Current Topics in Pharmacology
Vol. 24, 2020

Original Article

Appraisal of time-dependent ROS-mediated mitochondrial damage induced by arsenic and its alleviation by catechin in epithelial cells

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ABSTRACT

Mitochondria play an important role in cellular energetics and balance. High levels of reactive oxygen species induce oxidative stress in living cells. Arsenic is known to induce reactive oxygen species (ROS), and oxidative stress is the major cause of mitochondrial impairment leading to cell death. It also explains the preneoplastic events and cancer of the bladder. Polyphenols are known to forestall chronic disorders. Catechins (flavan-3-ols) are a subgroup of flavonoids. Here in the current study to evaluate the amelioration of arsenate-induced cytotoxicity by catechin, parameters such as ROS, mitochondrial membrane potential (MMP) apoptosis, and DNA damage were considered and evaluated in epithelial cells. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was performed to evaluate cell viability. ROS was measured at different time points (30 min, 1 h, and 2 h) to ensure the quenching activity of catechin. Pre-treatment of the cells with catechin ameliorated arsenic-induced cytotoxicity in epithelial cells and the results were significant ($p < 0.05$).

KEYWORDS: ROS, MMP, SCGE, catechin, arsenate.

INTRODUCTION

The antioxidant activity of flavonoids against oxidative stress is of high interest. Attempts have been made in the current study to attenuate the arsenic-induced oxidative damage. These ROS spill out of the mitochondria into the cell where they induce oxidative harm to a wide assortment of organically significant particles, including DNA, proteins, and polyunsaturated unsaturated fats (PUFAs) on cell boundaries [1]. This metalloid exists in various oxidative states, which includes arsenate and arsenite. Warm-blooded animals can methylate to monomethylarsonic and dimethylarsinic acids [2]. Reactive oxygen species (ROS) play an important causal role in the genotoxicity of arsenic in mammalian cells. Reactive oxygen species, which are generated during metabolic activation of the metalloid, and the ensuing oxidative stress are hypothesized to be critical initiating events in arsenic-induced cytotoxicity [3]. The production of ROS inhibits the activity of antioxidant enzymes and results in oxidative stress [4]. A report also suggests an increase in apoptosis in the experimental model after exposure to sodium arsenate [5]. An increase in DNA oxidation was observed in individuals exposed to arsenic in the environment [6]. Catechin derivative isomers show great potential for use in human health [7]. In this experimental study, the benefits of catechin are explored in managing the adverse effects of arsenate in the epithelial cell. The intracellular ROS levels were evaluated at different time points, and altered MMP, and apoptosis percentage reduction was evaluated.

MATERIALS AND METHODS

Cell culture

CHO cell line was procured from National Centre for Cell Sciences-Pune, India. The cells were grown in medium T-25 flasks (Falcon, Becton Dickinson, USA), provided with Dulbecco's modified eagle medium (DMEM) including 10% fetal bovine serum (FBS), 1% antibiotic, at 37 °C in a carbon dioxide incubator for a known period (NuAire, Plymouth, MN, USA) with 5% carbon dioxide in 95% air. The cells were maintained at 85% confluence in flasks and utilized. Trypan blue assay was used to evaluate the viability of CHO cells.

MTT assay

The toxicity of the arsenate was estimated using MTT assay. 85% of confluent cells were harvested through trypsinization and used at a density of 1 x 10^4 cells per chamber and supplied with arsenate, catechin and a combination of catechin and arsenate to estimate the cytotoxicity. 0.1 mL of dimethyl sulfoxide (DMSO) was mixed at the end to liquify formazan crystals, and reading was taken at 550 nm wavelength [8].

Estimation of superoxide dismutase (SOD)

3 x 10^5 cells were aliquoted in a single well (6 well plate) kept overnight in carbon dioxide incubator at 37 °C, then supplied with sodium arsenate, catechin, and a combination of catechin and arsenate, allowed to stand for 24 h, later cells were harvested in PBS. 1.85 mL of sodium carbonate buffer was mixed with 50 µL of the sample. Further 0.1 mL of epinephrine was mixed and the increase in the absorbance was recorded immediately at 480 nm using a colorimeter [9].

