Cytological And Toxicological Properties Of A Medicinal Mushroom

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ABSTRACT

The cytological and toxicological potentials of an extract of a medicinal mushroom, *Daldinia concentrica,* were evaluated using the *Allium cepa* model. The extract induced macroscopic and microscopic changes causing a concentration-dependent root growth inhibition and chromosomal aberrations in *A. cepa*. Compared to the control, treatment with the extract resulted in significant ($p<0.05$) inhibition of root growth with EC_{50} value of 1%. The onion root cells showed reduced mitotic indices with corresponding increase in concentration of the mushroom extract. The presence of chromosomal aberrations such as fragments, breaks, bridges and sticky chromosomes in significant ($p<0.05$) amounts indicates a high rate of genotoxicity in the mushroom extracts. No chromosomal aberration was observed in the control. These observations call for caution in the indiscriminate consumption of *Daldinia concentria*. Low concentrations and wide spacing of dosage is suggested for the intake of the medicinal mushroom.

KEY WORDS: *Daldinia concentrica*, *Allium cepa*, chromosome aberration, cytotoxicity, medicinal mushroom, genotoxicity

INTRODUCTION

The use of some mushrooms for curing ailments cuts across different geographical domains. Reports on the tradomedical use of some mushrooms in Nigeria and elsewhere is well documented (Oso 1975, 1977, Stamets 2002, Akpaja *et al.* 2003, 2005, Olila *et al.* 2006, Idu *et al.* 2006, 2009).

It is now generally accepted that scientific studies have in recent times begun to confirm what many indigenous societies have known for ages and with this empirical confirmation come the concomitant broadening of the spectrum of the medicinal applications these mushrooms can be used for. According to Stamets (2002), medicinal mushrooms have extraordinarily low toxicity on humans probably because fungi share a more recent common ancestry with animals than with plants, protozoans and bacteria. Fungal medicines are therefore active against many diseases that afflict humans.

The earliest reports on the indigenous use of the mushroom *Daldinia concentric* in Nigeria appears to be that of Akpaja *et al.* (2003, 2005) on the ethnomycology and usage of the mushroom among the Igbo and Bini-speaking people of Southern Nigeria respectively. Subsequent reports on the *in vitro* antimicrobial activity of the mushroom against some human pathogens have been documented (Gbolagade *et al.* 2006). The antimicrobial activity of the mushroom

suggests that the mushroom has active principles that can impair normal cellular division and this impairment may eventually translate into the inability of the target organism(s) to reproduce.

Despite the discovery of about twenty new metabolites including aromatic steroids cytochalasins (Buchanan *et al.*, 1996), daldinones (Qung *et al.,* 2002) from the mushroom, cytological investigations into its effects on pathogenic organism is yet to be deciphered. This is important, more so, as the mushroom has been confirmed to have a novel anti-HIV agent benzofuranlactone named concentricolide (Qin *et al*., 2006). In the light of the anticipated widespread medicinal use of this mushroom, it has become necessary to investigate the effect of the mushroom in a living system to validate its safe usage.

The *Allium cepa* plant assay has undoubtedly proved to be extremely useful and reliable for the evaluation of cytotoxic and anti-mitotic activity of various compounds in aqueous extracts of medical plants (Akinboro and Bakare, 2007, Oloyede *et al*., 2009). The *Allium* test produces similar results to other test systems such as eukaryotes and prokaryotes (Fiskesjö, 1988). In this study, we seek to evaluate the toxicity of aqueous extracts of *Daldinia concentrica* on cell division and chromosomes in a eukaryotic organism like *A. cepa* (onion) with the hope that the results would provide useful information on the genetic safety assessment of the herbal mushroom.

MATERIALS AND METHODS

Plant and preparation of decoction of mushroom

The medicinal mushroom, *Daldinia concentrica*, was collected in December 2009 from some dead logs of wood within the Ugbowo campus of the University of Benin, Benin City situated between latitudes 6^o 06' N, 6^o 30' N and longitudes 5^o 30' E, 5^o 45' E. The decoction was prepared as described by Dede *et al.* (2006). 10g of the mushroom was ground to powder and soaked in 100ml of ambient water in a beaker followed by another 500ml of warm water thereafter for 1h. The extract obtained was filtered with muslin cloth.

