Research Article CODEN: IJPRNK Impact Factor: 5.567 ISSN: 2277-8713
Manoharan Mohana, IJPRBS, 2016; Volume 5(2): 75-88 IJPRBS

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

EFFECT OF THE BACOSIDE FRACTION FROM *Bacopa monnieri* AGAINST OXIDATIVE STRESS-INDUCED APOPTOSIS IN UNTRANSFORMED (BUCCAL) AND TRANSFORMED (KB ORAL CARCINOMA) CELLS

MANOHARAN MOHANA[1], PALGHAT RAGHUNATHAN PADMA[1]

Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore-641 043, Tamil Nadu, India.

Accepted Date: 31/03/2016; Published Date: 27/04/2016

Abstract: Apoptosis poses a barrier against cancer formation by eliminating pre-cancerous cells from survival and multiplication. Herbal medicines are of special interest because of their lower toxicities. The plant chosen for the present study is *Bacopa monnieri*. *Bacopa monnieri* is a creeping perennial with small oblong leaves and purple flowers, found in warm wetlands. The entire plant is used medicinally. In the present study, the effect of bacoside fraction at a concentration of 50µg/ml was studied on the apoptotic events under oxidative stress induced conditions. The anticancer effect of the bacoside fraction was studied in an oral carcinoma (KB) cell line. The primary cultures of human buccal cells were as a control. The extent of cell death, and the events associated with it, was studied, both in the presence and the absence of an oxidizing standard chemotherapeutic agent, namely etoposide. The results clearly proved that bacoside fraction exhibits a differential effect between the non-cancerous and cancerous cells, in that, it protects the non-cancerous buccal cells from etoposide-induced cell death, while rendering the cancerous KB cells more susceptible to the chemotherapeutic agent.

Keywords: Apoptosis – *Bacopa monnieri* – oxidative stress – buccal cells – KB cells

PAPER-QR CODE

Corresponding Author: MR. MANOHARAN MOHANA

Access Online On:

www.ijprbs.com

How to Cite This Article:

Manoharan Mohana, IJPRBS, 2016; Volume 5(2): 75-88

INTRODUCTION

Cancer is a public health problem worldwide, affecting all categories of persons, and a major cause of death in developed and developing countries [1]. It is the major cause of human death because of high incidence and mortality [2]. The International Agency for Research on Cancer (IARC) estimate of the incidence of mortality and prevalence from major types of cancer for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide. By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year [3]. Oral cancer is a major problem in Asia, especially in the Indian subcontinent. It is the third most common type of cancer, which accounts for over 30% of all cancers in the country. Two-third of the oral cancer is prevalent in males, whereas there is much variation in females [4].

Millions of cells in our body undergo a regulated form of cell death called apoptosis. This process plays a vital role in keeping us healthy, not only in protecting us from conditions like cancer, but also maintains tissue homeostasis. Many oncogenic stresses, including uncontrolled proliferation or DNA damage trigger apoptosis and, consequently, kill the cells at risk of transformation, thereby effectively preventing cancer. Apoptosis also suppresses tumourigenesis [5]. Decreased cell death by apoptosis can contribute to carcinogenesis as well as to progression of cancer once it has been established. Defects in apoptosis programs can lead to resistance to treatment, since most anticancer therapies, including chemotherapy, radiation or immunotherapy, primarily act by engaging this intrinsic cell death program in cancer cells [6].

Cancer is not only a result of uncontrolled proliferation, but also of reduced apoptosis. Therefore, inducing cell apoptosis is one of the key strategies in anticancer therapy [7]. Natural compounds in medicinal plants have diverse pharmacological activities and also advantages over synthetic drugs, such as moderate action and better tolerance [8]. Natural plant extracts offer a promising hope in the treatment of cancer arising from genetic mutations [9]. Present modes of treatment based on synthetic drugs have limited potential, because they are toxic and expensive and also alter cell signaling pathways. Natural drugs that are safe, affordable, and effective are needed to control cancer development and progression. Natural products have been used for thousands of years in the management of several diseases including various types of cancer [10].

