Genotoxic Effects of *Ganoderma lucidum* (Curtis) Karst and *Pleurotus ostreatus* (Jacq. Fr.) Kummer using the *Allium* Test

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Abstract

This study determined the genotoxic effects of *Ganoderma lucidium* and *Pleurotus ostreatus* on *Allium cepa* root tips. Small bulbs of *Allium cepa* were grown in different concentrations of aqueous extracts of milled *Ganoderma lucidium* (0.3g/ml, 0.6g/ml, 0.8g/ml, 1.0g/ml) and *Pleurotus ostreatus* (0.25g/ml, 0.75g/ml, 1.25g/ml, 1.75g/ml). Five root tips from each bulb were harvested after 48 hours and processed for cytological studies using aceto-orcein squash techniques. The average length of root bundles was determined after 96 hours. Treatment with *P. ostreatus* extracts seemed to inhibit root growth in a concentration-dependent manner while that of *G. lucidium* did not inhibit root growth. Extract mitotic index (M.I.) was calculated for each extract concentration. The M.I. for *Ganoderma lucidium* extracts were: 7.1 (0.3g/ml), 5.7 (0.6g/ml), 6.2 (0.8g/ml), and 4.5 (0.1g/ml), whereas M.I. for *Pleurotus ostreatus* extracts were 6.7 (0.25g/ml), 6.3 (0.75g/ml), 6.0 (1.25g/ml) and 5.4 (1.75g/ml). In the root cells, extracts from both test samples revealed chromosome stickiness, vagrant chromosomes, spindle multipolarity, c-mitosis, and bridged fragments.

Keywords: *Allium cepa,* Chromosomal aberrations, *Ganoderma lucidium*, Genotoxic effects, *Pleurotus ostreatus*

Introduction

Mushrooms are macro fungi with a distinctive fruiting body that can be hypogeous or epigeous and large enough to be seen and picked with the naked eyes. Mushrooms are eaten and used as spices and meat in vegetable soups in West African tropical countries. Because of their subtle flavour, aroma, physical taste and appeal, they have been regarded as dainties for centuries. Mushrooms are not only tasty, but also serve as good sources of proteins, minerals, and vitamins (1). Macroscopic fungi have been shown to have great potentials in the production of bioactive metabolites, ethno-medicinal resources, and pharmacological products. On a dry mass basis, mushrooms have 19-35% protein, compared to 7.3% in rice, 13.2% in wheat, and 25.2% in milk. They also have high deposits of trace and essential minerals (2).

Pleurotus ostreatus (Jacq. Fr.) Kummer (Basidiomycota) – known as the oyster mushroom – is a species distributed on all continents, except for Antarctica (3). *Pleurotus* spp. is a promising medicinal mushroom, exhibiting hematological, antiviral, antitumor, antibiotic,

antibacterial, hypocholesterolic, and immunomodulation activities. For instance, polysaccharide extracted from *P. ostreatus* was found to have significantly higher antitumor activity against HT-29 colon cancer cells *in vitro*, and this activity was dose-dependent. Hence, *Pleurotus ostreatus* was considered as a possible candidate for developing a novel antitumor agent with low toxicity (4). The high iron content of dried oyster mushrooms highlights its use as a potential blood builder. Oyster mushroom naturally produces compounds known as statins, which stimulate receptors in the liver, which clear cholesterol from the body (5, 6, 7). Varying antimicrobial activities of *P. ostreatus* against *Escherichia coli, Bacillus subtilis, Streptococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Fusarium proliferatum* and *Salmonella typhi* have been reported (8, 9, 10).

Ganoderma lucidum is a popular medicinal mushroom known as lingzhi in Chinese, reishi in Japanese, and yeonghi in Korea (11). Fruit bodies in the genus *Ganoderma* are sessile or stipitate, with a lustrous upper surface and distinct cortex. They come in a variety of colours, including yellow, black, white, and reddish purple (12). *Ganoderma lucidum* has two distinct growth forms: one, found in North America, is sessile and rather large with only a small or no stalk, and the other, found primarily in the tropics, is smaller and has a longer narrow stalk. It grows as a parasite or saprotroph on a wide variety of trees and has a global distribution in both tropical and temperate regions. Over the last two millennia, *G. lucidium* has been used as a home remedy in traditional medicine in many Asian countries. It was thought that consuming *G. lucidium* in the form of tea or mushroom powder on a regular basis would help to preserve human vitality, promote longevity and prevent or treat a wide range of illnesses, including cancer. Herbal doctors in yoruba land, Nigeria refer to *Ganoderma* species as 'olu iju' meaning mushroom for the treatment of fibroid (13). The mycelial culture of *G. lucidum* was active against microorganisms such as *Escherichia coli, Staphylococcus aureus, Kllebsiella pneumoniae and Pseudomonas aeruginosa* (14). *Ganoderma* species was reported to be used in the treatment of asthma and neoplasia, inhibit platelet aggregation, lower blood pressure, cholesterol, and blood sugar levels (6, 15, 16, 17).