Allium cepa Test

Procurement and preparation of onion bulbs

Onion bulbs *(Allium cepa* L., 2n=16*)* bulbs of the purple variety of average size (15-22 mm diameter) were purchased from Lagos Street in Ring Road, Benin City. They were sun-dried for two weeks and those that were not dried, or attacked by fungi, or had started shooting were all discarded at the beginning of the experiment. The outer scales were carefully removed, without tampering with the primordial root ring.

Macroscopic evaluation

For the root growth inhibition evaluation, the *Daldinia concentrica* extract was diluted to obtain 10%, 5%, 2.5% and 1.25%. To account for a number of bulbs in the population that would be naturally slow or poor growing, the bases of seven equally sized bulbs were suspended on the extract samples inside 100 ml beakers and kept in the dark for 72 h (Rank and Nielsen, 1998). Test extracts were changed daily. The negative control was set up with tap water of good quality only (Fiskesjö, 1985). At the end of the exposure period, the lengths of at least 20 best growing roots of roots from each of 5 onion bulbs at each concentration were measured (in cm) with a metre rule. From the

weighted averages for each concentration and the control, the percentage root growth inhibition in relation to the negative control was determined:

$$\text{Over all mean root length of test solution} = \frac{\text{Total length of roots}}{\text{Total number of roots}}$$

$$\%\ \text{root growth of control} = \frac{\text{Overall mean root length of test solution}}{\text{Overall mean root length of control}} \times 100$$

The EC_{50} (the effective concentration at which 50% root growth of control was inhibited) was also calculated by plotting a graph of percentage root growth of control against sample concentrations. The effect of each sample on the morphology of growing roots was also examined.

Microscopic Evaluation

For the evaluation of induction of chromosomal aberration, the onion bulbs were grown on 5%, 1%, 0.1%, 0.01% extract concentrations (v/v) and the control for 48 h at the end of which root tips from these bulbs were cut and fixed in ethanol: glacial acetic acid (3:1, v/v) inside universal bottles and stored at 4°C for 24 h before use. The already fixed root tips were hydrolyzed in 1N HCl at 60°C for 5 min after which they were washed several times with distilled water. Two root tips were squashed on each slide, stained with aceto-orcein for 10 min. Excess stains were removed, and the cover slips carefully lowered on to exclude air bubble. The edges of the cover slips were sealed on the slides with clear fingernail polish as suggested by Grant (1982). Six slides were prepared for each concentration and the control out of which five (at 1000 cells per slide) were analyzed at ×1000 magnification for induction of chromosomal aberration using Nikon Eclipse (E400) light microscope. The mitotic index (MI) and frequency of chromosomal aberrations (CA) were calculated as in previous studies (Olorunfemi *et al*. 2011).

$$\textbf{Mitotic index} = \frac{\textbf{Number of dividing cells}}{\textbf{Total number of cells}} \times 100$$

$$\%\ \textbf{Aberrant cells} = \frac{\textbf{Number of Aberrations}}{\textbf{Total number of cells}} \times 100$$

Statistical Analysis

The SPSS 10.0 statistical package was used for this analysis. The means, with 95% confidence limits and the standard errors for each of the quantitative sets of data were calculated. Differences between the control and the individual dosage group of each extract were analyzed by means of the Students' *t*-test of significance at the $P < 0.05$ level.

RESULTS

Table 1 shows the results of the effects of the extract of *Daldinia concentrica* on root growth of *Allium cepa*. Good root growth was achieved in the control. At tested concentrations, root growth was highest at the 1.25% concentration of all the extracts and least at 10%. Inhibition of root growth was concentration dependent and statistically significant ($P < 0.05$) at tested concentrations. The EC_{50} for the decoction of *Daldinia concentrica* was 1% (Fig. 1).

Table 1: Effects of *Daldinia concentrica* extract of on root growth of *Allium cepa*.