Comet assay

Single strand DNA breaks were quantified by comet assay in an alkaline medium. $7x10^5$ cells were seeded in 6 cm^2 plates. Cells were kept for 24 h, later cells were trypsinized and collected in PBS. The slides were dipped in the lysing solution after covering with suspended cells, maintained at 4 °C overnight, and the lysed cells were subjected to electrophoresis for 28 min (300 mA, 20V). The slides were washed with the neutralizing buffer. Ethidium bromide-stained slides were immediately observed under the fluorescence microscope and photographed, and analyzed using a software [10].

Reactive oxygen species (ROS)

Briefly, $3x10^5$ cells were aliquoted into a Petri plate and allowed to settle overnight in a culture medium. Epithelial cells were exposed to arsenate or antioxidant for 30 min, 1 h, and 2 h. All experiments were performed in quadruplets. After incubation, cells were washed and exposed to 5 µM DCFDA at three different time points [11]. Cells were collected in cold phosphate buffer solution (PBS) and analyzed using flow cytometry.

Mitochondrial membrane potential ($\Delta\Psi m$)

Briefly, $3x10^5$ cells were aliquoted into a Petri plate and allowed to settle overnight in a culture medium. Cells were incubated for 24 h. Epithelial cells were exposed to arsenate and catechin. Rhodamine 123 was supplemented and incubated for 30 min in a carbon dioxide incubator [12]. Cells were harvested in cold PBS and analyzed using fluorescence activated cell sorting (FACS).

Acridine orange/Ethidium bromide dual staining for apoptosis

Briefly, $3x10^5$ cells were aliquoted in to a 6-well plate and allowed to settle overnight in the culture medium. Epithelial cells were treated with arsenate or antioxidant of different concentrations. Acridine orange/EtBr was aliquoted to each well (250 µl) and incubated for 40 min. Post incubation cells were washed with PBS and analyzed under the Fluorescence microscope [13].

RESULTS

MTT assay was employed to estimate the cell metabolic activity in the presence of arsenate. The IC_{50} value of 45 µM was obtained. Reactive oxygen species levels increased in a time-dependent manner. Three-time points were considered for ROS estimation in the presence of arsenate alone and with catechin. 30 min incubation with arsenate alone (260 ± 18), control (50 ± 7), and catechin (152 ± 11) showed a significant decrease in ROS after treatment. 1 h incubation, with arsenate alone (366 ± 18), control (55 ± 7) and with catechin (210 ± 19) showed a significant decrease in ROS after treatment. 2 h incubation, with arsenate alone

(520 ± 18), control (58 ± 7) and with catechin (340 ± 11) showed a significant decrease in ROS after treatment. Catechin caused a time-dependent decrease ($p < 0.001$) in the ROS levels (Fig. 1). Results indicate the biological activity of catechin molecules harmonizing the adverse effect of arsenic.

Mitochondrial membrane potential was recorded using the rhodamine 123 dye. After 24 h incubation the change in the transmembrane potential was evaluated. Catechin-pre-treated cells after exposure to arsenic showed a significant ($p < 0.05$) decrease in the MMP shift in epithelial cells (Fig. 2). SOD activity decreased in the arsenic-exposed group indicating the overproduction of ROS (Fig. 3). Catechin-pre-treated cells showed a significant ($p < 0.01$) increase in SOD activity compared to arsenate-alone-exposed cells.

