ANTICANCER ACTIVITY OF COLOCASIA GIGANTEA (BLUME) HOOK. f. IN CULTURED CELL LINES

Nambam Bonika Devi[1], Prof. Ganesh Chandra Jagetia[2]
[1,2]Department of Zoology, Mizoram University, Aizawl, Mizoram, India

ABSTRACT

Evaluation of cytotoxic effects of the ethanol extract of *Colocasia gigantea* (CGE) in *HeLa* and *V79* cells by MTT assay showed a concentrations dependent rise in the cytotoxicity. The maximum cytotoxicity was observed at 300 and 200 µg/ml ethanol extract of *Colocasia gigantea* for HeLa and V79 cells, respectively. Assessment of treatment duration revealed that cytotoxic effect of ethanol extract of *Colocasia gigantea* was marginal increased with treatment duration. Treatment of *HeLa* cells with different concentrations of ethanol extract of *Colocasia gigantea* reduced the clonogenicity of cells in a concentration dependent manner, which reached a nadir at 300 µg/ml. To understand the biochemical mechanism of action, the *HeLa* cells were treated with different concentrations of ethanol extract of *Colocasia gigantea* and contents of glutathione and activities of the glutathione-s-transferase and catalase and lipid peroxidation were determined. The ethanol extract of *Colocasia gigantea* reduced the glutathione concentration and activities of the glutathione-s-transferase and catalase in a concentration and time dependent manner and greatest reduction was observed at 6 h post treatment, whereas lipid peroxidation increased in a concentration and time dependent manner. The ethanol extract of *Colocasia gigantea* induced cytotoxicity and reduced the reproductive integrity of HeLa cells. The cytotoxicity of ethanol extract of *Colocasia gigantean* may be due to elevated lipid peroxidation and reduced concentration of glutathione and glutathione-s-transferase and catalase activities.

Key words: Colocasia gigantea, MTT, clonogenic, GSH, GST and catalase.

INTRODUCTION

Despite availability of state or art treatment regimen cancer still remains one of the leading causes of death in both the developed as well as in the under developed countries [1]. It is estimated that one in every eighth deaths is due to cancer [2]. Cancer is a multifaceted disease and with improving health facilities and increased life span more cancer cases are coming to light than ever before. This has also increased the mortality rates and cancer deaths are of major concern globally[3]. Apart from many synthetic drugs the natural products have also immensely contributed to the paraphernalia of chemotherapeutic drugs. The fact is that 80% of the global population still depend on plants and natural product for their healthcare proves the importance of plants as a major source of medicine. The one third of the all drugs approved by Federal Drug Administration, USA has been natural products [4]. This reemphasizes the importance of plants and natural products in healthcare and new drug discovery. The evaluation of natural products could provide a new breakthrough in cancer treatment and new technologies are being explored for obtaining novel compounds from biodiversity of nature. The pharmaceutical industry has a continuing need to find new and better chemical compounds to develop as drugs for human healthcare [5]. Many drugs used for the treatment of different diseases including cancer are obtained from natural products [6] and plants provide a major platform for design and new drug discovery. About 75 % of the registered small anticancer molecules since the 1940s have drug discover form the complex secondary metabolites

synthesized by plants. Therefore it is necessary to screen diverse plants for their anticancer activity in the hope that there will be a time one it may be possible to come across some biomolecules that will treat cancer effectively with lesser adverse side effects. *Colocasia gigantea* (family Araceae), also known as Giant Elephant ear or Indian taro, is a stemless plant producing large leaves with underground rhizomes. The rhizomes and the stalks are eaten as a vegetable and the leaves are eaten raw with pomegranate in India. In Thailand, *C. gigantea* tubers are heated over fire and consumed as a medicine[7]. It is used to treat drowsiness and to reduce internal heat. The tuber reduces stomach problems, cures infection and heals wounds. Fresh or dries tubers are being used in the treatment of phlegm along with honey [8]. It is also used in the treatment of tuberculosis and constipation in Hawai [9]. Juice of taro are said to arrest arterial hemorrhage[10]. The information regarding the anticancer activity of *Colocasia gigantea* is lacking and it is used as a diet, which indicates that if is found to kill cancerous cells it may be a useful paradigm to fight cancer. Therefore, the present study was undertaken to study the antitumour activity of *Colocasia gigantea* in cultured HeLa cells.

MATERIALS AND METHODS

Chemicals

Doxorubicin was supplied by Getwell Pharmaceuticals, Gurgaon, India. Reduced glutathione (GSH), 1-chloro-2,4-dinitronbezene (CDNB), 5,5'dithio 2-nitrobenzoic acid (DTNB), ethylenediamine tetra-acetic acid (EDTA), bovine serum albumin (BSA), thiobarbituric acid (TBA), crystal violet were obtained from Sigma Chemical Co. (Bangalore, India).Trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2)were procured from SD Fine Chemicals, Mumbai, India, whereas disodium hydrogen phosphate (Na_2HPO_4), Tris buffer (Tris (hydroxymethyl) aminomethane and ammonium oxalate were purchased from Merck India Limited, Mumbai, India. Trypsin EDTA 1X, MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide), MEM medium, fetal bovine serum (FBS), and reduced nicotinamide adenine dinucleotide (NADH) were requisitioned from HiMedia, Mumbai, India.

