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Research Article

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Antigenotoxic potential of the aqueous basidiocarp extracts of *Calocybe indica*

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ABSTRACT

Calocybe indica, commonly known as the milky white mushroom, is an edible mushroom native to India. The antigenotoxic potential of the aqueous basidiocarp extract (25 g/L and 50 g/L) of *C. indica* was investigated using the *Allium cepa* test system. Meristematic cells of root tips treated with 100% Coca-Cola served as a positive control. A significant reduction in the mitotic index (7.83 ± 0.60) was observed in the positive control (100% Coca-Cola) compared to the negative control. The highest percentage (65.25 ± 4.58) of chromosomal abnormalities was observed in the positive control. Chromosomal abnormalities were significantly reduced in root tip cells treated with Coca-Cola followed by treatment with basidiocarp extracts such as 25 g/L and 50 g/L (30.32 ± 4.44 and 14.20 ± 2.41, respectively). Coca-Cola induced chromosomal abnormalities were reduced by treatment with basidiocarp extracts, demonstrating the antimutagenic potential of *C. indica*. The present study indicates that the aqueous basidiocarp extracts of *C. indica* have anti-genotoxic effects. The clastogenic abnormalities caused by Coca-Cola are competently restored in the root meristem cells of *A. cepa* treated with the extract.

KEYWORDS: *Allium cepa*, *Calocybe indica*, Chromosome abnormalities, Coca-Cola, Mitotic index

INTRODUCTION

Soft drinks are flavored, non-alcoholic water-based beverages that can be sweetened, acidified, carbonated and flavored with fruit juice and salts. Plant extracts or other fragrant components may contribute to the flavor of these beverages (Majewska *et al.*, 2003; Chandraker *et al.*, 2014). The third most popular beverage is carbonated soft drinks (Tahmassebi & BaniHani, 2020). Soft drinks are produced and consumed on a large scale nowadays. According to Yang *et al.* (2017), the annual per capita consumption of carbonated soft drinks is about four times higher than that of fruit drinks. It is not known what the exact ratio of ingredients and concentration in soft drinks. Genetic problems may result from the higher sugar content and chemical ingredients (Nseir *et al.*, 2010). There are reports on the pesticidal effects of soft drinks, although there is not enough research on this topic (Garcia-Reyes *et al.*, 2008; Castilla-Fernández *et al.*, 2021).

Since the beginning of time, people have regarded mushrooms as a gastronomic marvel and widely utilized them in traditional medicine (Gariboldi *et al.*, 2023). More and more studies are being conducted on mushrooms to determine how they affect the action of environmental genotoxicants (Wasser, 2011). The scientific community has shown tremendous interest in the medicinal potential of mushrooms within the past ten years (Gariboldi *et al.*, 2023). According to De Flora *et al.* (2001) and Patel and Goyal (2012), the main medical applications of mushrooms that have been found thus far include antioxidant, anti-diabetic, hypocholesterolemic, anti-tumor, anti-cancer, immunomodulatory, anti-allergic, nephroprotective, and anti-microbial properties. Unquestionably, the growing body of evidence from numerous research organizations worldwide about the anti-tumor application of mushroom extracts makes this a rapidly developing field of study deserving of widespread attention.

The topic of this communication is the anti-genotoxic properties of aqueous extracts from *Calocybe indica*, an edible fungus, also referred to as milky white mushroom. It is rich in protein, fiber, calcium, phosphorus and iron (Subbiah & Balan, 2015; Nagaraj *et al.*, 2021). In addition, the mushroom is medicinally beneficial due to its diverse secondary metabolites (Sumathy *et al.*, 2015). Ghosh (2015) and Gurunathan *et al.* (2015) reported the anti-cancerous properties of *C. indica*. For the investigation, an *Allium cepa* root tip-based test system was employed. This study, which focuses on the root tips of *A. cepa* plants, is an initial step towards investigating the broader implications of soft drink effects on animal cells.