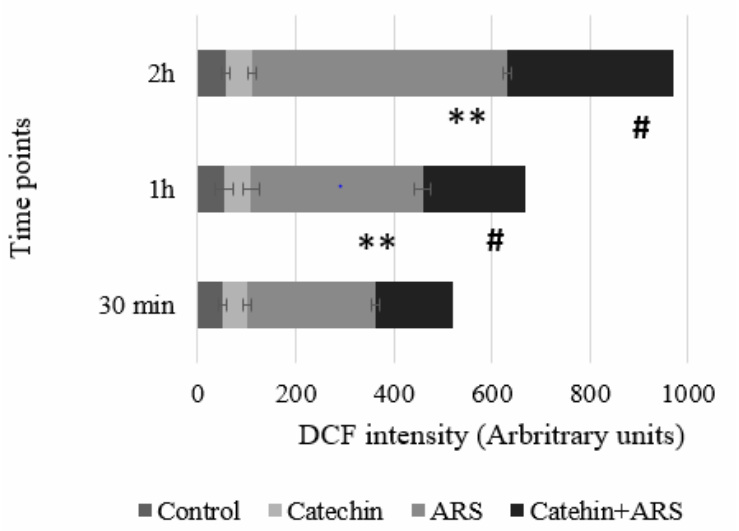

Fig. 1. ROS in epithelial cells, exposed to arsenate and catechin at different time points (30 min, 1 h and 2 h). Results are expressed as Mean ±SE; changes were found to be significant ($p < 0.01$). **$p < 0.01$ compared to the arsenate-alone-treated group. $^{\#}p < 0.05$ compared to the combination of catechin and arsenate.

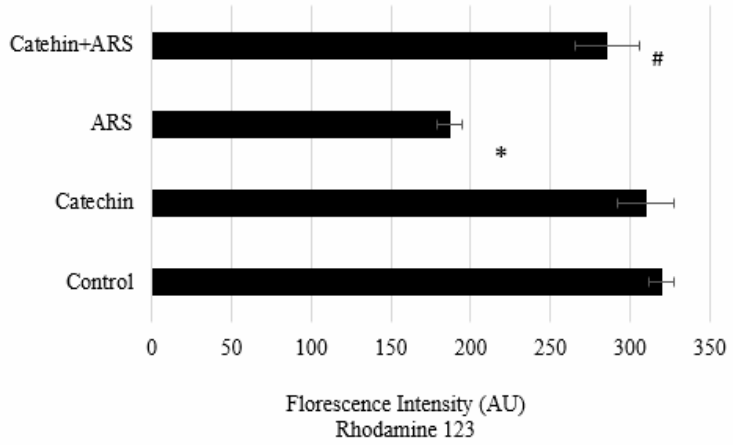

Fig. 2. Mitochondrial membrane potential measurement depending on the rhodamine 123 intensity. Graphical results indicate the shift in the membrane potential in catechin-pretreated cells. *$p < 0.05$ compared to the arsenate-alone-treated group. Results are expressed as Mean ± SE and significant ($p < 0.05$). $^{\#}p < 0.05$ compared to the combination of catechin and arsenate.

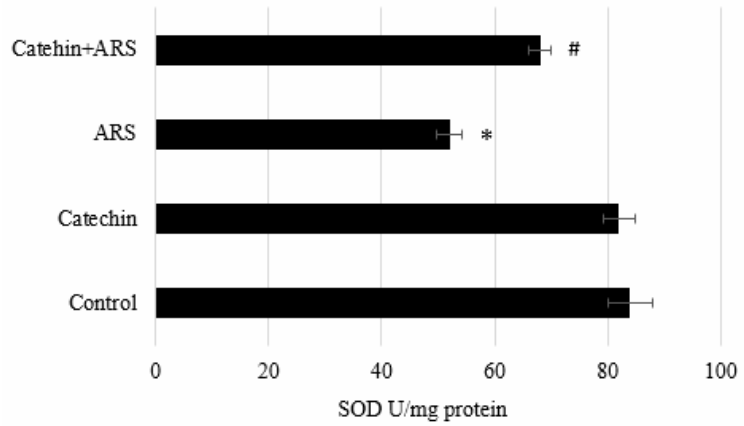

Fig. 3. Super oxide dismutase (SOD) activity restored in catechin-treated group ($p < 0.05$). *$p < 0.05$ compared to the arsenate-alone-treated group. #$p < 0.05$ compared to the combination of catechin and arsenate.