With this background, the candidate plant chosen for the present study was *Bacopa monnieri*. *Bacopa monnieri*, a nootropic plant, belongs to Scrophulariaceae family, and is found in wet, damp and marshy areas of tropical regions. The presence of active saponins like bacosides A and B has been reported in *Bacopa monnieri*, which act as antioxidant and memory enhancers [11]. *Bacopa monnieri* is an important Ayurvedic drug and, traditionally, it is reported to be used for skin diseases, fever, inflammation, anaemia, urinary disorder and psychiatric disorders. It is also

considered to be a cardiotonic, a potent nerve tonic, for the treatment of asthma, hoarseness, insanity and epilepsy. Ethnobotanically, the leaves are used in speech disorders, in premature ejaculation, flatulence, abdominal pain, cough and cold. The major therapeutically important chemical constituents of this plant are the triterpenoid saponins, bacosides [12].

Many studies have been conducted on the memory enhancing property of *Bacopa monnieri*. Limited studies have been reported for the anticancer effect of *Bacopa monnieri.* Earlier studies reported that methanolic extract of *Bacopa monnieri* exhibited good antioxidant and anticancer property in Hep2 cell line (laryngeal carcinoma) [13]. In the present study, the anticancer effect of the bacoside fraction was studied in an oral carcinoma (KB) cell line. In order to understand the effect of the fraction on non-cancerous oral cells, as a control, primary cultures of human buccal cells were used. The protocol for the collection and use of the buccal cells from healthy human volunteers was scrutinized and approved by the Institutional Human Ethics Committee (Approval No. AUW/IHEC-14-15/XPD-08).

In these cell types (KB and buccal cells), the extent of cell death, and the events associated with it, were studied, both in the presence and the absence of an oxidizing standard chemotherapeutic agent, namely etoposide. Etoposide was used at a final concentration of 200 µM for the duration of 24 hours.

METHODODLOGY

Preparation of bacoside fraction

The bacoside fraction was prepared from the shade dried aerial parts of *Bacopa monnieri* [14]. The concentration of the bacoside fraction used in the present study is 50µg/ml.

Culturing of buccal cells

Buccal cells are the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products [15]. Buccal cells are a source of material that can be obtained from non-invasive collection methods. Buccal cells isolated from healthy individuals were cultured in DMEM, with minor modifications of the method [16].

The cells were washed twice with PBS. The cells were resuspended in DMEM supplemented with 2% FBS, 50U/ml penicillin and 50µg/ml streptomycin solution with 1.2µg/ml flucanazole in the culture flask. The cells were incubated overnight at 37ºC in a CO_2 incubator (Napco, UK). Following this, the medium was replaced with one containing 5% FBS, antibiotics and flucanazole. The cells were incubated overnight at 37ºC in a CO_2 incubator (Napco, UK). The cells (10^5 cells/ml) were seeded in a 96-well plate and the suspension culture was incubated at 37ºC for 24 hours. At the end of the incubation period, the cells were treated with etoposide, in the presence and

the absence of the bacoside fraction. The exposure of etoposide was given for 24 hours at 37ºC. The treated cells were harvested by rapid pipetting. The collected cells were centrifuged at 4000 rpm for 3 minutes, washed twice with PBS and finally suspended in PBS. The cell suspension was then used for the viability assays and staining analysis.

Culturing of KB Cell Line

The cell line was procured from National Centre for Cell Science, Pune, India. The cells were maintained in a CO_2 incubator (Innova, UK) with 5% CO_2 and 95% humidity, in DMEM supplemented with 10% FBS. Penicillin and streptomycin (MP Biomedicals, USA) were also added to the medium to 1X final concentration from a 100X stock. Once the cells had attained confluent growth, the cells were trypsinized using Trypsin–EDTA (MP Biomedicals, USA) and 10^5 cells were seeded into sterile 6-well and 96 well plates. In each well of the 6-well plate, a sterile coverslip was placed before the cells were seeded. Then the plates were incubated in a CO_2 incubator (Innova, UK) with 5% CO_2 and 95% humidity.

The cells were treated with etoposide, both in the presence and the absence of the bacoside fraction. The exposure of etoposide was given for 24 hours at 37ºC. The time points were arrived at by conducting a time related response analysis of each cell type. After treatment, the coverslips from the 6-well plates were removed and placed on a glass slide and sealed with vaseline. These slides were used for various staining techniques, whereas in 96-well plates, the medium was removed and replaced with fresh medium to study cell viability.

