

CYTO-DEPRESSIVE POTENTIAL OF *GANODERMA LUCIDUM*, *PLEUROTUS OSTREATUS*, AND THEIR BINARY COMBINATIONS AGAINST MERISTEMATIC CELLS OF *ALLIUM CEPA* L.MOYEN UDDIN PK^{1,2,3}

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Received: 16 August 2018, Revised and Accepted: 29 October 2018

ABSTRACT

Objective: The current study is designed to evaluate the cyto-depressive effects of *Ganoderma lucidum* (GL) and *Pleurotus ostreatus* (PO) and their binary amalgams (GL+PO) on meristematic cells of *Allium cepa* L.

Methods: Experiments were assessed by utilizing *A. cepa* bioassay.

Results: Onion knobs were exposed to 500 µg/mL, 1000 µg/mL, and 2000 µg/mL groupings of the concentrates for naturally visible and minute examination. The concentrates essentially repressed the root development of *A. cepa* L. contrasted with the control in a period of treatment and concentration-dependent manner ($p < 0.05$). In addition, photomicrographs revealed chromosomal aberrations extending from c-mitosis, stickiness, and bridges in the root tip meristematic cells of *A. cepa*. All mushroom extracts demonstrated remarkable cyto-depressive impacts on meristematic cells of *A. cepa* L. with some changeability.

Conclusion: In view of the results come out, GL and PO could be considered as potential sources of anticancer mixes. Notwithstanding, additionally thinks about are essential for disengagement and portrayal of the bioactive mixes and more examination on their pharmacological properties is required. It is first report in Bangladesh where we studied cyto-depressive role of edible mushrooms, *Ganoderma lucidum* (GL) and *Pleurotus ostreatus* (PO).

Keywords: Mitosis inhibition, Chromosome aberration, Mitotic index, Hydromethanolic extract, Anticancer.

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INTRODUCTION

Ganoderma lucidum (GL), which is commonly called as "Reishii" in Bangladesh, shows various pharmacological effects, namely antioxidative, antitumor, and antiviral [1-4]. González-Palma *et al.* have been shown that the fruiting bodies of *Pleurotus ostreatus* (PO) have exhibited signal antioxidant activity [5].

Recently, we have found that a numerous higher and medicinal plants have exerted antimitotic effects *in vivo* and *in vitro* assays, and these findings authenticated based on their content of potential mutagenic substances [6-8]. Few studies have been carried out including cyto-depressive of mushrooms [9] and no record found on cyto-depressive study on the binary combination of GL and PO.

In this study, we have evaluated the binary combinations of GL and PO for its cytotoxic effects on *Allium cepa* L. root tip meristematic cells at different concentrations of mushroom extracts. This is the first report in Bangladesh where the cyto-depressive activity of GL, PO, and their binary combinations was reported.

METHODS**Mushrooms collection and preparation of extracts**

Edible mushrooms, GL and PO, were collected from Mushroom Development Institute belong to the Department of Agriculture Extension, Ministry of Agriculture, Bangladesh, Savar, Dhaka-1340. Mushrooms were identified and authenticated by Research officer from Mushroom Development Institute, Bangladesh. About 250 g of powder of each mushroom was shocked in 80% methanol and was kept at 25°C for 7 days in the shade. Fractions were collected, concentrated, and dried and were stored in the refrigerator for future uses.

Dose preparation

The hydromethanolic extracts of GL, PO, and their combinations were used to screen cyto-depressive effects with three different concentrations, namely 500, 1000, and 2000 µg/ml, for *A. cepa* assay. Copper sulfate (PubChem CID: 24463) was used as a standard compound in *Allium cepa* test. At room temperature, the *A. cepa* bulbs were grown in tap water.

Sample application

After 3-4 days, when the roots were 2-5 cm in length, the bulbs were treated with mushrooms extract, copper sulfate, and distilled water. Total 195 onions comprising 2-5 cm root length were used in this experiment for 24, 48, 72, 96, and 120 h of consecutive treatments. Group 1: Used 9×5 onions for the treating with positive control (C+), 500, 1000, and 2000 µg/ml, Group 2: Used 3×5 onions for negative control (C-), 2000 µl, Group 3: Used 9×5 onions for GL, 500, 1000, and 2000 µg/ml, Group 4: Used 9×5 onions for PO, 500, 1000, and 2000 µg/ml, and Group 5: Used 9×5 onions for GL+PO, 500, 1000, and 2000 µg/ml. Solutions of each treatment were changed with appropriate concentrations (500, 1000, and 2000 µg/ml).