Collection of rhizomes and extraction

The matured and non-infected rhizomes of *Colocasia gigantea* (family- Araceae) were collected from Manipur. The plant was identified by PG Department of Botany, DM College, Imphal-West, Manipur, India and it was further authenticated by the Botanical Survey of India, Shillong, Meghalaya, India. The matured rhizomes were cleaned shade dried, their skinremoved and chopped into thin slices for easy and quick drying. The dried rhizomes were powered using an electrical grinder at room temperature. A known amount of powdered rhizome of *C. gigantea* was sequentially extracted in petroleum ether, chloroform, ethanol and distilled water in order of increasing polarity using a Soxhlet apparatus. Each extracts, except petroleum ether was concentrated in vacuo and stored at -70°C until further use. The ethanol extract was used for the entire study and henceforth it will be called as CGE.

Drug/s dissolution

Doxorubicin was freshly dissolved in MEM and the ethanol extract of *Colocasia gigantea* were freshly dissolved in distilled water and diluted and filter sterilized immediately before use.

Cell culture

HeLa and V79 cells were procuredfrom the National Centre for Cell Science, Pune, India. The cells were grown in 25 cm^2 culture flasks (Corning Inc., Corning, NY, USA) containing 5 ml Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, 1% L-glutamine and 50 µg/ml gentamicin. The cells were incubated at 37°C in an atmosphere of 5% CO_2 in humidified air in a CO_2 incubator (Eppendorf AG, Hamburg, Germany).

Experimental Design

A fixed amount of cells were seeded into 96 well plates or culture flasks that were divided into the different groups depending on the experimental protocol:

MEM group: The cells of this group served as negative control group and did not receive any treatment.

CGE group: This group of cells was treated with different concentrations of CGE.

DOX group: The cell cultures of this group were treated with5, 10 and 20 µg/ml of doxorubicin (DOX) and served as positive control.

Determination of cytotoxicity by MTT assay

The cytotoxic effects of different concentrations of ethanol extract of *Colocasia gigantea* was studied by MTT assay in HeLa and V79cells as described by Mosmann (1983). Usually 10^4 cells were seeded into 96 well plates (HiMedia, Mumbai, India) in 100 µl minimum essential medium (MEM). The microplates were kept at 37ºC in a CO_2 incubator in an atmosphere of 5% CO_2 in 95 % humidified air and the cells were allowed to attach for 24 h. Next day different concentrations of CGE or doxorubicin were added into each well of the microplates and incubated in the CO_2 incubator. After 48 hours, MTT was added into each well and the microplates were incubated for another 2 hours. The drug containing media were removed and the insoluble purple formazan formed was dissolved using lysis buffer and incubated once again for 4 hours after which the absorbance was recorded at 560 nm using a microplate reader (Spectramax M2, Molecular Devices, Sunnyvale, CA, USA). The cytotoxicity was calculated using the formula: Control-Treatment/Control X 100.

Determination of optimum exposure time for cytotoxicity

A separate experiment was conducted to study the effect of treatment duration of CGE on the cytotoxicity, where grouping and other conditions were essentially similar to that described above except that the cells were exposed to CGE for 2, 4 and 6 h and the cytotoxicity was determined by MTT assay as described above.

Determination of anticancer activity

Another experiment was performed to evaluate the anticancer activity of CGE, where grouping and other conditions were similar to that described in the experimental design. The anticancer activity of CGE was determined by inoculating 10^6 exponentially growing HeLa cells into several culture flasks. The cells were allowed to attach for 24 h and were treated with 100, 200 and 300 µg/ml CGE.

After 2 hours of drug treatment the media were removed and the flasks were washed twice with sterile PBS, and dislodged by trypsin EDTA treatment and the Clonogenicity of cells was determined by clonogenic assay [11]. Usually 200 HeLa cells were seeded into several individual petridishes containing 5 ml MEM and left undisturbed for colony formation for another 11 days. After the end of day 11 the resultant colonies of cells were stained with 1 % crystal violet in methanol and scored. Plating efficiency (PE) of the cells was determined and surviving fraction (SF) calculated.

PE = (Number of colonies counted x 100) / (Number of cells seeded)

SF = (Number of colonies counted) / (Number of cells seeded) x (mean plating efficiency).

BIOCHEMICAL ASSAYS

A separate experiment was carried out to estimate the effect of 100, 200 and 300 µg/ml CGE on the activities of various antioxidants in HeLa cells at 2, 4 and 6h post drug treatment.The drug containing media were removed; the cells were washed with sterile PBS and displaced using trypsin EDTA treatment. The cells were pelleted and weighed and 5% homogenate of the cells was prepared in PBS (pH 7.4) using ultrasonicator (PCI Analytics Pvt. Ltd., Mumbai, India). The following assays were carried out:

Glutathione estimation

Glutathione estimation was carried out as described earlier [12]. Briefly, 1.8 ml of 0.2M Na_2HPO_4 was mixed with 40 µl 10 mM DTNB and 160 µl of cell homogenate. The mixture was allowed to stand for 2 minutes at room temperature and the absorbance was read against the blank at 412 nm in a UV-VIS Biospectrophotometer (Eppendorf India Limited, Kolkata, India).The blank consisted of distilled water instead of cell homogenate.