Concentration (%)	Mean root length (cm) ±S.E*	Root Growth (%) of control
Control	4.9 ± 0.17	-
1.25	2.0 ± 0.22	40.8
2.5	0.8± 0.09	16.3
5	0.4 ± 0.11	10.2
10	0.2 ± 0.03	4.1

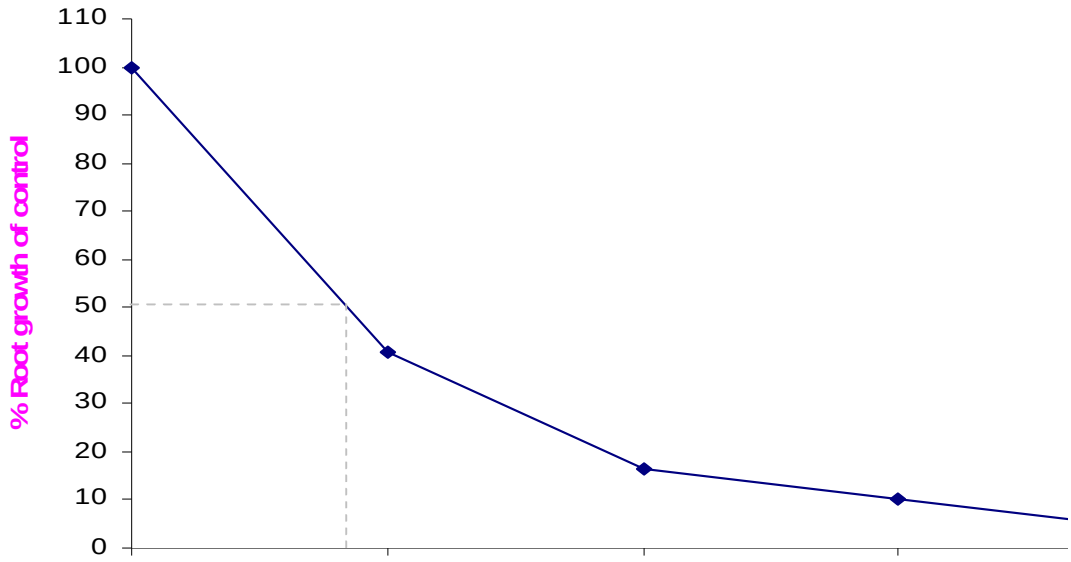


Fig. 1: Growth inhibition curve of Allium cepa roots grown in the mushroom extract

The effect of *Daldinia concentrica* extract on cell division and chromosome behaviour of *Allium cepa* are presented in Table 2. Mitosis was observed to be normal in the cells of the control and had a mitotic index (MI) value of 46. With increasing concentration of the extracts however, there was concentration-dependent decrease in the mitotic index. There were no dividing cells at the 10% concentration of the extracts. The frequency of these aberrations was, however, not concentration dependent. The incidence of aberrant cells was observed to be lowest (4%) in cells of roots treated with 5% of mushroom extract and highest (38%) in cells of roots treated with 0.01% of the extract.

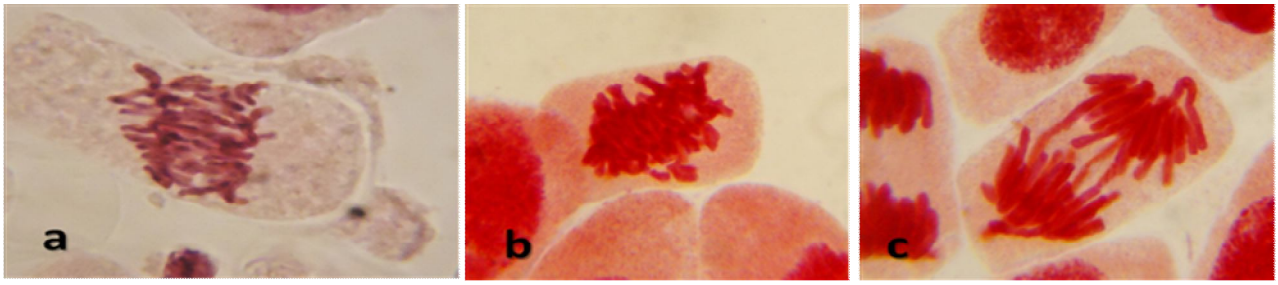
Table 2: Mitotic activities of the root cells of A. cepa treated with Daldinia concentrica extract

Extract concentration (%)	No of dividing cells	Mitotic index	% Aberrant cells	Mitotic inhibition
Control	229	46	-	-
0.01	192	38	0.06	17.39
0.1	146	29	0.12	36.96
1	106	21	0.14	54.35
5	20	4	0.16	91.30

* 5000 cells/per concentration of each extract and the control.