MATERIALS AND METHODS

Basidiocarp Collection and Extraction

The dried basidiocarp of the edible mushroom *Calocybe indica* Purkay. & A. Chandra, commonly known as milky white mushroom, was purchased from the market and powdered. The

mushroom powder (25 g and 50 g) was extracted with 1000 mL of boiling water for 10 minutes. The extract was filtered with filter paper (Whatman No. 1) and used for the treatment.

Allium Test

Two separate studies were conducted under the same conditions to evaluate the antimitotic and genotoxic effects of Coca-Cola and the antigenotoxic properties of *C. indica* extracts. In the first experiment, two treatments were carried out. Eight commercial onions (*Allium cepa*) weighing between three and four grams were used for each treatment. They were properly decalcified, placed on test tubes containing distilled water and left to germinate in the dark. For each treatment, they were removed after 24 hours. The bulbs were then placed in test tubes containing two different concentrations of *C. indica* extract (BC1 - 25 g/L (*C. indica* basidiocarp extract 25 g/L) and BC2 - 50 g/L (*C. indica* basidiocarp extract)) and left for 24 hours. After 24 hours, the roots were washed and the bulbs were placed on test tubes filled with water for 24 hours.

Sixteen identical-sized, commercially available *A. cepa* bulbs weighing 3-5 g were used for the second experiment (8 for each treatment). Following a thorough deshelling process, they were put on test tubes with distilled water inside and given a full day to germinate in the dark. After a day, two bulbs with underdeveloped roots were taken out of each treatment. For an hour, the remaining bulbs were treated with 100% Coca-Cola. Following the Coca-Cola treatment, the bulbs were exposed to two different extract concentrations (25 or 50 g/L; CBC1 (Coca-Cola treatment followed by BC1) and CBC2 (Coca-Cola treatment followed by BC2)) for a full day. Next, the roots were cleansed. After that, the bulbs were left on the water-filled test tubes for 24 hours.

Onion bulbs germinated in distilled water for 72 hours and then treated with 100% Coca-Cola for one hour served as a positive control (C+ve), while onion bulbs germinated in distilled water for 72 hours served as a negative control (C–ve). Following the 72-hour treatment period, each bulb's root length was measured, and the number of roots was counted. The roots were fixed using Carnoy's solution, which is ethanol and acetic acid mixed in a 3:1 ratio. Following fixation, the roots underwent a light microscope examination after being crushed with 2% acetocarmine, and hydrolyzed in 1 mol/L HCl at 60 °C for a minute. The ratio of divided cells to total cells counted was used to express the mitotic index. Cells exhibiting clumps, bridges, giant cells, bands, lesions, and tropokinesis were scored in four randomly chosen zones per slide to identify chromosomal aberrations. For every treatment group, four slides containing eight onion bulbs were examined.

Statistical Analysis

Data on different chromosomal abnormalities, root number, root length, and mitotic index were subjected to statistical analysis. A one-way analysis of variance was used to determine the significance of the treatments (ANOVA). Using SPSS 20.0 software, the mean separation was carried out by Duncan's multiple range test (DMRT) ($P < 0.05$).

RESULTS AND DISCUSSION

The present investigation assessed the cytotoxic and antimitotic potential of the soft drink Coca-Cola and the anti-cytotoxic effect of the aqueous extracts (25 g/L and 50 g/L) of the edible mushroom *C. indica*. The anti-cytotoxic potential of extracts was evaluated using the *A. cepa* test system. The results obtained reflect the antigenotoxic activities of *C. indica* on *A. cepa* root cells.

Root length and number were significantly affected ($P < 0.001$) by extracts of *C. indica* at concentrations of 25 and 50 g/L. For both the positive and negative controls, the mean root numbers were 7.66 ± 0.58 and 22.33 ± 1.20, respectively. The average root length was determined to be 1.89 ± 0.49 cm for the positive control and 0.86 ± 0.03 cm for the negative control, respectively. The number and length of roots differed significantly ($P < 0.001$) between the treatment and control groups (Table 1).

The average root length in the treatment groups BC1, BC2, CBC1 and CBC2 (treatments with basidiocarp extract following Coca-Cola treatments) was significantly ($P < 0.001$) greater than that of the positive control. For BC1, BC2 and the negative control, there were no significant differences in the mean root length of the treatment groups (Table 1). The reduction in root length and root number in the positive control indicates the antimitotic effect of Coca-Cola.

