onyenwe 1983).

C. jagus, which is in the same family as *A. cepa* (Amaryllidaceae), was used and gave very good results which are comparable to

Table 2. Chromosome aberrations of the *C. jagus* root tip cells treated with different concentrations of *B. diffusa* root extracts.

Concentration	Total cell number	Number of dividing cells	Stickiness	C-mitosis	Vagrant	Bridges fragment	Multipolar anaphase
Control	512	27 ($P_{11}M_{13}A_1T_2$)	0	0	0	0	0
0.1%	311	15 ($P_7M_6A_1T_1$)	0	1	1	0	1
0.2%	362	13 ($P_4M_3A_3T_3$)	1	1	0	1	0
0.4%	288	10 ($P_7M_1A_1T_1$)	1	0	2	1	1
0.8%	357	12 ($P_7M_2A_1T_2$)	4	0	0	2	0

those obtained by other researchers who have used *A. cepa* root tips (El-Bayoumi et al.1979, Kabarity and Malallah 1980, Ilevbare 1983). This innovation is particularly important against the background of the fact that *A. cepa* is an edible and hence economic plant to humans. *C. jagus* on the other hand is not edible and grows in the wild.

The results from this work strongly suggest that *C. jagus* can now be used as an alternative to the hitherto-used edible and economic plants - *A. cepa* for cytological work. Furthermore, owing to the ability of the root extracts of *B. diffusa* to accumulate metaphase and hence inhibit mitosis, it is possible to use these extracts as an alternative to the rather expensive colchicine for cytological studies.

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Boerhaavia diffusa Kök Özütlerinin *Crinum jagus* Kök Uçlari Üzerine Sitolojik Etkileri

Özet

Boerhaavia diffusa'nin kök özütlerinin, *Crinum jagus* kök uçlari üzerine mitotik etkileri incelendi. Bu çalismanin sonuçlari, kromozom yapiskanligi (hem metafaz hem de anafazda), C-metafaz, izole kromozomlar ve yapiskan köprüler gibi kromozomal anormallikler ortaya koydu. Sonuçlar; uygulanan özütlerin konsantrasyonu arttikça, mitoz üzerinde daha büyük bir inhibitor etkinin gittikçe artan kromozomal bozukluklarla birlikte ortaya çiktigini göstermektedir. *B. diffusa* bitkisinin kök özütlerinin %0.1, %0.2, %0.4 ve %0.8 konsantrasyonlarda aktif oldugu bulundu. Özütün, metafazi biriktirdigi ve bu özelligi nedeniyle sitolojik çalismalarda son derece faydali olabilecegi bulundu. Bu sonuçlar; kanser terapisinde kullanim, geleneksel tiptaki diger hastaliklarin tedavisinde kullanim ve sitolojik çalismalarda kolsisine bir muhtemel bir alternatif olarak kullanim isiginda tartisildi.

Anahtar Kelimeler: *Boerhaavia diffusa*, *Crinum jagus*, kromozom, mitoz, sitolojik etki.