The mechanism of action through which *P. oestratus* and *G. lucidium* exhibit their medicinal effects are not fully understood. However, it has been suggested that genotoxic activity may be one of the major mechanisms of action (17, 18). *Allium cepa* root tips have been used to evaluate biological effects caused by chemicals since Levan introduced the first *Allium* test in 1938. There are many other root tip systems such as *Vicia faba, Tradescantia* and *Crinum jagus* which along with *Allium cepa*, constitute classical models (19, 20, 21, 22). This paper presents the genotoxic effects of *Ganoderma lucidium* and *Pleurotus ostreatus* using the *Allium* test. The aim is to provide some possible explanation for the antimicrobial, anti-dermatitis, anti-cancer and other medicinal effects of these macro fungi.

Materials and Methods

Mushroom Samples

Ganoderma lucidum and *Pleurotus ostreatus* samples were obtained from the

Center for Mushroom Research and Cultivation, Yaba College of Technology, Lagos, Nigeria. The ornamental truncate spores of *Ganoderma lucidum*, which are oblong with one narrowing end and other features, were used to identify it (23, 24). The identification of *Pleurotus oestratus*, was done by a Mycologist at the Mushroom Research and Training Laboratory, Yaba College of Technology, Lagos, Nigeria. Mushroom samples were dried at room temperature and milled to powder form. *Ganoderma lucidium* and *P. ostreatus* (50g) each were soaked separately in 500 ml of boiling water overnight and filtered using Whattman filter papers to obtain the hot water extract. The extracts of *G. lucidium* and *P. ostreatus* were reconstituted using gravimetric methods (21) into 0.30g/ml, 0.60g/ml, 0.80g/ml, 1.0g/ml; and 0.25g/ml, 0.75g/ml, 1.25g/ml, 1.75g/ml; respectively. One *Allium cepa* onion bulb was placed in a beaker containing each concentration of *G. lucidium* and *P. ostreatus*, while water was used as control treatment. The treatments were replicated four times. After 48 hours, five root tips were carefully harvested from each bulb (by cutting about 1 - 2cm of the root length from the apex). These were used to prepare slides based on the aceto-orcein squash technique. Then after 96 hours, mean length of root bundles were measured (20, 21). To mimic natural conditions, the onion bulbs were placed directly on the test liquids (18, 19, 25, 26).

Squash Technique

Five root tips were randomly selected by cutting them off from each concentration of *Allium* treatment with a sharp razor blade and fixed in freshly prepared 1:3 acetic acid: 95% alcohol (V/V) for at least 24 hours. Then, they were stored in 70% alcohol until when required (20). Fixation was carried out following the methods of (20). These root tips were hydrolyzed in 1N HCl at 60^{o}C for five minutes (27). This was done to soften the root tissues (28) and facilitate the disintegration of the middle lamella of the cells before staining. The roots were placed on a glass slide and the terminal root tips (1-2mm) removed (29, 30) before slide preparation (20). For examination of mitotic chromosomes, root tips were squashed in an FLP-orcein, following the method of (31). These materials were squashed directly, by tapping with the blunt end of a ball point pen to cause the cells to spread out properly.

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 (32). The effect of different concentrations and duration of treatment on the frequencies of the four phases of mitosis were determined. Microphotographs of chromosomal aberrations were taken from the temporary slides following the method of (33).

Data Analysis

Data were analyzed using General Linear Model (GLM) which incorporates the univariate analysis (ANOVA) and the pair wise test comparison at $p<0.05$ level of significance.

Results

Mild to strong root inhibitions were observed in all concentrations of extracts of *P. oestratus*, and they were concentration

dependent (Table 1). The effect was not severe at low concentration (0.25g/ml). However, severe effects were observed at higher concentrations (0.75g/ml, 1.25g/ml and 1.75g/ml). On the other hand, *G. lucidium* did not inhibit root length growth. The mitotic index for all concentrations were greater than that of control (9.4) in a concentration-dependent manner (Tables 1 and Table 2). The chromosomes of the control treatment were normal (Plate 1 and 2), while chromosomal aberrations induced by *G. lucidium* on *Allium cepa* roots included sticky chromosomes, bridges and fragments, as well as vagrant and attached chromosomes (Plate 3 and 4). Also, *P. ostreatus* caused chromosome stickiness, c-mitosis, bridges and fragments as well as vagrant and binuclei chromosomes in *Allium cepa* roots (Plate 5 and 6). The mitotic indices of all extracts of *G. lucidium* and *P. ostreatus* were lower than that of the control (9.4) suggesting genotoxic action of both extracts on *Allium cepa* root tip cells.