Glutathione - S – transferase estimation

Glutathione-s-transferase activity was estimated by the method of Habig *et al.,* (1974). Briefly, 0.5 ml of 0.1 M phosphate buffer pH 6.5, 0.1ml of 20mM CDNB, and 8.8 ml distilled water were incubated at 37°C for 10 min. After incubation, 0.5 ml of 20 mM GSH and 0.1 ml of cell homogenate were added. The absorbance was read at 340 nm at 1 min intervals for 6 minutes in UV-VIS Biospectrophotometer.

Catalase

The method of Aebi (1984) was followed for catalase estimation. Briefly, 20 µl of sample was

diluted with 1.98 ml of 50 mM phosphate buffer (pH 7.0) in a 3 ml cuvette and the reaction (maintained at 20ºC) was started by adding 1 ml of 30 mM H_2O_2. The decrease in absorbance was monitored at 240 nm for 60 seconds in a UV-VIS Biospectrophotometer.

Estimation of lipid peroxidation

Lipid peroxidation (LOO) assay was carried out by the method of Buege and Aust (1978). Briefly, 1 ml of cell homogenate was mixed with 2 ml of TCA-TBA-HCl reagent and mixed thoroughly. The reaction mixture was heated in a boiling water bath for 15 minutes, cooled immediately to room temperature, centrifuged at 1000 rpm for 10 min and supernatant was collected and its absorbance was read at 535 nm against blank in a UV-VIS Biospectrophotometer.

STATISTICAL ANALYSES

The statistical analyses were performed using Origin Pro 8. All the results are expressed as Mean ± Standard Error of the Mean (SEM). Experimental data were analyzed by one way ANOVA followed by Tukey's test for multiple comparisons for different parameters between the groups. A P value of < 0.05 was considered as significant. The experiments were repeated for confirmation and since the difference between the original and repeat experiments was statistically non-significant the data of both the experiments were combined and presented in tables and figures.

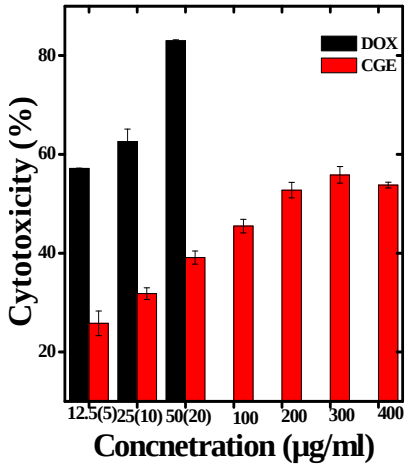

Figure 1: The cytotoxic effect of different concentrations of ethanol extract of *Colocasia gigantea* & doxorubicin on HeLa cells assessed by MTT assay. CGE- Ethanol extract of *Colocasia gigantea*, DOX- Doxorubicin. Figures in brackets indicate concentration of doxorubicin. The data represent Mean±SEM, N=5.

RESULTS

The results are expressed in fig. 1-9 as Mean ± Standard Error of the Mean (SEM).

Determination of Cytotoxicity

Treatment of Helga and V79 cells with different concentrations of CGE caused a concentration dependent increase in the cytotoxicity and the maximum cytotoxic effect was recorded for the highest concentrations of 300 µg/ml CGE. The cytotoxicity between of 200 and 300 µg/ml CGE was not statistically significant the former was chosen for other experiments (Figure 1). Similarly, CGE induced maximum cytotoxicity at 200 µg/ml in V79 cells (Figure 2). The positive control DOX also showed a similar pattern (Fig. 1-2).

Determination of treatment duration

The optimum CGE treatment duration for cytotoxic effect was also evaluated by MTT assay at 2, 4 and 6 hours. The CGE treatment resulted in a time dependent increase in the cytotoxicity in HeLa and V79 cells and maximum cytotoxic effect was observed in the cells treated with CGE for h respectively (Fig. 3-4). However, this increase was not statistically significant hence 2 h treatment duration was selected for further experiments.

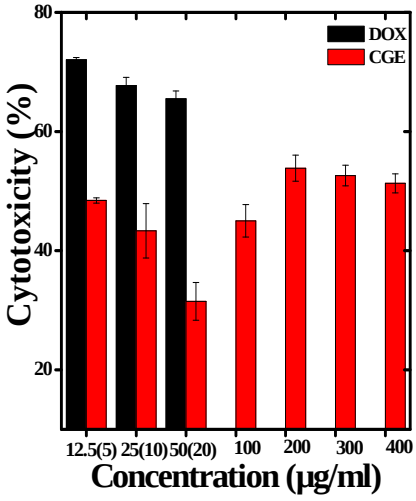

Figure 2: The cytotoxic effect of different concentrations of ethanol extract of *Colocasia gigantea* & doxorubicin on V79 cells assessed by MTT assay. CGE- Ethanol extract of *Colocasia*

gigantea, DOX- Doxorubicin. Figures in brackets indicate concentration of doxorubicin. The data represent Mean±SEM, N=5.

