AS MEN OF HONOUR
WE JOIN HANDS
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Science Article

Chromosomal Aberrations Induced in Root Tips of Allium cepa by Phaeogyroporus portentosus (Mushroom) extract

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Abstract:

Scientific information regarding the cytotoxicity of mushrooms used as medicinal agents is essential. *Phaeogyroporus portentosus* (giant mushroom) , commonly used as an antioxidant, is being investigated for its cytotoxic and genotoxic potential. The freeze-dried aqueous extract was evaluated using the root dip treatment of *Allium cepa* meristematic cells assay. Chromosomal abnormalites induced in the *Allium cepa* roots were observed and mitotic index was determined upon exposure to three aqueous concentrations **(**6µg/ml**,** 12 µg/ml and 18 µg/ml **)** of the mushroom extract and control. Results showed that the aqueous extract of the mushroom, *P. portentosus,* induced a concentration-dependent root growth inhibition and chromosomal aberrations behaviour in *A. cepa* (onion) cells. The aqueous extract of *P. portentosus* revealed significant effects on the cell division of *A. cepa* cells inducing chromosomal aberrations such as bridges, fragments and sticky chromosomes when compared with the control. A decrease in mitotic index was observed with an increase in concentration of mushroom extract. All observations including dose-dependent reduction in mitotic index (MI) in the extract were significantly different from that of the control. The ability of the aqueous extract of *P. portentosus,* to cause a decrease in the mitotic index of *A. cepa* cells shows that it should be used with caution. High concentrations should be discouraged and it may stand promising in the development of anticancer drugs in the future.

Key words: *Phaeogyroporus portentosus***,** *chromosomal aberrations, Allium cepa, mitotic index*

Introduction

Mushrooms are widely consumed in Nigeria due to their high nutritional value. They are mostly used for tasty delicacies in cuisines and meat in home made soups. Medicinal mushrooms are fleshy spore bearing fruiting bodies of fungi which produce significant metabolites having both toxic (cytotoxic mushrooms cause death to the cell of an organ in a living organism).and medicinal properties. It is advisable to exercise caution with mushrooms of which there is a dearth of information. The *Allium cepa* assay is a rapid and sensitive method that determines the extent of the destructive capacity of a substance or an agent. This method has been frequently used to determine the cytotoxic, mutagenic and genotoxic effects of several substances [1],[2]. It is also called root chromosomal aberration assay which consists of the onion meristematic cells in order to identify chromosomal alterations [1].

Edible and medicinal mushrooms are widely used all over the world but there is need for their cytotoxic properties to be evaluated. *Morchella esculentus* (morel) is an example of an edible mushroom popularly eaten in Portugal and Serbia. It was investigated for its antimutagenic property, and it revealed high antimutagenic activity in its extracts [3] .*Phaeogyroporus portentosus* is a rare, large mycorrhizal mushroom in the order Boletales and family Basidiomycetes. It belongs to the kingdom Boletus, and contains species whose extracts are known to inhibit tumour proliferation [4]. *Portentosus* is a latin word meaning "marvellous or amazing". Until 1845, *P. portentosus* was previously known by scientific names such as *Boletus marginatus*, *Phlebopus marginatus* and *Boletus portentosus*. Common names of this mushroom in Nigeria are "*Olu ekiika*" meaning giant mushroom in yoruba language (Western Nigeria) and "*Akamba udip*" Calabar language (Eastern Nigeria). This group of fungi have also been reported as good sources of medicines by the traditional medical practitioners of Nigeria [5]. Extracts of *P. portentosus* have been reported to exhibit antioxidant activity, high total phenolic contents and effective inhibition against the alpha-glucosidase enzyme. It may help in the treatment of type 2 diabetes [6]. Onions are believed to be effective in the treatment of diabetes.

The *Allium cepa* test is used for screening and monitoring environmental chemicals with mutagenic and carcinogenic potential. The decrease in the mitotic index (MI) of *Allium cepa* meristem cells can be considered as a reliable method to determine the presence of cytotoxic agents in the environment *Allium cepa* chromosomal root assay was used in this study to determine the mitotic index, which is used in cell division for the ratio between the number of cells undergoing mitosis in a sample to the total number of cells in that sample. Cancerous cells have a high mitotic index, hence identification of antigenotoxic factors is expected to lead to the development of cancer preventing agents [7],[8]. This study was undertaken to determine the ability of the aqueous extract of *P. Portentosus* to cause any chromosomal aberrations or differences in the normal growth of *Allium cepa* roots.