Pre-treatment

Root tips from every treatment were gathered and settled in a solution of acidic acid and alcohol (Carnoy's fixative; 1:3 of acidic acid and ethanol). After specific treatment, knobs from each group were protected in 70% alcohol. In the wake of washing the root tips (utilizing refined water), root knobs were hydrolyzed with 1 N HCl at 65°C for 5 min, and after that, bulbs were washed once more.

Slides preparation

Around 1-2 mm of the bulbs were cut and set on the 10 slides for every treatment. Thereafter observing root length enlargement for each

group, 2000 µg/ml concentration-treated groups were chosen for slide arrangement since at this dosage (after 48 h), no root development was watched. At last, root tip was submerged in aceto-orcein and kept for 3 min and, at that point, compressed the root tips utilizing the metal pole. Afterward, 50 µl of aceto-orcein was included for appropriate recoloring of cell core.

Microscopic observations

Slides were seen under a light magnifying instrument at ×400 to see clear picture of the chromosome deviations and least 100 cells for every slide were counted (Nine slides watched every treatment). The mitotic index (MI) was learned by detaching the amount of cells in mitosis with the total number of cells [10]. All analyses in this examination were completed in triplicate.

Statistical analysis

GraphPad Prism 6 (Pad Software, Inc., USA) was used to analyze data, and results are expressed as mean ± standard deviation. Error bars were expressed as 95% CI. $p < 0.05$ was measured to specify statistical significance connotation.

RESULTS

MI calculation

Table 1 shows MI, which is the measure of cytotoxicity in all living organisms. The level of cytological effects can be calculated by assessing the decreased rate of MI. The MI of mushroom extracts was concentration-dependent manner and the lowest MI value was found at 2000 µg/ml treated with mushrooms and standard compound. The MI for GL+PO extract declined significantly ($p < 0.001$) from 500 µg/ml to 2000 µg/ml. The MI values were 0.126, 0.177, and 0.240, respectively,

Table 1: Mitotic index of control and different treatments

Treatments		Total cells±SE	Dividing cells±SE	Mitotic index±SE
C+	500 µg/ml	1022±9.31	236±9.22	0.230±2.00 [#]
	1000 µg/ml	1139±10.71	254±10.01	0.223±1.08 [#]
	2000 µg/ml	1105±7.90	158±11.34	0.142±1.47**
C-	2000 µl	1239±12.34	463±7.89	0.372±1.91
GL	500 µg/ml	987±15.11	184±9.00	0.187±2.03*
	1000 µg/ml	1046±14.33	190±7.19	0.182±1.48*
	2000 µg/ml	1158±17.91	205±8.02	0.177±2.74*
PO	500 µg/ml	976±9.03	262±12.11	0.268±2.00
	1000 µg/ml	1028±10.02	256±15.01	0.249±2.01
	2000 µg/ml	1035±8.27	249±13.70	0.240±1.46
GL+PO	500 µg/ml	1065±11.30	158±7.02	0.149±1.46*
	1000 µg/ml	1133±12.72	155±9.02	0.137±2.01*
	2000 µg/ml	1089±9.23	138±6.02	0.126±1.09**

C+: Positive control (CuSO₄), C-: Negative control (distilled water), GL: *Ganoderma lucidum*, PO: *Pleurotus ostreatus*, GL+PO: Binary combination. Three fields were counted for calculating a total number of cells. Nine slides for each treatment×100 cell in each slide. * $p < 0.05$, ** $p < 0.01$ (treatments vs. distilled water), [#]Non-significant

for GL+PO, GL, and PO at a concentration of 2000 µg/ml, whereas the MI value of positive control (C+) and distilled water (C-) was calculated with 0.142 and 0.372 at the same concentration. The column statistics of MI of control and treatments are shown in Table 2. Column statistics explicate that cytological effects of mushrooms on root tips of *A. cepa* depend on the concentrations of the sample ($p < 0.05$).

Chromosome aberrations analysis

Chromosome aberrations and incidences of chromosome aberrations encouraged by the treatment of mushrooms extract (Fig. 1) and obtained data revealed that the frequencies of chromosomal aberrations were increased with dose-dependent treatments. At 2000 µg/ml, stickiness, bridges, and c-mitosis of the chromosome were the most common aberrations observed (Fig. 1a-d). The stickiness of chromosome was found to be the most frequent aberration obtained for all treatments. The total percentage of chromosomal aberration of C+ was calculated with 4.34% while 4.14%, 3.47%, and 5.14% were found, respectively, for GL, PO, and GL+PO at a concentration of 2000 µg/ml (Fig. 1e).