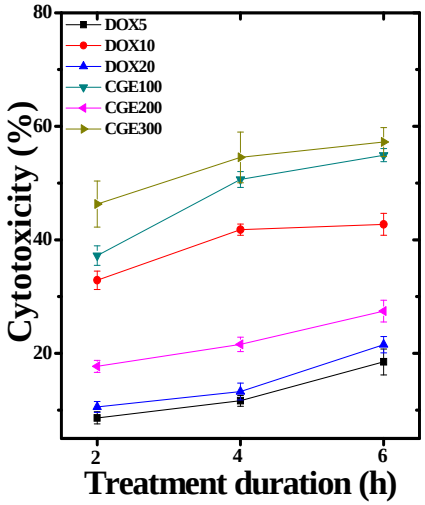

Figure 3: The effect of different concentration of the ethanol extract of *Colocasia gigantea* & doxorubicin on HeLa cells determined by MTT assay. Ethanol extract of *Colocasia gigantea* (CGE), Doxorubicin (DOX). The data represent Mean ± SEM, N=5.

Clonogenic Assay

Treatment of HeLa cells with different concentrations of CGE caused a concentration dependent decline in the clonogenicity of cells (Fig. 5). A maximum decline in the clonogenicity was observed for 300 µg/ml CGE, where the survivin g fraction of HeLa cell reached a nadir (0.22) less than half of 200 µg/ml (Fig. 5).

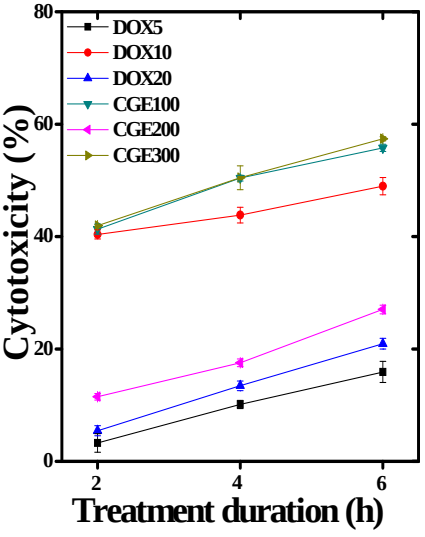

Figure 4: The effect of different concentration of the ethanol extract of *Colocasia gigantea* & Doxorubicin on V79 cells determined by MTT assay. Ethanol extract of *Colocasia gigantea* (CGE), Doxorubicin (DOX). The data represent Mean ± SEM, N=5.

g fraction of HeLa cell reached a nadir (0.22) less than half of 200 µg/ml (Fig. 5).

Glutathione

Treatment of HeLa cells with different concentrations of CGE caused a concentration dependent but significant depletion in glutathione contents at all the post-treatment times (Figure 6). The GSH concentration also declined in a time dependent manner and maximum decline was observed at 6 h post treatment (Fig. 5). The concentration of glutathione also declined in a similar as DOX treated group (Fig. 6).

Glutathione-s-transferases

GST actvity declined in a concentratoin depenent manner and it was significant lower than the MEM treated group. The acivit of GSt also reducted with time in the HeLa cells treated with diffrent oncentrations of CGE and a greatest decline was observed at 6 h post-treatment and for 300 µg/ml (Fig. 7).

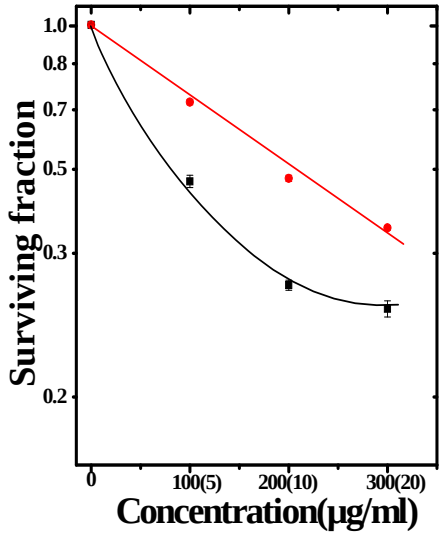

Figure 5: Effect of different concentrations of the ethanol extract of *Colocasia gigantea* & doxorubucin (DOX) treatment on the survival of HeLa cells. Figures in brackets on X-axis indicate concentration of doxorubicin. The results are expressed as Mean ± SEM. N=3. Squares: doxorubicin & Circles: ethanol extract of *Colocasia gigantea*

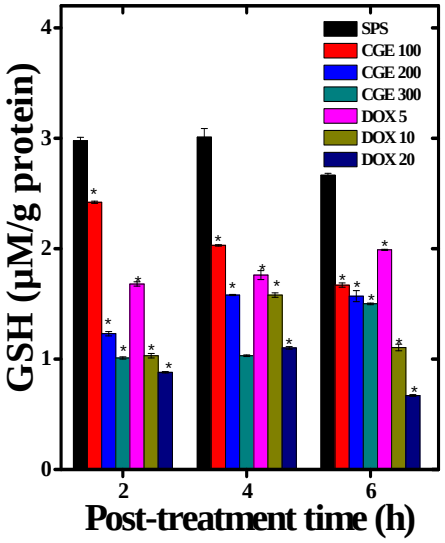

Figure 6: Alteration in the GSH activity of cultured HeLa cells treated with different concentrations of CGE and DOX. Minimum essential media (MEM), Ethanol extract of *Colocasia gigantea* (CGE) & doxorubicin (DOX). The data represent Mean±SEM, N=5. *p<0.01 when the treatment group are compared to MEM group. Standard error of the mean (SEM).