Parameters analyzed

The cell viability was assessed by MTT and SRB assays. The characteristic features of apoptosis were studied by analyzing various parameters like morphological changes (Giemsa staining), apoptotic index (propidium staining) and nuclear events (EtBr, AO/EtBr and DAPI staining).

The following treatment groups were set up in both the cell types to study the extent of death and the morphological and nuclear changes associated with it.

- Untreated (negative) control cells
- Etoposide treated (positive control) cells
- Cells treated with bacoside fraction
- Cells treated with bacoside fraction + etoposide

Cell viability assays

MTT dye reduction assay

The MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay, was employed to elucidate the cytotoxicity of the sample[17].

Sulphorhodamine B (SRB) assay

The SRB assay was employed to determine the cell viability in the presence and the absence of bacoside fraction in the oxidant-treated cells[18].

Morphological changes of apoptosis

The treated cells were stained with Giemsa and the morphological changes were viewed under phase contrast microscope (Nikon, Japan) [19].

Nuclear changes of apoptosis

The etoposide-treated cells with or without the bacoside fraction and the untreated control cells were scored by propidium iodide staining[20], ethidium bromide staining[21], DAPI staining[22] and acridine orange / ethidium bromide staining[23] using an inverted fluorescent microscope (Motic, Hong Kong).

Satistical analysis

The parameters analyzed were subjected to statistical analysis using SigmaStat (Version 3.1). Statistical significance was determined by one-way ANOVA, with $p<0.05$ considered significant.

RESULTS

Effect of the bacoside fraction on the viability of primary cultured buccal cells and KB oral carcinoma cells

The per cent viability obtained in the various treatment groups in buccal cells and KB cells are represented schematically in Figures 1 and 2 respectively for the MTT assay, and Figures 3 and 4 respectively for the SRB assay.

The etoposide treatment caused a steep decrease in the survival of both buccal and KB cells, indicating that it does not discriminate between the two types of cells. The viability of the buccal cells improved markedly in the presence of the bacoside fraction in the buccal cells. The fraction, by itself, showed mild toxicity to these (normal) cells.

On the other hand, in KB cells, the fraction, by itself, caused a steep decrease in the viability, which was comparable to that of etoposide treatment. This observation implies the anticancer effect of the bacoside fraction. It was interesting to note that the bacoside fraction decreased the survival of only the KB cells to such an extent, and not the buccal cells.

When etoposide and the bacoside fraction were co-administered to the KB cells, there was a further reduction in the survival of the cells, which was more evident in the SRB assay. This showed that the bacoside fraction exhibits a differential effect between the non-cancerous and cancerous cells, in that, it protects the non-cancerous buccal cells from etoposide-induced cell death, while rendering the cancerous KB cells more susceptible to the chemotherapeutic agent.

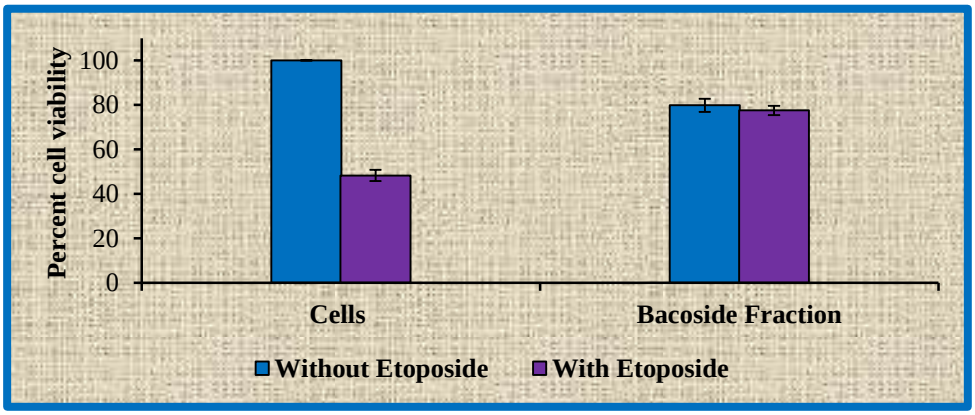
Effect of the bacoside fraction on the morphological and nuclear changes in etoposide induced stress in primary buccal cells and KB cells

The morphological changes observed in primary buccal cell culture and KB cells stained with Giemsa and the nuclear changes were analysed using the various nuclear stains, namely EtBr, PI, DAPI and AO/EtBr. The number of apoptotic cells was counted and the apoptotic ratio was calculated for each treatment group. The results are shown in the Table 1 and Figures 5 and 6.