Physicochemical analysis

Table 3 shows that mushroom extracts exhibit significant inhibition of *A. cepa* roots tip growth in compared to negative and positive control. The cyto-depressive effects of treatments depend on the increasing concentration of mushrooms extract and duration of each treatment. The average root lengths of negative control (2000 µl) and positive control (2000 µg/ml) were 2.71 cm and 3.01 cm, respectively, at day 0 of treatment. After 48 h of treatment, root length was increased by negative control with 44.58% and positive control with 3.5%, whereas GL with 7.6%, PO with 3.62%, and 2.86% for binary combination of GL and PO were found correspondingly. Subsequent treatment of *A. cepa* root tip with 72 h or more, increasing of root length was terminated by positive control and GL+PO ($p < 0.05$). From Fig. 2, survival of the meristematic root cells was affected by concentrations and durations of treatment. Meristematic cells division was terminated by mushrooms at 2000 µg/ml, 1000 µg/ml, and 500 µg/ml, respectively, after 72 h, 96 h, and 120 h correspondingly. During termination of cell divisions, *A. cepa* roots color was changed to brownish from white appearance of roots. Results are shown in Fig. 2a-d.

DISCUSSION

Here, we studied cytological effects of edible mushrooms at a defined concentration of mushroom extracts on fast-growing cells (*A. cepa* root tip) as like of cancer cells, and these were evaluated by analyzing root growth and root morphology. The higher concentration of treatments exhibited an inhibition of root length, and the result obtained was statistically significant differences when compared with negative control. Cytotoxicity was determined by detecting chromosomal aberrations as like stickiness, bridges, c-mitosis, micronucleus, and MI. The MI of *A. cepa* roots tip treated with 2000 µg/ml of GL+PO was reduced significantly in comparison to negative control (0.126% for GL+PO and 0.372% for negative control) while significant inhibition of root growth by copper sulfate was documented after 48 h of treatment (0.142% for

Table 2: Column statistics of MI values of control and treatments

Descriptive statistics	C-	C+	GL	PO	GL+PO
25%	0.349	0.142	0.177	0.24	0.126
Median	0.36	0.223	0.182	0.249	0.137
10%	0.349	0.142	0.177	0.24	0.126
75%	0.372	0.23	0.187	0.268	0.149
90%	0.372	0.23	0.187	0.268	0.149
Lower 95% CI of mean	0.3318	0.07683	0.1696	0.2168	0.1088
Upper 95% CI of mean	0.3889	0.3198	0.1944	0.2878	0.1659
Discrepancy	-0.3603	-0.1983	-0.182	-0.2523	-0.1373
95% CI of discrepancy	0.3318-0.3889	0.07682-0.3198	0.1696-0.1944	0.2168-0.2878	0.1088-0.1659
p value (two-tailed)	0.0003	0.0197	0.0003	0.0011	0.0023

C+: Positive control (CuSO₄), C-: Negative control (distilled water), GL: *Ganoderma lucidum*, PO: *Pleurotus ostreatus*, GL+PO: Binary combination, MI: Mitotic index, CI: Confidence interval

Table 3: The average root numbers and root lengths in controls and treatment concentrations

Concentration (µg/ml)	Average root (mean±SD)	Average root lengths (mean±SD)				
		Day 0	Day 2	Day 3	Day 4	Day 5
C+						
500	28.4±7.89	3.34±2.98	3.56±0.75	3.73±0.71	3.83±0.83	-
1000	25.8±5.65	2.98±2.76	3.00±0.87	3.17±0.69	-	-
2000	22.4±3.46	3.01±1.34	3.12±0.91	-	-	-
C-						
(2000 µl)	27.6±6.89	2.71±2.45	4.89±1.87	5.11±1.22	5.43±1.45	5.69±1.80
GL						
500	26.6±3.56	2.84±1.92	2.89±0.82	2.96±0.72	3.07±0.87	3.23±0.67
1000	23.9±2.98	3.15±4.98	3.21±0.65	3.42±0.66	3.53±0.64	-
2000	29.3±5.87	2.67±2.56	2.89±0.93	-	-	-
PO						
500	25.6±2.46	2.74±1.08	2.79±0.80	2.83±0.84	2.92±0.86	3.11±0.84
1000	28.9±2.09	3.25±4.33	3.32±0.68	3.56±0.57	3.62±0.34	-
2000	24.3±4.11	2.66±2.59	2.76±0.91	2.86±0.82	-	-
GL+PO						
500	26.6±2.99	2.64±2.02	2.69±0.63	2.74±0.53	2.81±0.47	-
1000	27.9±2.04	3.85±3.90	3.89±0.81	3.94±0.87	-	-
2000	23.3±5.01	2.37±2.05	2.44±1.03	-	-	-