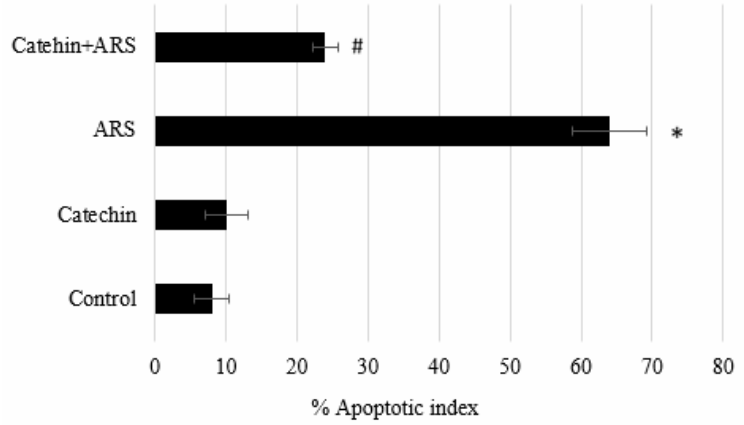

Fig. 4. Acridine orange/Ethidium bromide staining for evaluating percentage apoptosis. Catechin reduced apoptotic cells significantly ($p < 0.05$). *$p < 0.05$ compared to the arsenate-alone-treated group. #$p < 0.05$ compared to the combination of catechin and arsenate.

Acridine orange and ethidium bromide dual marker helped in counting apoptotic cell percentage. Arsenic exposure increased the apoptotic cell population (Fig. 4), but a significant decrease was observed in the percentage of cells undergoing apoptosis in catechin-pretreated cells ($p<0.01$). Percentage tail length in comet assay is a measurement of the extent of DNA damage in cells. Catechin significantly protected the DNA damage in epithelial cells (Fig. 5), when treated prior to arsenic exposure ($p < 0.01$).

DISCUSSION

Catechin exhibits superoxide scavenging activity [14]. Arsenic exposure initiated reactive oxygen species-dependent apoptosis in the hepatocytes involving loss of mitochondrial membrane potential [15]. The current study revealed catechin's role in decreasing the adverse effects of arsenic. ROS was found to be increased in a time-dependent manner. Catechin treatment for 2 h prior to arsenic exposure was found to be effective. All time point (30 min, 1 h, and 2h) experiments showed the ROS quenching role of the catechin. Catechins function as antioxidants in biological systems being prone to oxidation [16]. Electron transfer from catechins plays an important role in singlet-oxygen quenching, free-radical scavenging, and recycling. Catechins showed the highest ABTS•-scavenging capacity [17]. Interaction of antioxidants with the

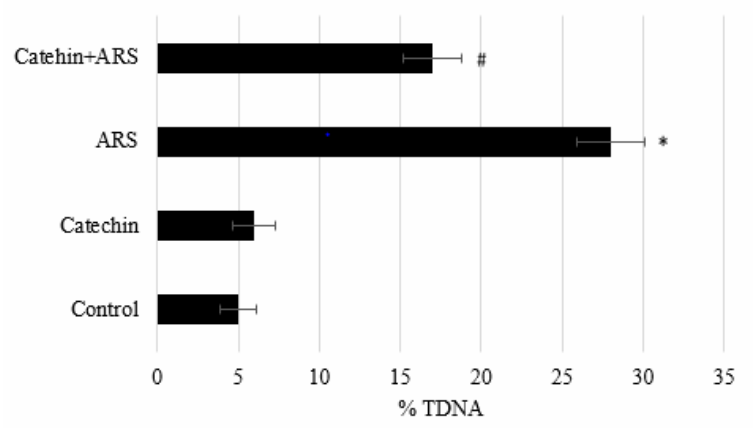

Fig. 5. Single strand breaks of DNA represented as percentage tail DNA. DNA damage reduced significantly in catechin-treated cells ($p<0.05$). *$p < 0.05$ compared to the arsenate-alone-treated group. $^{\#}p < 0.05$ compared to the combination of catechin and arsenate.

membrane surface protects the membrane from attack by oxidants present in the aqueous phase while interaction with both the membrane surface and hydrophobic interior protects the membrane from hydrophilic and hydrophobic oxidants [18]. Our results are similar to those found in previous studies. Catechin ameliorates arsenic-induced toxicity in epithelial cells. Decreased SOD activity in cells exposed to arsenic is due to the overproduction of ROS. The current experimental study indicates the superoxide quenching role of catechin. This behavior can be attributed to the catechol moiety in the catechin molecule [19]. Arsenate is known to induce DNA damage through oxidative stress in cells [20]. Catechin pre-treatment decreased the ROS levels thereby indirectly reducing the single-strand DNA breaks.