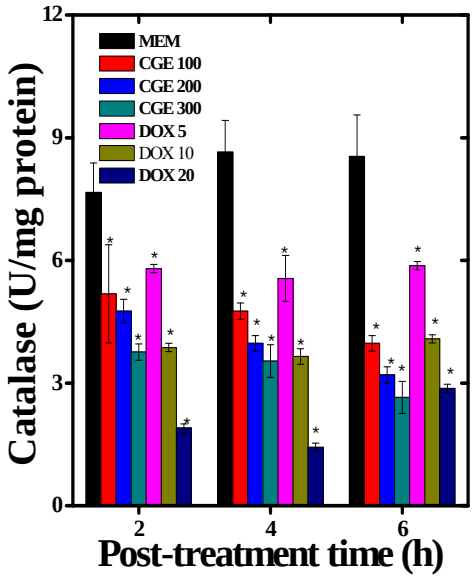

Figure 8: Alteration in the Catalase activity of cultured HeLa cells treated with different concentrations of CGE & DOX. Minimum essential media (MEM), Ethanol extract of *Colocasia gigantea* (CGE) & doxorubicin (DOX). The data represent Mean±SEM, N=5. *p<0.01 when the treatment group are compared to MEM group. Standard error of the mean (SEM).

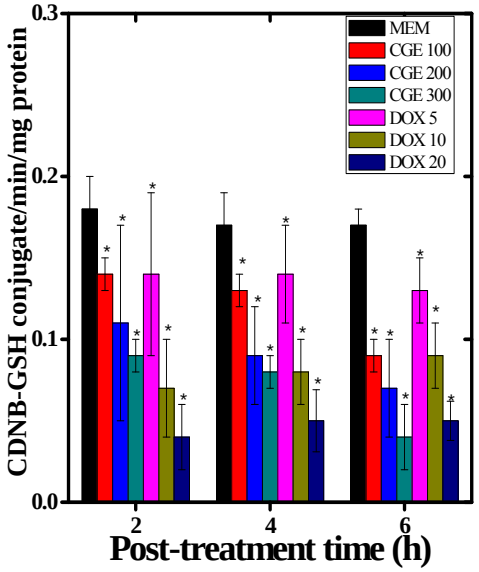

Figure 7: Alteration in the GST activity of cultured HeLa cells treated with different concentrations of CGE & DOX. Minimum essential media (MEM), Ethanol extract of *Colocasia gigantea* (CGE) & doxorubicin (DOX). The data represent Mean±SEM, N=5. *p<0.01 when the treatment group are compared to MEM group. Standard error of the mean (SEM).

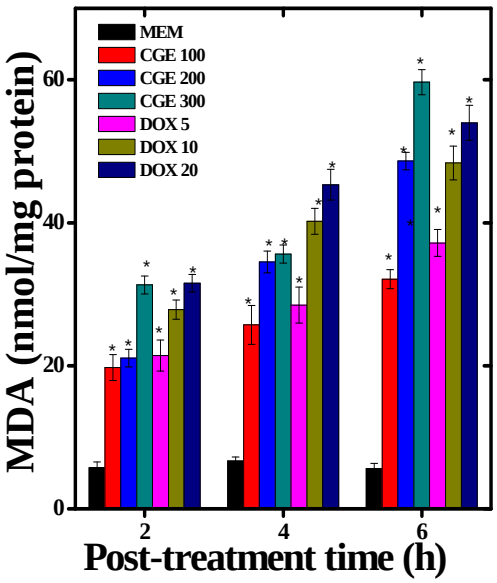

Figure 9: Alteration in the Lipid peroxidation activity of cultured HeLa cells treated with different concentrations of CGE & DOX. Minimum essential media (MEM), Ethanol extract of *Colocasia gigantea* (CGE) & doxorubicin (DOX). The data represent Mean±SEM, N=5. *p<0.01 when the treatment group are compared to MEM group. Standard error of the mean (SEM).

Catalase

The catalase activity also alleviated with increasing CGE concentration and there was significant reduction in the catalase activity at all post-treatment assay time when compared to MEM treatment. The analysis of catalase activity with time showed a time dependent decline in the catalase activity for all CGE concentrations and it was lowest at 6 h post treatment Fig. 8) The DOX treatment also resulted in an identical decline in catalase activity at all post treatment times (Figure 8).

Lipid Peroxidation

The treatment of HeLa cells with different concentrations of CGE induced LOO efficiently as indicated by a concentration dependent rise in the LOO at all post-treatment times (Fig. 9). This increase in LOO was significantly higher and it was at least 6 folds higher at 6 h post treatment in CGE treated group. The maximum LOO was detected at 6 h post treatment in all the groups (Fig. 9). The DOX treatment also showed a pattern similar to that of CGE treatment (Fig. 9).

DISCUSSION

The adverse effects induced by modern chemotherapeutic regimens and development of therapy resistance are the major stumbling block for successful treatment of tumors [13,-16]. The other disadvantage of systemic chemotherapy is induction of second malignancies due to genomic damge in the normal cells [17]. Therefore screening of newer paradigms that do not trigger the development of adverse effects and second malignancies are of crucial importance. The natural products and plants can provide the opportunity to develop non-toxic and effective drug molecules to treat cancer. Therefore the present study was undertaken to evaluate the anticancer potential of *Colocasia gigantea* in cultured HeLa cells.