C+: Positive control (CuSO₄), C-: Negative control (distilled water), GL: *Ganoderma lucidum*, PO: *Pleurotus ostreatus*, GL+PO: Binary combination, -: Indicates no root length increased, SD Standard deviation

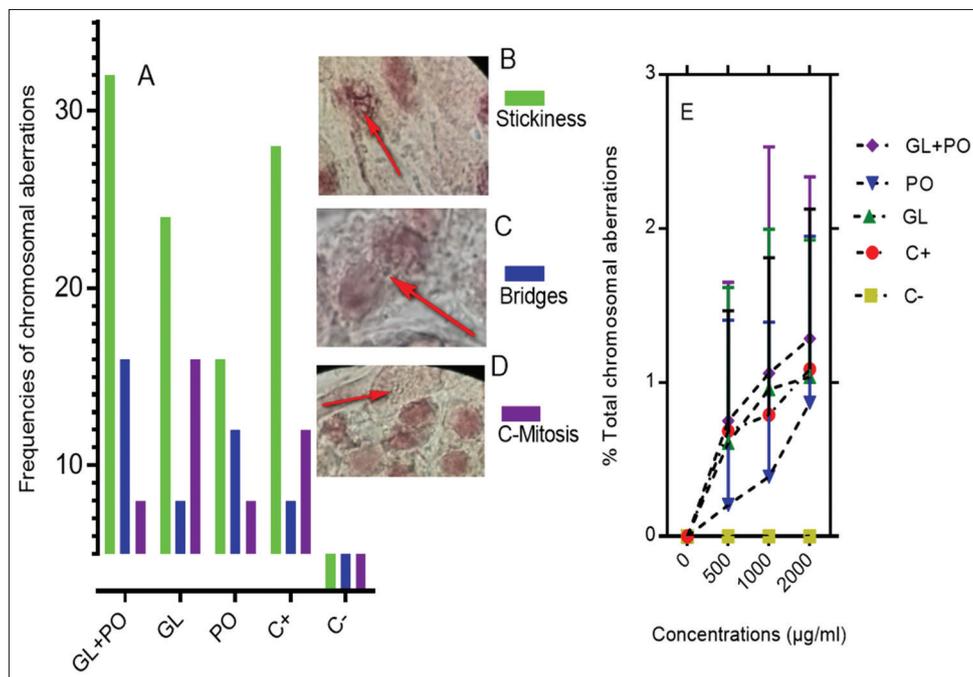


Fig. 1. Chromosomal aberrations - (a) Frequencies of chromosomal aberrations, (b) stickiness of chromosome, (c) bridges of chromosome, (d) c-mitosis, disturbance of the spindle activity, (e) percentage of the total chromosomal aberration. Three fields were totaled for computing of total number of chromosomal aberrations. Nine slides for each treatment × 100 cell in each slide. *C+ positive control (CuSO₄), C- negative control (distilled water), GL: *Ganoderma lucidum*, PO: *Pleurotus ostreatus*, GL+PO: Binary combination

2000 µg/ml, 0.223% for 1000 µg/ml, and 0.230% for 500 µg/ml). It is demonstrated that the decreased of MI below 22% in comparison to negative control can have a lethal impact on the living organisms, on the other hands declined value <50% usually has sublethal effects, and it is called by cytotoxic limit value [11-13].

From Rojas *et al.*, 1993, it is clear that MI measures the cellular death or delay in the cell proliferation kinetics [14] and declining in the mitotic activity could be due to termination of DNA synthesis or a hindering in the G phase of cell cycle resulting inhibiting the cell from entering mitosis [15]. In earlier, cytological effects through blocking the DNA synthesis of some herbal extracts were reported by Chen and Watkins [16] and Hoffmann *et al.* [17] likewise cytological effects

of chemicals [18-20]. Other studies have been reported that there are several herbals inhibit mitosis as well [21]. In our studies, the decreased MI values of *A. cepa* roots tip explicate the interaction of mushrooms extract with cell DNA, resulting cell division and root growth became inhibited and also terminated at define time and concentration of treatment. Mushroom extracts, a binary combination of GL and PO, revealed the strongest mitosis inhibition effects in the root meristem cells by observing of stickiness, bridges of chromosome emphasize the premise of the toxic effects on fast-growing cells. The physicochemical properties of DNA, protein, or both have changed due to the effect of the environmental pollutants and chemical compounds causing sticky chromosome [22]. Our results presented the initiation of chromosome aberrations in the *A. cepa* root tip cells treated with the mushroom