CONCLUSION

The results of the present study demonstrate the benefits of catechin in alleviating the toxic effects of arsenate in cells. Catechin also exhibited mito-protective property in epithelial cells. Pre-treatment of catechin resolved the accumulation of excess ROS in cells, indicating the reduction of DNA oxidation in the experimental model. Catechins are widely present in many plants including tea leaf which is a regular beverage. In summary, the dietary supplementation of catechins helps in combating the oxidative stress induced by the metalloid.

ACKNOWLEDGMENTS

The authors are grateful to MAHE for providing the facilities to conduct the study.

CONFLICT OF INTEREST STATEMENT

None.

REFERENCES

1. Phillips, M., Cataneo, R. N., Greenberg, J., Grodman, R., Gunawardena, R. and Naidu, A. 2003, European Respiratory Journal, 21, 48-51.
2. Guillamet, E., Creus, A., Ponti, J., Sabbioni, E., Fortaner, S. and Marcos, R. 2004, Mutagenesis, 19, 129-135.
3. Gong, X., Ivanov, V. N., Davidson, M. M., and Hei, T. K. 2015, Archives of Toxicology, 89, 1057-1070.
4. Kharroubi, W., Dhibi, M., Mekni, M., Haouas, Z., Chreif, I., Neffati, F. and Sakly, R. 2014, Environmental Science and Pollution Research, 21, 12040-12049.
5. Salmataj, S. A., Kamath, S. U., Murty, V. R. and Pai, S. R. 2018, 3 Biotech, 8, 446.
6. Lantz, R. C. and Hays, A. M. 2006, Drug metabolism reviews, 38, 791-804.
7. Hui, C. K., Majid, N. I., Yusof, H. M., Zainol, K. M., Mohamad, H. and Zin, Z. M. 2020, Heliyon, 6, e04337.
8. Sladowski, D., Steer, S. J., Clothier, R. H., and Balls, M. 1993, Journal of immunological methods, 157, 203-207.

9. Dhote, V. and Balaraman, R. 2008, Clinical and Experimental Pharmacology and Physiology, 35, 630-637.
10. Singh, N. P., Danner, D. B., Tice, R. R., Pearson, J. D., Brant, L. J., Morrell, C. H. and Schneider, E. L. 1991, Mutation Research/DNAging, 256, 1-6.
11. James, J., Fiji, N., Roy, D., MG, D. A., Shihabudeen, M. S., Chattopadhyay, D. and Thirumurugan, K. 2015, Analytical Methods, 7, 8572-8575.
12. Vander Heiden, M. G., Chandel, N. S., Williamson, E. K., Schumacker, P. T. and Thompson, C. B. 1997, Cell, 91, 627-637.
13. Elumalai, P., Gunadharini, D. N., Senthilkumar, K., Banudevi, S., Arunkumar, R., Benson, C. S. and Arunakaran, J. 2012, Toxicology letters, 215, 131-142.
14. Grzesik, M., Naparło, K., Bartosz, G. and Sadowska-Bartosz, I. 2018, Food chemistry, 241, 480-492.
15. Choudhury, S., Ghosh, S., Mukherjee, S., Gupta, P., Bhattacharya, S., Adhikary, A. and Chattopadhyay, S. 2016, The Journal of nutritional biochemistry, 38, 25-40.
16. Nagaoka, S. I., Nakayama, N., Teramae, H. and Nagashima, U. 2019, Chemical Physics, 522, 77-83.
17. Chandrasekar, V., Belur, P. D. and Regupathi, I. 2016, Resource-Efficient Technologies, 2, S114-S118.
18. Oteiza, P. I., Erlejman, A. G., Verstraeten, S. V., Keen, C. L. and Fraga, C. G. 2005, Clinical and Developmental Immunology, 12.
19. Chen, D., Wang, C. Y., Lambert, J. D., Ai, N., Welsh, W. J. and Yang, C. S. 2005, Biochemical pharmacology, 69, 1523-1531.
20. Ghaffar, A., Hussain, R., Abbas, G., Ahmad, M. N., Abbas, A., Rahim, Y. and Mohiuddin, M. 2018, Toxin reviews, 37, 206-215.