The statistical analyses of the different concentrations of the mushroom extracts showed significant ($p<0.05$) between the treatments and the different values of mitotic indices and chromosomal aberration percentages.

The types of chromosomal aberrations induced in the mushroom extract is presented in Plate 1. The chromosomal aberrations induced by extract at various concentrations were spindle disturbance, fragments, breaks, bridges and sticky chromosomes.



DISCUSSION

The potential cytotoxic and genotoxic effects of aqueous extracts of *Daldinia concentrica* on *Allium cepa* were evaluated. Studies have shown that in *Allium cepa*, (Fiskesjo, 1997, Babatunde and Bakare, 2006, Akinboro and Bakare, 2007) whenever there is root growth inhibition (macroscopic parameter), there is always reduction in the number of dividing cells (microscopic parameter). Results of our study are in agreement with this assertion. The inhibition of root growth in *A. cepa* is in consonance index varied from 46 in control and to 4 in 5% extract. The decreased mitotic index values in the treated onion roots is an indication of the with the assertion of Nielsen and Rank (1994) that the sigmoid curve is a typical dose response for toxic substances whose negative effects is an indication of the cytotoxic effects of the mushroom extract. This observation is consistent with earlier report on the mushroom *Ganoderma lucidum* (Curtis) P. Karst, (Olorunfemi *et al.* 2011).

The mitodepressive ability of the mushroom extract (the ability to block the synthesis of DNA and nucleus proteins) is demonstrated by the reduction in the number of dividing cells at tested concentrations. The mitotic presence of cytotoxic substances in the *D. concentrica* extract, which caused inhibition of mitotic activities, while the observation of

aberrant cells in the treated onion root tip meristems indicates genotoxic effects (Akinboro and Bakare, 2007) of the mushroom extract. The chromosomal aberrations induced by the extract at various concentrations were spindle disturbance, fragments, breaks, bridges and sticky chromosomes which arise from changes in structure of the chromosomal material. Chromosome bridges and fragments are clastogenic effects, both resulting from chromosomal and chromatid breaks (Kovalchuk *et al*. 1998) while stickiness reflects high toxicity of a substance as well as irreversibility of the change (Turkoglu, 2007). Acentric fragments in anaphase is the result of chromosome or chromatids interruptions indicating interference with DNA while bridges probably occur by the interruption and joining chromosomes or chromatids or as a result of chromosome stickiness, or it may be ascribed to unequal translocation or inversion of chromosome segments (Turkoglu, 2007, Gömürgen, 2005).

The presence of alkaloids in the extracts of *Borreria filiformis* and *Vinca rosea (*Ene and Osuala, 1990) and *Azadirachta indica*, *Morinda lucida*, *Cymbopogon citratus*, *Mangifera indica* and *Carica papaya* (Akinboro and Bakare, 2007) have been reported to induce chromosomal aberrations. The spindle disturbances detected in this study might have been due to the presence of alkaloids in the tested extract. As opined by Akinboro and Bakare, (2007), the presence of chemicals with turbagenic potentials may be responsible for the complete arrest of cell division at high concentration of the mushroom extract. These observations may explain the ability of the mushroom extract action as an antimicrobial agent as reported by Gbolagade (2007). Although the present study has not been able to ascertain the actual ingredients responsible for the observed chromosomal aberrations; they are however obviously consumed as part of the diet in the mushroom.

The results suggest that *Daldinia concentrica* extract possess mitodepressive effects on the growth of the meristematic root cells, as well as cell division property of *A. cepa.* The widespread tradomedical use of this mushroom by many cultures in Nigeria (Akpaja, 2003, Idu *et al.,* 2007, Osemwegie *et al.,* 2005) needs to be properly studied. Moreso, increase in the cytotoxicity and genotoxicity as the concentration increase suggests that there is need to succinctly determine the dose at which it would be effective and at the same time, safe. Regulation backed by education and research is needed to improve the quality and quality use of traditional Chinese medicine (Li *et al*. 2003). This assertion also holds true for the Nigerian traditional medicine. While the traditional use of this medicinal mushroom cannot be stopped, it may be possible to conduct studies into the precautionary measures that can be observed in the use of the mushroom for treating the many health problems it is known for having answers to. In conclusion, the results call for caution in the indiscriminate consumption of *Daldinia concentrica*. Low concentrations and wide spacing of dosage is suggested for the intake of the medicinal mushroom.

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