Materials and Methods

Plant Extraction

A large fresh fruiting body of the mushroom, *P. portentosus,* was handpicked from the premises of the campus staff quarters in University of Lagos, Akoka, Lagos state. It was authenticated at the Herbarium, Department of Botany, University of Lagos, where a voucher specimen (voucher number LUH 0001M) was deposited. The cap and stalk were weighed, cut and macerated in water using ceramic mortar and pestle, then homogenized with a blender, until an even fluffy mesh (1.765 kg) was obtained. The mesh of *P. portentosus* (1kg) was extracted in fresh tap water seperately for 3 days, filtered and concentrated using a freeze drier to yield 242.16g of aqueous extract. The mushroom extract was kept in the freezer (40°C) till further use.

Allium cepa Assay

Twelve (12) healthy and dried onions (42-53mm), were

purchased from Bariga market, Lagos state, Nigeria. The outer scales were carefully removed, leaving the primordial root ring intact. The base of each *A. Cepa* bulb was treated with different concentrations (6 µg/ml, 12 µg/ml and 18 µg/ml) of *P. portentosus* aqueous extracts in duplicates while fresh tap water was used as control (Figures 1a and 1b). The root growth was measured for seven days and extracts were changed daily, after which the roots of onion bulbs with the best growth at each concentration was removed, and their lengths measured in cm with a metre rule. The root length data was then calculated as mean ± SE. Root tips from onions were cut and fixed in ethanol/acetic acid fixative (3:1, v/v) then stained with lactic acetic orcein and squashed. Chromosomal aberrations were observed under the microscope (Bresser WF10X, United kingdom) The mitotic index and the frequency of aberrant cells (%) were calculated as the number of dividing cells per 500 observed and based on the proportion of aberrant cells scored at each concentration, respectively [8]. The mitotic index, MI was determined, for root growth inhibition evaluation according to the method reported by Awodele *et al.*, (2010) [9]

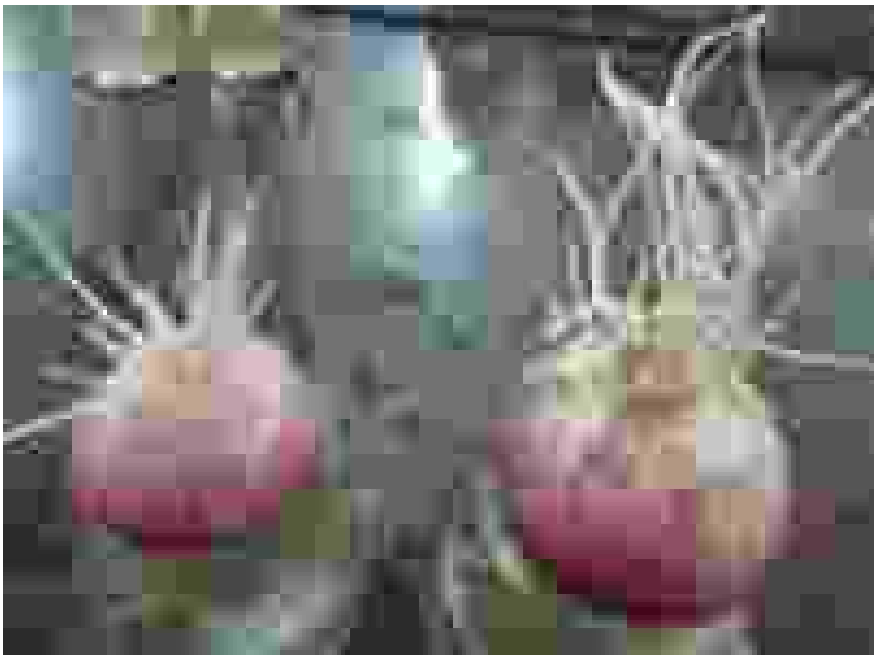

Figure 1a: control samples in water

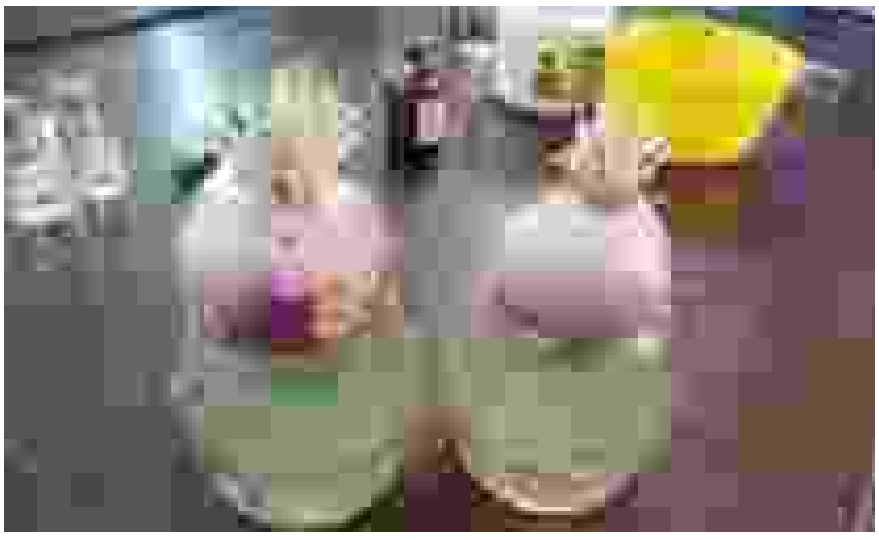

Figure 1b: aqueous extract of *P. portentosus*

$$MI = \frac{\text{the number of dividing cells}}{\text{the total number of cells}}$$