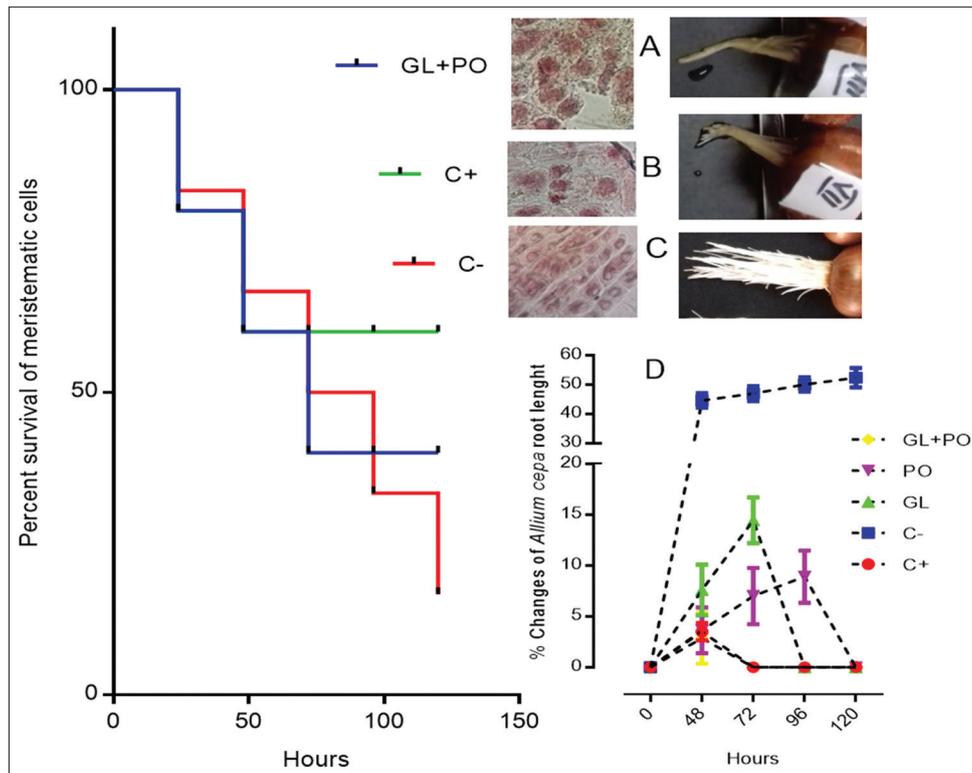


Fig. 2: Survival curve of meristematic cells (root tip cells) - (a and b) increasing of roots length was terminated by GL+PO and positive control; (c) distilled water - no effects on cell division, and (d) percent changes of *Allium cepa* roots length. *C+: Positive control (CuSO_4), C-: Negative control (distilled water), GL: *Ganoderma lucidum*, PO: *Pleurotus ostreatus*, GL+PO: Binary combination

extracts. Chromosome aberrations in roots tip cells increased significantly on experience to mushrooms extract, which designate mutagenic activity (Tables 1-3 and Figs. 1 and 2). These findings are relevant to the results of many scientific researches which examined the cytological effects of diverse herbal medicines [23].

CONCLUSION

The binary combinations of GL and PO extracts induced cytotoxicity (Mitosis inhibition) in *A. cepa* root tip meristematic cells development. Henceforward, the mix blend of GL and PO extricates is a good wellspring of medications to treat ceaseless illnesses.

ACKNOWLEDGMENT

Md. Moyen Uddin PK (MPK) is grateful to Primeasia University for providing necessary laboratory facilities for this work. The author is thankful to Rabiul Islam Talukder (RIT, Department of Biochemistry), Mohammad Sayful Islam (MSI, Department of Pharmacy), Primeasia University, Bangladesh, and Mohammad Khairul Islam Sarker, Independent University of Bangladesh, for technical and laboratory support.

AUTHORS' CONTRIBUTION

MPK conceived, designed, data analyzed, wrote original draft, and finally revised manuscript. MSI and RIT helped as volunteer researcher(s) during the period of work.

CONFLICTS OF INTEREST

The authors have declared that no competing interest exists.

FUNDING

The authors received no specific funding for this work.

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