Table 2 indicates that Coca-Cola had a significant ($P < 0.001$) reduction in the mitotic index. In onion root cells treated with Coca-Cola (C+), CBC1 and CBC2, the mitotic index (MI) values were significantly ($P < 0.001$) lower than the MI of the negative control. This implies that Coca-Cola suppresses the mitotic activity of *A. cepa*. The decrease in mitotic index is caused by the ingredients in Coca-Cola, which have cytotoxic effects in turn. There was no significant difference in the mitotic index between the negative control and treatments with the aqueous extracts (25 and 50 g/L) of *C. indica* (Table 1).

Genotoxicity assay revealed many clastogenic aberrations in interphase, prophase, metaphase and anaphase of root tips of

Table 1: Root length and root number of onion bulbs in control and extract-treated groups

Treatments	Average root number ± SE	Average root length (cm) ± SE	Mitotic index ± SE
C–ve	22.33 ± 1.20^{d}	1.89 ± 0.49^{d}	21.0 ± 0.58^{c}
C+ve	7.66 ± 0.58^{a}	0.86 ± 0.03^{a}	7.83 ± 0.60^{a}
BC1	19.67 ± 0.88^{c}	1.82 ± 0.44^{d}	20.08 ± 0.19^{c}
BC2	20.33 ± 0.33^{cd}	1.79 ± 0.02^{d}	19.64 ± 0.23^{c}
CBC1	13.67 ± 0.88^{b}	1.17 ± 0.23^{c}	14.7 ± 0.72^{b}
CBC2	14.67 ± 0.88^{b}	1.02 ± 0.04^{b}	15.63 ± 0.71^{b}

C–ve - negative control; C+ve - positive control (100% Coca-Cola); BC1-25 g/L *C. indica* Basidiocarp extract; BC2-50 g/L *C. indica* Basidiocarp extract; C 100% Coca-Cola; $P < 0.001$. Means within column followed by the same letters are not significantly ($P<0.05$) different as determined by DMRT.

A. *cepa* when treated with the Coca-Cola. The aberrations were found to be significantly higher ($P < 0.001$) in the positive control (67.25 ± 4.58) than in the negative control (5.74 ± 1.87) and other treatments (Table 2). The negative control showed normal mitotic divisions. The clastogenic abnormalities observed included chromosome clumping, chromosome bridges, strap shaped nuclei, giant cells, nuclear lesions and tropokinesis (Figures 1a-1f). Nuclear lesions, giant cells and strap shaped nuclei were the interphase aberrations. Nuclear lesions and chromosome bridges were observed in the prophase and anaphase respectively. Chromosome clumping and tropokinesis were observed in the metaphase. Telophase aberrations were not observed. The aqueous extract of *C. indica* reduced a significant ($P < 0.001$) number of clastogenic aberrations (Table 2).

Several studies have been published to evaluate the antigenotoxicity and antimutagenicity using the A. *cepa* test system in the last years (Prajitha & Thoppil, 2016; Chandra *et al.*, 2022), and the current one demonstrates how effective this test technique is in confirming the protective effects of substances and natural compounds. In all living organisms, the mitotic index is a reliable quantitative measure of cytotoxicity (Smaka-Kincl *et al.*, 1996; Sreeranjini & Siril, 2011). Treatment with Coca-Cola showed a mitodepressive effect on cell division similar to previous works (Chandraker *et al.*, 2014; George & George, 2017; Bonciu *et al.*, 2022). The reduction in MI implies that Coca-Cola suppresses mitotic activity in A. *cepa*. The reduced rate of MI can be used to calculate the cytotoxicity level (Prajitha & Thoppil, 2016). A lower amount of ATP to supply energy for spindle elongation, microtubule dynamics, and chromosomal movement, or poor nucleoprotein synthesis, could be the cause of reduced mitotic activity (Sudhakar *et al.*, 2001; Majewska *et al.*, 2003).