Statistical Analysis

Differences between the control and the individual dosage grconcentrations of P.portentosus extract were analyzed by means of students' t-test of significance at the p⊠0.05 level

RESULTS

The effects of various concentrations of *P. portentosus* extract on the cytology and root growth of *Allium cepa* cells are presented in Table 1. The mitotic index decreased with increasing concentrations of the extracts. In figure 1c, the root tip lengths of *A. cepa* bulbs in the three extracts of *P. portentosus* were seen to be lower than that of the control sample in figure 1a. Furthermore,

the mushroom extract was seen to have reduced the mitotic index in onion root meristems in Table 1, indicating the presence of bioactive substances with the potential for pharmacological use in chemotherapy. The reduction of the mitotic index might be explained as being due to the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis [8]. Figure 1d shows all the chromosomal aberrations observed from (a-f) in the *A.cepa* cells. Inhibition of root growth was concentration dependent and statistically significant at the P 0.05 level

Figure 1c: Root tip lengths of *A.cepa* bulbs in three (3) different concentrations of aqueous extracts of *P. portentosus* in duplicates.

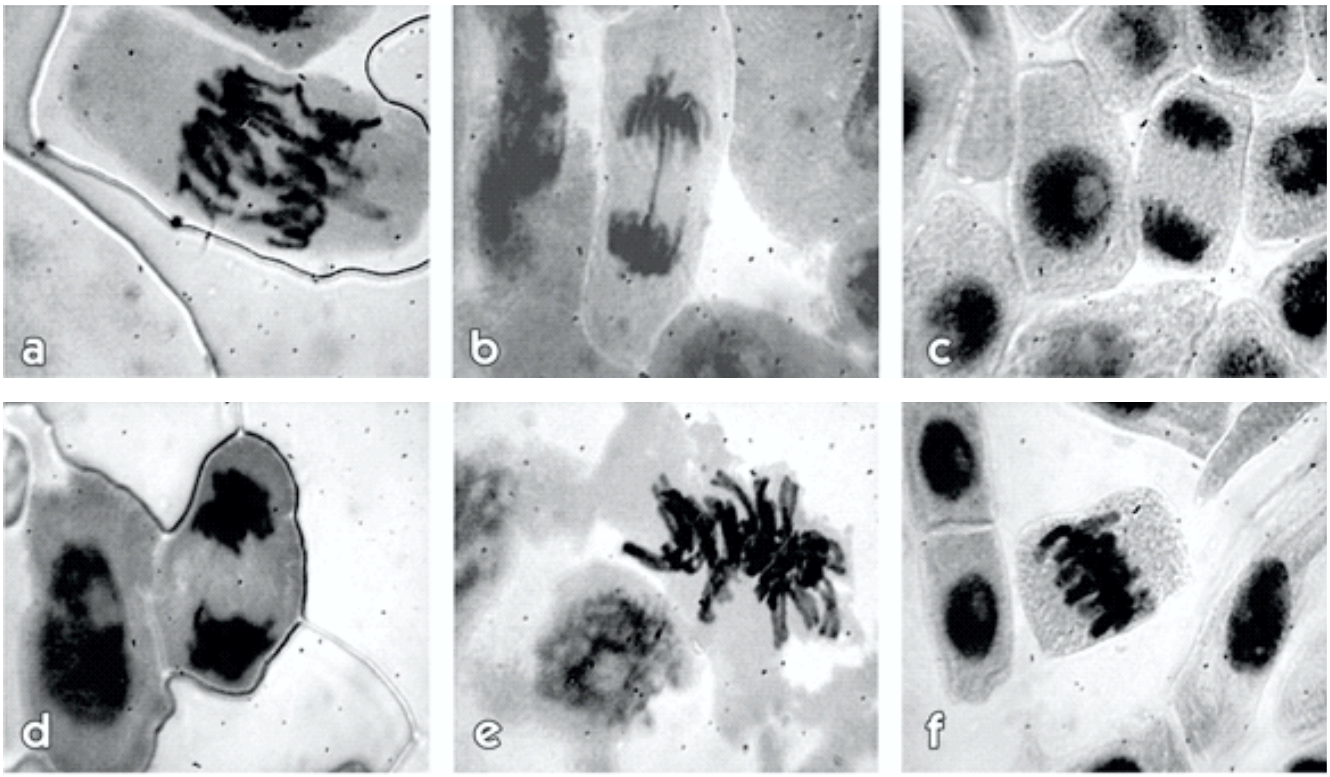

Figure 1d: Chromosome aberrations observed in *A.cepa* meristematic cells exposed to aqueous extracts of *P. portentosus*. (a) normal anaphase (b) chromosome bridged anaphase (c) normal telophase (d) sticky telophase aberration (e) vagrant chromosome (f) sticky chromosome