The cytotoxic effect of any drug candidate/s can be ascertained by MTT assay, which is a rapid and standard technique to determine the cytotoxicity of any drug/treatment. The viable cells or metabolically active cells are able to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT into formazan by the action of mitochondrial succinate dehydrogenase enzyme and the level of activity is a measure of the viability of the cells. The weaker the color formed, the more are the dead cells. MTT assay has been used to test cytotoxicity of numerous drugs in various cell lines since its discovery [18]. The treatment of HeLa and V79 cells with ethanol extract of *Colocasia gigantea* caused a concentration dependent rise in the cytotoxicity. There are no reports regarding the evaluation of cytotoxicity of ethanol extract of *Colocasia gigantea.* However MTT assay has been used to investigate the cytotoxic effects of other plants in vitro [19-21]. The cytotoxic effect of CGE was further confirmed by clonogenic assay, whch is long term assay to study the toxicity of any agent. The CGE treatment led to a concentration dependent decline in the clonogenicity of *HeLa* cells. The cytotoxic effect of ethanol extract of *Colocasia gigantea*has not been studied yet. However the other medicinal plants like *Tinospora cordifolia*, and *Aphanmixis polystchya* and synthetic molecules including doxorubicin, daunorubicin and cytarabine have been reported to alleviate the clonognonic potential of cultured cells earlier [22-25]. Almost all cancer cells are at increased oxidative stress, which may be essential for progression and development of tumor.

The tumors also express high level of antioxidants to balance the increased oxidative stress and this increased antioxidant level is linked with the survival advantage in the tumor cells and also it helps to develop resistance to chemotherapy [26,27]. The excess oxidative stress induced by chemotherapeutic drugs is responsible for cell death as it stimulates various mechanism of cell death including non-apoptotic form of cell death [28]. Lipid peroxidation is a measure of oxidative stress as the products of lipid peroxidation damage the important macromolecules like proteins and nucleic acid which final lead to death of the cell [29,-31]. The CGE increased the oxidative stress in a concentration and time dependent manner and this may be the reason for effective cell killing in the present study. Most of the chemotherapeutic agents kill neoplastic cells by increasing oxidative stress in the tumor cells [32,33]. Malondialdehyde (MDA) is a major product of lipid peroxidation [34]. MDA has the ability to react with nucleic acid bases and form adducts to dG, dA, and dC [35]. Lipid peroxidation has been implicated in the pathogenesis of a number of diseases including cancer due to its ability to damage DNA and subsequent mutations in the tumor suppressor genes [30,36]. This property of

lipid peroxidation may be responsible for killing tumor cells in the present study. The glutathione is the most abundant non-protein intracellular antioxidant that has diverse role in numerous physiological processes [37].

The increase in glutathione has been implicated in tumor progression and resistance to chemotherapy and reduced glutathione levels have been reported to kill tumor cells more effectively [38-42]. A similar mechanism seems to operational in the present study where the treatment of HeLa cells with CGE has reduced the GSH concentration in a time and concentration dependent manner. The enzyme GST catalyzes the nucleophilic attack of glutathione (GSH) on electrophilic substrates by binding with glutathione on its hydrophilic G-site and its adjacent H-site with the electrophilic substrates to bring them in a close proximity. They also activate the sulfhydryl group on GSH, thereby allowing for nucleophilic attack of GSH on the electrophilic substrate [43].

Elevated levels of GST in tumor cells are associated with increased resistance to apoptosis [44,45]. The CGE reduced the GST activity in a concentration and time dependent fashion that may have induced effective killing of HeLa cells. Various GST inhibitors have been shown to modulate drug resistance by sensitizing tumor cells to anticancer drugs [46,47]. Catalase or oxidoreductase is present in all organisms and it detoxify H_2O_2 into water and oxygen and it is also involved in various other processes. High levels of catalase have been reported in patients with lung cancer, whereas decreased levels of catalase were indicated in breast cancer, head and neck cancer, gynaecological cancer, lymphoma, prostate cancer and urological cancer [48]. The over expression of catalase has been reported to reduce the apoptosis in tumor cells after chemotherapy [49]. The treatment of HeLa cells with CGE depleted the activity of catalase in concentration and time dependent manner, which would killed the HeLa cells effectively.

The mechanisms of cell killing by CGE are mostly not understood. However present study makes it very clear that CGE administration has increased the lipid peroxidation more than 6 fold thereby leading to a rise in the oxidative stress, which would have damaged the cellular DNA, other biomolecules and membranes killing the cells. The alleviated levels of GST, catalase and GSH would have further increased the oxidative stress and added insult to injury killing the HeLa cells effectively. The cancer and cancer cell lines over express the COX-II and nuclear transcription factors NF-κB and Nrf2 and they are also involved in resistance to tumor therapy [50-52]. The suppression of transcriptional activation of these genes by CGE may have played an important role in effectively killing the cells. The induction of apoptosis and activation of p53 and related proteins may have also contributed their share in bringing cell death.

CONCLUSIONS

The present study clearly demonstrates the cell killing ability of CGE and the cell killing may be due to the increased LOO, accompanied by a decline in the GSH, GST and catalase, that would have increased the oxidative stress that may have triggered the DNA, protein and membrane damage killing the cells effectively. CGE may have also suppressed the activation of COX-II, NF-κB and Nrf2 elements that may have induced apoptotic cell death. The over expression of p53 and related proteins may have also contributed to cell death in the present study.