A chromosomal break or exchange can result in chromosomal aberrations, which are changes in the chromosome structure. While most chromosomal abnormalities found in A. *cepa* meristem cells are fatal, some corresponding aberrations can still be viable and can have genetic effects (Çelik & Aslantürk, 2007). Prophase aberrations were the most prevalent type of anomaly observed in A. *cepa* cells. Its anti-mutagenic potential on the A. *cepa* test system is demonstrated by the percentage

Table 2: Chromosomal abnormalities in controls and extract-treated root tips of *Allium cepa*

		Aberrant Cells ± SE					
Treatments	Dividing cells ± SE	Interphase	Prophase	Metaphase	Anaphase	Telophase	Percentage of Aberration ± SE
C–ve	46.0 ± 1.15^{d}	2.0 ± 0.58^{a}	0.67 ± 0.33^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	5.74 ± 1.87^{a}
C+ve	34.67 ± 1.45^{c}	8.67 ± 0.88^{c}	4.67 ± 1.20^{b}	6.0 ± 0.58^{d}	6.0 ± 0.58^{c}	0.0 ± 0.0^{a}	67.25 ± 4.58^{c}
BC1	42.67 ± 0.88^{cd}	1.33 ± 0.58^{ab}	1.0 ± 0.57^{a}	0.0 ± 0.0^{a}	0.33 ± 0.33^{ab}	0.0 ± 0.0^{a}	6.23 ± 0.67^{a}
BC2	42.0 ± 1.15^{c}	0.0 ± 0.0^{a}	0.67 ± 0.33^{a}	0.67 ± 0.33^{ab}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	7.91 ± 0.60^{a}
CBC1	16.67 ± 0.67^{a}	1.33 ± 0.33^{ab}	0.0 ± 0.0^{a}	2.33 ± 0.33^{c}	1.33 ± 0.33^{ab}	0.0 ± 0.0^{a}	30.32 ± 4.44^{b}
CBC2	16.67 ± 1.20^{a}	1.33 ± 0.33^{ab}	0.0 ± 0.0^{a}	1.33 ± 0.33^{bc}	1.67 ± 0.47^{b}	0.0 ± 0.0^{a}	14.20 ± 2.41^{a}

C–ve - negative control; C+ve - positive control (100% Coca-Cola); BC1-25 g/L *C. indica* basidiocarp extract; BC2-50 g/L *C. indica* basidiocarp extract; C+ve 100% Coca-Cola; $P < 0.001$. Means within column followed by the same letters are not significantly ($P<0.05$) different as determined by DMRT