Table 1: Effects of various concentrations of mushroom extract, *P. portentosus* on the cytology and root growth of *Allium cepa.*

Conc.	Number of cells	Number of dividi ng cells	Mitotic Index	Mean root length±S.E	Bridges & fragments	Stickiness	Vagrant
C ontrol	500	39	78	4.43 0±0.008	0	0	0
6ug/ml	437	22	50	2.08 6±0.005	2	7	4
12ug/ml	404	19	47	2.057 ±0.004	3	6	6
18ug/ml	392	17	43	2.064 ±0.00 5	3	5	4

$p < 0.05$ versus control (fresh tap water)

DISCUSSION

Due to the growing interest in mushrooms as medicinal agents, their safety in consumption and efficacy as a natural antioxidant treatment must be examined. Higher plants such as *Allium cepa* can be used as genetic models to evaluate genotoxic effects such as chromosome aberrations and disturbances in the mitotic cycle. [8]. The aqueous extract of *P. portentosus* was observed to have significantly reduced the length of *Allium cepa* root tips when compared with the control in Figures 1a and 1b. Reduced mitotic root length, mitotic cycle inhibition and induced chromosomal aberrations such as bridges, fragments and sticky chromosomes were also observed (Figures 1d, 1e and 1f). These observations were significantly different from those of the control of *Allium cepa* from day 2-7 throughout the assay (Table 1). *Allium cepa* assay is one of the widely used assays for genotoxicity and antimutagenicity testing of substances [10]. This assay is used to identify the harmful effect of any substance, in this case, the mushroom *P. portentosus* in different concentrations and time of exposure. The result above showed that the aqueous extract of *P. portentosus* exhibited a dose-dependent inhibition of root growth cell division, mitotic index on the root tip and chromosomes of *Allium cepa* cells. Cytotoxic level shows that the aqueous extract of this mushroom may stand promising in the development of anticancer drugs due to its ability to cause a decrease in the mitotic index of onion meristematic cells which could be interpreted as cellular death [8]. In addition, Table 1 shows that stickiness and bridges were the most common aberrations observed (Fig 1d). Other abnormalities such as sticky telophase and vagrant chromosomes were also observed at all concentrations of extract tested (6µg/mL, 12 µg/mL) (see Figures 1e and 1f).

The aqueous extract of *P. portentosus* in Nigeria has been found to have high ferric reducing properties [6] which makes it useful as an antioxidant agent. In Northern Thailand, it is also reported as source of food after proper boiling and a source of antioxidants for the pharmaceutical and food industry [11]. Previously, extracts of *P. portentosus* have been reported to exhibit antioxidant activity and high total phenolic contents. The genotoxic potential observed in biological assays like this one with *A. cepa* may be due to the presence of phenolic compounds, because it is known that phytotoxic potentials of many phenols may affect membrane permeability hereby causing damage to DNA and proteins, leading to cell's death [12].

Mitotic index is used as an indicator of cell proliferation biomarker which measures the proportion of cells in the mitotic phase of the cell cycle. Hence, the decrease in the mitotic index of *A. cepa* meristematic cells could be interpreted as cellular death [8]. On the other hand, this root inhibition and chromosomal aberration ability may be related to other classes of secondary metabolites, or may be due to synergistic action between the present phenolics [3]. Further studies are needed to elucidate the structures of chemical constituents in the extract.

However, the cytotoxic and genotoxic effects exhibited by this mushroom extract in *A. cepa* meristem cells clearly indicate that at certain concentrations, this mushroom is not safe for human consumption. At very low concentration, it is recommended for use in order not to exclude the efficacy of *P. portentosus* as herbal remedy for the community. However high concentrations or continuous use, should be discouraged, due to the reduced mitotic index, chromosomal aberrations, alterations and modifications observed in this research.

Conclusion

The result from this assay, revealed that the aqueous extract of the mushroom, *P. Portentosus* showed significant chromosomal aberrations in the root tips of *A. cepa,* which are different from the control.. This extract posseses cytotoxic effects on the growth and cell division of *Allium cepa* root cells, which were concentration dependent. Hence caution should be exercised by tradomedical doctors in its use all over. High concentrations of the aqueous mushroom extract should be discouraged. However, this information may have therapeutic applications that can indirectly help in the prevention and/or treatment of degenerative diseases such as cancer.

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