Discussion

Ganoderma lucidium and *P. ostreatus* exhibited some level of toxicity. An increase in *Pleurotus ostreatus* concentration had a negative effect on the growth of *Allium cepa* roots (Table 1). However, this trend of root length inhibition was not evident in root tips exposed to *G. lucidium* extracts. Cell growth depends on mitosis, which when inhibited causes cessation of growth in an organism. Inhibition of mitosis may be due to the accumulation of prophase (prophase arrest) or nucleotoxic action of the extracts. It may also be caused by the disturbance of the formation of spindle fibres during cell division which leads to chromosomal aberrations (20). This results in stunting of both stem and roots. This mechanism might be used by the macro fungi to exert toxic effects on microorganisms which cause disease to humans. The other possible mechanism is to cause the production of aberrant chromosomes during the mitotic cycle. Hence aberrant metaphase such as c-mitosis, vagrant chromosomes, anaphase bridges and fragments or sticky chromosomes may be used to disrupt the activities of the target organism by the macro fungi (Table 3).

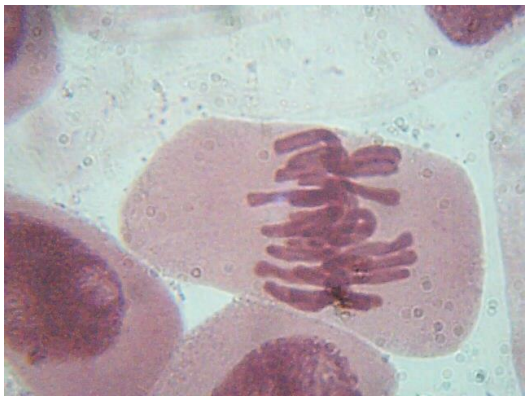
Table 1: Root length (mean±standard deviation) of *Allium cepa* treated with *Pleurotus ostreatus* and *Ganoderma lucidium* extracts

Concentration (g/ml)	*Pleurotus ostreatus*
Control	0.66+0.28
0.25	0.61+0.41
0.75	0.15+0.05
1.25	0.15+0.06
1.75	0.26+0.14
Concentration (g/ml)	*Ganoderma lucidium*
Control	1.11+0.40
0.3	3.42+1.07
0.6	3.19+0.80
0.8	2.24+0.73
1.0	2.01+0.62

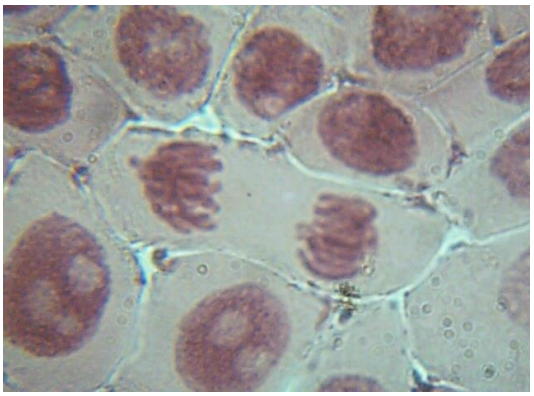
Table 2: Mitotic effects of *Ganoderma lucidium and Pleurotus ostreatus extracts* on the root tips of *Allium cepa*

Concentration	Numbers of Dividing cell	Numbers of dividing cells				Stickiness	C-Mitosis	Bridges	Vagrant fragment	Binu cleus	Multiple anaphase	Attached	Total aberration	Lagg	Mitotic Index
	Numbers of cell	Dividing cells	P	M	A	T									
Control	500	47	6	15	12	14	0	0	0	0	0	0	0	0	9.4
G.L 0.3g	479	34	2	12	7	13	11	0	4	8	0	0	0	23	0	7.1
G.L 0.6g	457	26	1	8	8	9	4	0	4	7	0	0	0	15	0	5.7
G.L 0.8g	435	27	1	8	9	9	7	0	5	6	0	0	2	20	0	6.2
G.L 1.0g	421	19	1	5	5	8	5	0	4	4	0	0	0	15	2	4.5
PL 0.25g	475	32	3	11	8	10	7	0	4	5	0	0	0	17	1	6.7
PL 0.75g	461	29	1	10	8	10	5	1	5	6	1	0	0	18	0	6.3
PL 1,25g	447	27	3	7	8	9	6	2	5	5	0	0	0	18	0	6.0
PL 1.75g	423	23	1	8	5	9	5	1	3	4	0	0	0	13	0	5.4