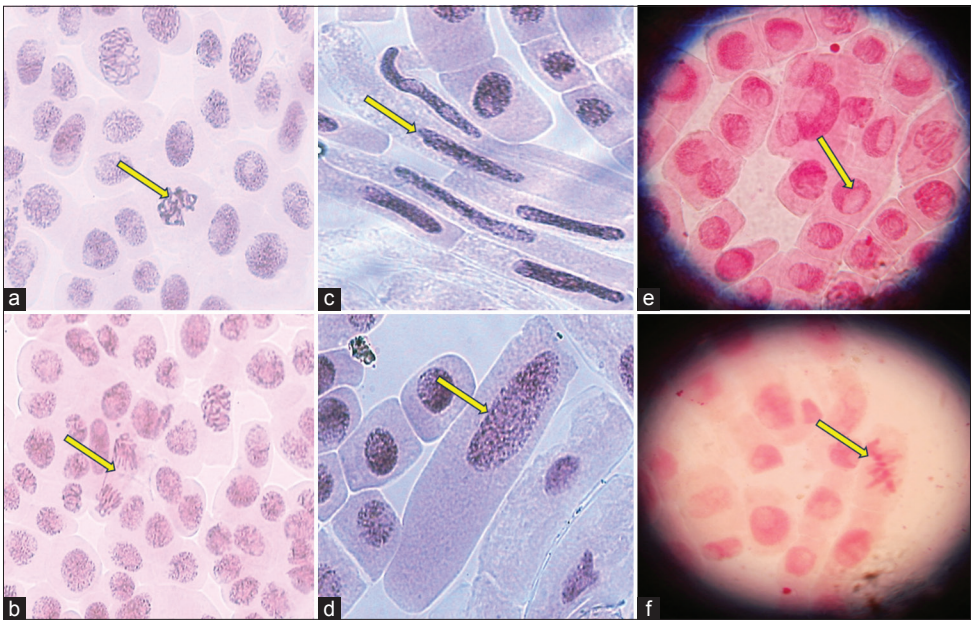

Figure 1: Mitotic aberrations in *Allium cepa* root cells after treatment with Coca-Cola. a) Chromosome clumping in metaphase, b) Chromosome bridge in anaphase, c) Strap shaped nucleus in interphase, d) Giant cell in interphase, e) Nuclear lesionin prophase and f) Tropokinesis in metaphase

of mitotic aberrations suppressed by *C. indica* extract on Coca-Cola produced chromosomal abnormalities in all groups. There was a statistically significant ($P < 0.001$) reduction in the overall number of aberrations caused by Coca-Cola as a result of the *C. indica* extract. According to the current investigation, *C. indica* extract significantly reduces the mutagenic potential of Coca-Cola in cells.

CONCLUSION

Since Cola soft drinks are very popular worldwide and can be harmful to health. The issue can occur when drinking becomes routine in daily life, which sadly many people do. The present investigation suggests the use of *C. indica* as a dietary component can reduce the harmful genotoxic effects of Cola soft drinks.

REFERENCES

Bonciu, E., Liman, R., & Ciğerci, İ. H. (2022). Assessment of cytotoxicity of a caffeinated soft drink using Allium Assay. *Annals of the University of Craiova-Agriculture Montanology Cadastre Series, 52*(1), 43-50. https://doi.org/10.52846/aamc.v52i1.1312

Castilla-Fernández, D., Moreno-González, D., Gilbert-López, B., Garcia-Reyes, J. F., & Molina-Díaz, A. (2021). Worldwide survey of pesticide residues in citrus-flavored soft drinks. *Food Chemistry, 365*, 130486. https://doi.org/10.1016/j.foodchem.2021.130486

Çelik, T. A., & Aslantürk, Ö. S. (2007). Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. *Biologia, 62*, 292-296. https://doi.org/10.2478/s11756-007-0051-2

Chandra, N., Gupta, S., Sirohi, A., Sengar, R. S., Gangwar, L. K., Singh, B., & Ahmad, M. (2022). Analysis of Antigenotoxicity induced by Tulsi extract on *Allium cepa* Grown in Slurry Water. *Biological Forum, 14*(4), 1086-1092.

Chandraker, S. K., Singh, P., & Pandey, B. (2014). Clastogenic effect of soft drink on root tip of *Allium cepa*. *International Journal of Current Microbiology and Applied Sciences, 3*(5), 200-206.

De Flora, S., Izzotti, A., D'Agostini, F., Balansky, R. M., Noonan, D., & Albini, A. (2001). Multiple points of intervention in the prevention of cancer and other mutation-related diseases. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 480-481*, 9-22. https://doi.org/10.1016/S0027-5107(01)00165-8

Garcia-Reyes, J. F., Gilbert-Lopez, B., Molina-Diaz, A., & Fernandez-Alba, A. R. (2008). Determination of pesticide residues in fruit-based soft drinks. *Analytical Chemistry, 80*(23), 8966-8974. https://doi.org/10.1021/ac8012708

Gariboldi, M. B., Marras, E., Ferrario N., Vivona, V., Prini, P., Vignati, F., & Perletti, G. (2023). Anti-Cancer Potential of Edible/Medicinal Mushrooms in Breast Cancer. *International Journal of Molecular Sciences, 24*(12), 10120. https://doi.org/10.3390/ijms241210120

George, M., & George, A. (2017). Genotoxicity of caffeinated soft drinks on meristematic root cells of *Allium cepa*. *International Journal of Innovative Research in Science, Engineering and Technology, 6*(11), 22032-22038.