ACKNOWLEDGEMENTS

The authors are thankful to the Indian Council of Medical Research, University Grants Commission and Department of Biotechnology, Government of India, New Delhi for providing financial assistance to carry out this study.

REFERENCES

[1] Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Tieulent, J.L., Jemal, A. (2012). Global Cancer Statistics. CA Cancer J Clin., 65, 87–108.

[2] Khazir, J., Mir, B.A., Pilcher, L., and Riley, D.L. (2014). Role of plants in anticancer drug discovery. Phytochemistry Letters, 7, 173-181.

[3] Siegel, R., Miller, K., Jemal, A. (2016). Cancer statistics. CA Cancer J Clin. 2015; 65.

[4] Patridge E, Gareiss P, Kinch MS, and Hoyer D: An analysis of FDA-approved drugs: natural products and their derivatives. Drug discovery today, 21(2), 204-207.

[5] Newman, D.J., Cragg, G.M. (2014). Marine-Sourced Anti-Cancer and Cancer

Pain Control Agents in Clinical and Late Preclinical Development. Mar Drugs, 12, 255-278.

[6] Mathieu, V., Chantome, A., Lefranc, F., Cimmino, A., Miklos, W., Paulitschke, V., Mohr, T., Maddau, L., Kornienko, A., Berger, W., Vandier, C., Evidente, A., Delpire, E., & Kiss, R. (2015). Cellular and Molecular Life Sciences. Sphaeropsidin A shows promising activity against drug-resistant cancer cells by targeting regulatory volume increase. DOI 10.1007/s00018-015-1902-6.

[7] Manner, H.I. (2011). Farm and forestry production and marketing profile for giant taro (Alocasiamacrorrhiza). In: Elevitch CR, editor. Specialty crops for pacific island agroforestry [Internet]. Holualoa, Hawaii: Permanent Agriculture Resources (PAR).

[8] Essence of the Agriculture. (2006). Songkla University.

[9] Kokua, N.L. (1977). Taro (Kalo): Uses and recipes. Honolulu Pacific Botanical Garden, Kuwai, Hawaii.

[10] Drury, H. (1973). Useful Plants of India with notices of their chief value in commerce, medicine and arts. William H. Allen & Co. London.

[11] Puck, T.T. & Marcus, P.I. (1955). Rapid method for viable cell titration and clone production with Hela cells on tissue culture: the use of X-irradiated cells to supply conditioning factors. Proc. Nat. Acad. Sci., 3, 432-437.

[12] Moron, M.S., Depierre, J.W., Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochim Biophys Acta. , 582, 67-78.

[13] Raji, M.A. (2005). Management of chemotherapy-induced side-effects. The lancet oncology, 6(6), 357.

[14] MacDonald, V. (2009). Chemotherapy: managing side effects and safe handling. The Canadian Veterinary Journal, 50(6), 665.

[15] Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug resistance in cancer: an overview. Cancers, 6(3), 1769-1792.

[16] Lee, H.H., Bellat, V., Law, B. (2017). Chemotherapy induces adaptive drug resistance and metastatic potentials via phenotypic CXCR4-expressing cell state transition in ovarian cancer. PLoS ONE 12(2): e0171044.

[17] Morton. L.M., Swerdlow. A.J., Schaapveld, M,, Ramadan S,, Hodgson, D.C., Radford, J., & van Leeuwen F.E. (2014) Current knowledge and future research directions in treatment-related second primary malignancies. EJC suppl 12: 5-17.

[18] Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological methods, 65(1-2), 55-63.

[19] Booth, G.M., Malmstrom, R.D., Kipp, E. & Paul, A. (2012). Cytotoxicity of selected medicinal and nonmedicinal plant extracts to microbial and cervical cancer cells. BioMed Research International.

[20] Nguta, J.M., Appiah-Opong, R., Nyarko, A.K., Yeboah-Manu, D., Addo, P.G., Otchere, I., and Kissi-Twum, A. (2016). Antimycobacterial and cytotoxic activity of selected medicinal plant extracts. Journal of ethnopharmacology, 182, 10-15.

[21] Kuete, V., Fokou, F.W., Karaosmanoğlu, O., Beng, V.P., & Sivas, H. (2017). Cytotoxicity of the methanol extracts of Elephantopusmollis, Kalanchoecrenata and 4 other Cameroonian medicinal plants towards human carcinoma cells. BMC complementary and alternative medicine, 17(1), 280.

[22] Williams, B.A., Wang, X.H., Keating, A. (2010). Clonogenic assays measure leukemia stem cell killing not detectable by chromium release and flow cytometric cytotoxicity assays. Cytotherapy, 12(7), 951-960.

[23] Jagetia, G.C., & Rao, S.K. (2011). Assessment of radiation-induced DNA damage by comet assay in cultured HeLa cells treated with guduchi (Tinospora cordifolia Miers) before exposure to different doses of g-radiation. Research in Pharmaceutical Biotechnology, 3(7), 93-103.

[24] Jagetia, G.C. & Rao, K.V.N. M. (2015). Hesperidin, A Citrus Bioflavonoid Reduces the Oxidative Stress in the Skin of Mouse Exposed to Partial Body γ-Radiation. Transcriptomics, 3, 111. doi:10.4172/2329-8936.1000111.