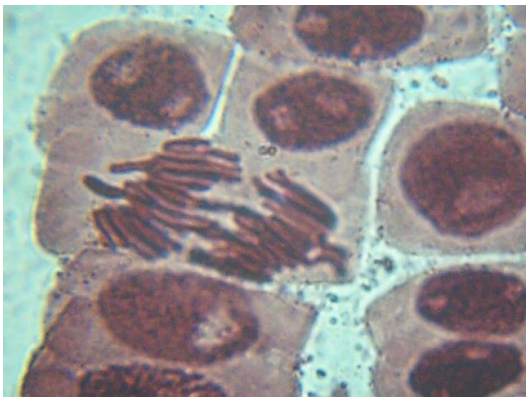
Key: P (prophase); M (Metaphase); A (Anaphase); T (Telophase); GL (*Ganoderma lucidium*): PL (*Pleurotus ostreatus).*



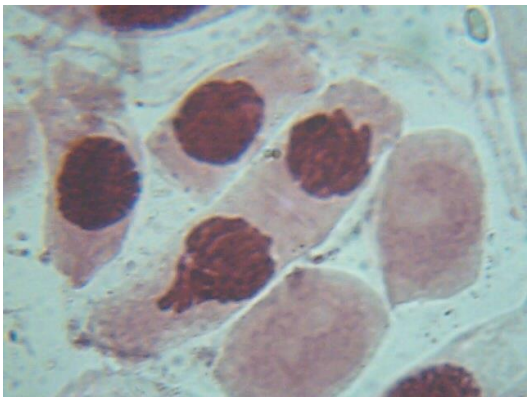
(1) Normal Metaphase from control



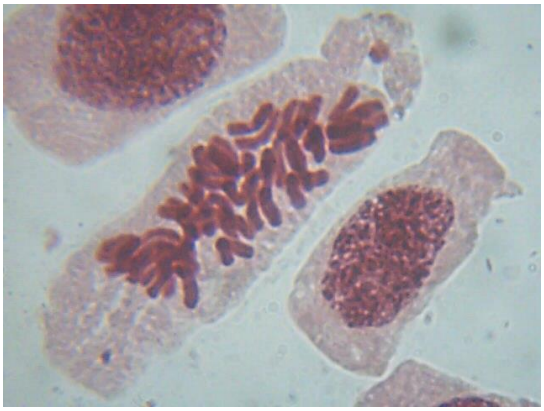
(2) Normal Anaphase from control



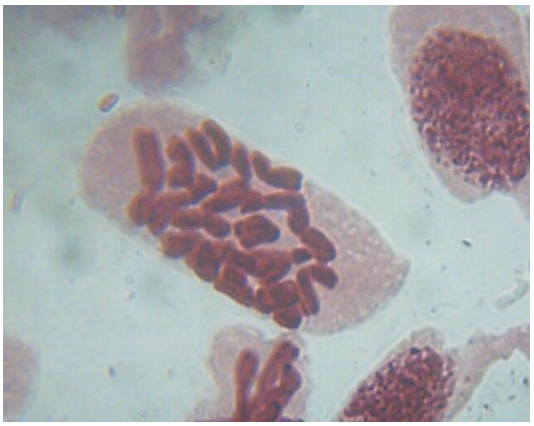
(3) Bridge Anaphase (0.6g/ml *G. lucidium*)



(4) Sticky Telophase (0.6g/ml *G. lucidium*)



(5) Vagrant Chromosome (1.25g/ml *P. ostreatus*)



(6) C-mitosis (1.75g/ml *P. ostreatus*)

Plates 1 - 6. Photomicrographs of microscopic/genotoxic effects of extracts of *Ganoderma lucidium* and *Pleurotus ostreatus* on *Allium cepa* root tips

This genotoxic abilities of the two macro fungi may be useful in the fight against micro-organisms that cause gastrointestinal disorders and even cancers. For instance, c-mitosis is indicative of a weak toxic effect which may be reversible; vagrant chromosomes are weak c-mitotic effects indicating the risk of aneuploidy; while sticky chromosomes indicate a high toxic, irreversible effect, probably leading to cell death (34, 35, 36). Furthermore, stickiness usually leads to the formation of anaphase and telophase bridges, which inhibit metaphase and cytokinesis, during cell division. Stickiness might be due to the ability of the extracts to cause DNA depolymerization and partial dissolution of nucleoproteins, breakage and exchange of the basic folded units of chromatids and the stripling of the protein covering of the DNA in chromosomes (37). The mitotic index (MI) decreased with increase in concentration of both *Ganoderma lucidium* and *Pleurotus ostreatus* extracts. Similar results have been observed for some other mushroom species (12, 38) and genotoxicity evaluations using *Allium* test (14, 19, 38, 39, 40).

Conclusion

In this study, the genotoxic effects of aqueous extracts of *Ganoderma lucidium* and *Pleurotus ostreatus* were detected in *Allium* test. Genotoxicity appeared to be a major mechanism for the two macro fungi to exert cellular disruptive action on the test plant. It would be beneficial to apply such genotoxicity screening to other mushroom species. This will provide information on the action of such extracts in general use as herbal medicines or as alternatives in drug therapy.

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