Ghosh, S. K. (2015). Study of anticancer effect of *Calocybe indica* mushroom on breast cancer cell line and human Ewings sarcoma cancer cell lines. *New York Science Journal, 8*(5), 10-15.

Gurunathan, S., Park, J. H., Han, J. W., & Kim, J. H. (2015). Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by *Bacillus tequilensis* and *Calocybe indica* in MDA-MB-231 human breast cancer cells: targeting p53 for anticancer therapy. *International Journal of Nanomedicine, 10*(1), 4203-4223. https://doi.org/10.2147/IJN.S83953

Majewska, A., Wolska, E., Śliwińska, E., Furmanowa, M., Urbańska, N., Pietrosiuk, A., Zobel, A., & Kuraś, M. (2003). Antimitotic effect, G2/M accumulation, chromosomal and ultrastructure changes in meristematic cells of *Allium cepa* L. root tips treated with the extract from *Rhodiola rosea* roots. *Caryologia, 56*(3), 337-351. https://doi.org/10.1080/00087114.2003.10589343

Nagaraj, A., Wilson, S. A., & Vaidyanathan, L. (2021). Anti-obesity properties of *Calocybe indica* in zebra fishes with short-term high-fat diet induction. *Biomedical and Pharmacology Journal, 14*(1), 411-423. https://doi.org/10.13005/bpj/2141

Nseir, W., Nassar, F., & Assy, N. (2010). Soft drinks consumption and nonalcoholic fatty liver disease. *World Journal of Gastroenterology, 16*(21), 2579-2588. https://doi.org/10.3748/wjg.v16.i21.2579

Patel, S., & Goyal, A. (2012). Recent developments in mushrooms as anti-cancer therapeutics: a review. *3 Biotech, 2*, 1-15. https://doi.org/10.1007/s13205-011-0036-2

Prajitha, V., & Thoppil, J. E. (2016). Genotoxic and antigenotoxic potential of the aqueous leaf extracts of *Amaranthus spinosus* Linn. using *Allium cepa* assay. *South African Journal of Botany, 102*, 18-25. https://doi.org/10.1016/j.sajb.2015.06.018

Smaka-Kincl, V., Stegnar, P., Lovka M., & Toman, M. J. (1996). The evaluation of waste, surface and ground water quality using the Allium test procedure. *Mutation Research/Genetic Toxicology, 368*(3-4), 171-179. https://doi.org/10.1016/S0165-1218(96)90059-2

Sreeranjini, S., & Siril, E. A. (2011). Evaluation of anti-genotoxicity of the leaf extracts of *Morinda citrifolia* Linn. *Plant, Soil and Environment, 57*(5), 222-227. https://doi.org/10.17221/376/2010-PSE

Subbiah, K. A., & Balan, V. A. (2015). Comprehensive Review of Tropical Milky White Mushroom (*Calocybe indica* P&C). *Mycobiology, 43*(3), 184-194. https://doi.org/10.5941/MYCO.2015.43.3.184

Sudhakar, R., Gowda, K. N. N., & Venu, G. (2001). Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. *Cytologia, 66*(3), 235-239. https://doi.org/10.1508/cytologia.66.235

Sumathy, R., Kumuthakalavalli, R., Krishnamoorthy, A. S., & Balan, V. (2015). Effect of phytochemicals and antioxidant compounds enriched extract from *Calocybe indica* var. APK2 on proliferation of human MCF-7 breast carcinoma cells. *Der Pharmacia Sinica, 6*(2), 6-11.

Tahmassebi, J. F., & BaniHani, A. (2020). Impact of soft drinks to health and economy: a critical review. *European Archives of Paediatric Dentistry, 21*, 109-117. https://doi.org/10.1007/s40368-019-00458-0

Wasser, S. P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Applied Microbiology and Biotechnology, 89*, 1323-1332. https://doi.org/10.1007/s00253-010-3067-4

Yang, L., Bovet, P., Liu, Y., Zhao, M., Ma, C., Liang, Y., & Xi, B. (2017). Consumption of carbonated soft drinks among young adolescents aged 12 to 15 years in 53 low-and middle-income countries. *American Journal of Public Health, 107*(7), 1095-1100.