[25] Jagetia, G.C. & Venkatesha, V.A. (2016). Determination of Antineoplastic Activity of Rohituka, Aphanamixis Polystachya (Wall) RN Parker in Hela Cells: Correlation with Clonogenicity and DNA Damage. Int J Complement Alt Med., 3(4).

[26] Liou, G.Y. & Storz, P. (2010). Reactive oxygen species in cancer. Free radical research, 44(5), 479- 496.

[27] Gill, J.G., Piskounova, E. & Morrison, S.J. (2016). Cancer, Oxidative Stress, and Metastasis. In Cold Spring Harbor symposia on quantitative biology, 81,163-175. Cold Spring Harbor Laboratory Press.

[28] Liu, J. & Wang, Z. (2015). Increased oxidative stress as a selective anticancer therapy. Oxidative medicine and cellular longevity.

[29] Barrera, G. (2012). Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy. ISRN Oncology, 21.

[30] Zhong, H. & Yin, H. (2015). Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: Focusing on mitochondria. Redox biology, 4, 193-9.

[31] Gaschler, M.M. & Stockwell, B.R. (2017). Lipid peroxidation in cell death. Bioochem Biophys Res Commun., 482(3), 419-425.

[32] Conklin, K.A. (2004). Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Inteqr Cancer Ther., 3(4), 294-300.

[33] Gorrini, C., Harris, I.S. & Mark, T.W. (2013). Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov., 12(12), 931-47.

[34] Rice-Evans, C. & Burdon, R. (1993). Free radical-lipid interactions and their pathological consequences. Proq Lipid Res. 32(1), 71-110.

[35] Marnett, L.J. (2002). Oxy radicals, lipid peroxidation and DNA damage. Toxicology, 181-182, 219-222.

[36] Cejas, P., Casado, E., Belda-Iniesta, C., De Castro, J., Espinosa, E., Redondo, A., Sereno, M., García-Cabezas, M.A., Vara, J.A., Domínguez-Cáceres, A., Perona, R., González-Barón, M. (2004). Implications of oxidative stress and cell membrane lipid peroxidation in human cancer (Spain). Cancer Causes Control,15(7), 707-719.

[37] Lushchak, V.I. (2012). Glutathione homeostasis and functions: potential targets for medical interventions. Journal of amino acids.

[38] Circu, M.L. & Aw, T.Y. (2012). Glutathione and modulation of cell apoptosis. Biochim Biophys Acta. , 1823(10), 1767-1777.

[39] Franco, R. & Cidlowski, J.A. (2012). Glutahione efflux and cell death. Antioxid Redox Signal., 17(12), 1694-713.

[40] Traverso, N., Ricciarelli, R., Nitti, M., Marengo, B., Furfaro, A.L., Pronzato, M.A., Marinari, U.M., Domenicotti, C. (2013). Role of glutathione in cancer progression and chemoresistance. Oxid Med Cell Longev. 972913. doi: 10.1155/2013/972913.

[41] Rocha, C.R.R., Garcia, C.C.M., Vieira, D.B., Quinet, A., de Andrade-Lima, L.C., Munford, V., Belizário, J.E. & Menck, C.F.M. (2014). Glutathione depletion sensitizes cisplatin-and temozolomide-resistant glioma cells in vitro and in vivo. Cell death & disease, 5(10), p.e1505.

[42] Ramsay, E.E. & Dilda, P.J. (2014). Glutathione S-conjugates as prodrugs to target drug-resistant tumors. Front Pharmacol, 5, 181.

[43] Armstrong, R.N. (1997). Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem Res Toxicol., 10, 2–18.

[44] McIlwain, C.C., Townsend, D.M. & Tew, K.D. (2006). Glutathione S-transferase polymorphisms: cancer incidence and therapy. Oncogene, 25, 1639–1648.

[45] Zeng, X., Morgenstern, R. & Nyström, A.M. (2014). Nanoparticle-directed sub-cellular localization of doxorubicin and

the sensitization breast cancer cells by circumventing GST-Mediated drug resistance. Biomaterials, 35, 1227-1239.

[46] Townsend, D.M. & Tew, K.D. (2003). The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene, 22(47), 7369-75.

[47] Laborde, E. (2010). Glutathione transferase as mediators of signalling pathways involved in cell proliferation and cell death. Cell Death Differ., 1373-1380.

[48] Kodydková, J., Vávrová, L., Kocík, M. & Žák, A. (2014). Human Catalase, Its Polymorphisms, Regulation and Changes of Its Activity in Different Diseases. Folia Biologica (Praha), 60, 153-167.

[49] Bechtel, W. & Bauer, G. (2009). Catalase Protects Tumor Cells from Apoptosis Induction by Intercellular ROS Signaling. Anticancer Research, 29, 4541-4558.

[50] Sobolewski, C., Cerella, C., Dicato, M., Ghibelli, L. & Diederich, M. (2010). The Role of Cyclooxygenase-2 in Cell Proliferation and Cell Death in Human Malignancies. International Journal of Cell Biology, 21. http://dx.doi.org/ 10.1155/ 2010/215158

[51] Lu, T. & Stark, G.R. (2015). NF-kB: Regulation by Methylation. Cancer Res., 75(18), 3692-5.

[52] Choi, B.H., & Kwak, M.K. (2016). Shadows of NRF2 in cancer: Resistance to chemotherapy. Curr. Opinion Toxicol.1: 20-28.