In both primary buccal cells and KB cells, there was a drastic increase in the number of apoptotic cells with apoptotic morphology in the etoposide treated groups compared to that of untreated control. In the buccal cells, the co-administration of the bacoside fraction with etoposide, caused a decrease in the number of apoptotic cells when compared to that of the etoposide treated group. However, in KB cells, this co-treatment increased the proportion of apoptotic cells. This showed the differential response of the bacoside fraction exhibited in untransformed and transformed cells.

Figure 1

Effect of the bacoside fraction on the viability of buccal cells subjected to oxidative stress as determined by MTT assay

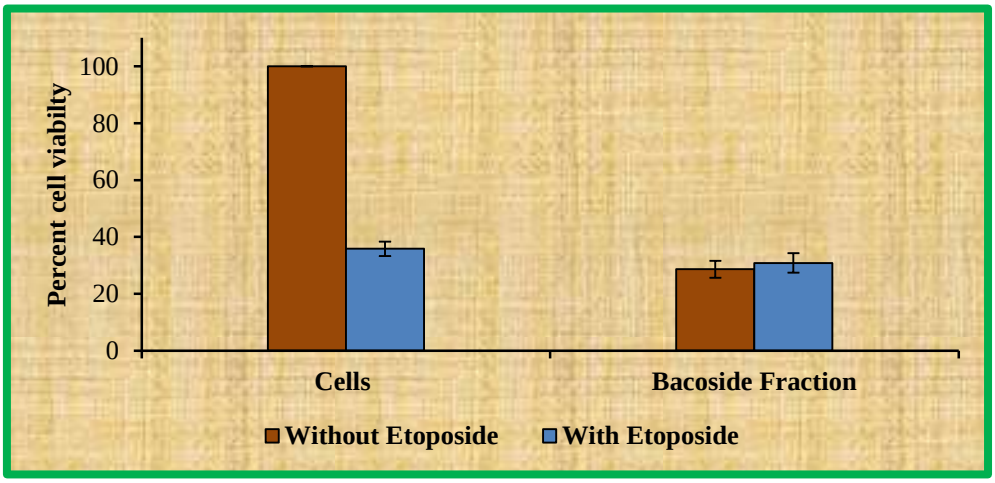


The values are mean ± SD of triplicates.

The viability of the untreated (negative) control group was fixed as 100% and the per cent viabilities in the other groups were calculated relative to this.

Figure 2

Effect of the bacoside fraction on the viability of KB cells subjected to oxidative stress as determined by MTT assay

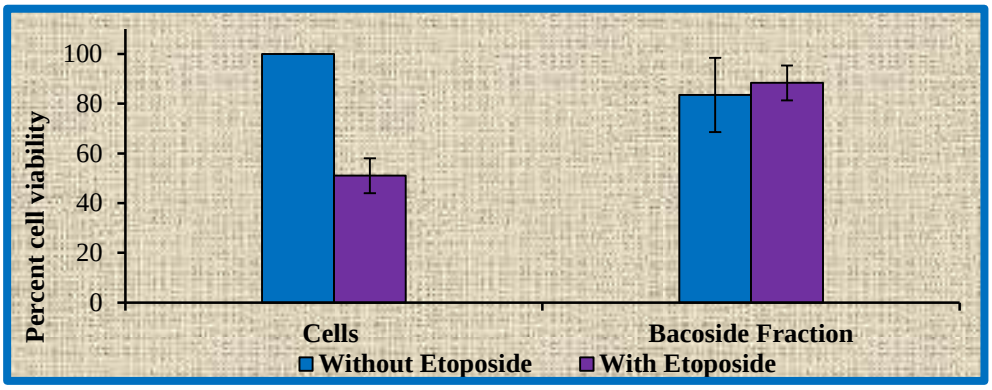


The values are mean ± SD of triplicates.

The viability of the untreated (negative) control group was fixed as 100% and the per cent viabilities in the other groups were calculated relative to this.

Figure 3

Effect of the bacoside fraction on the viability of buccal cells subjected to oxidative stress as determined by SRB assay

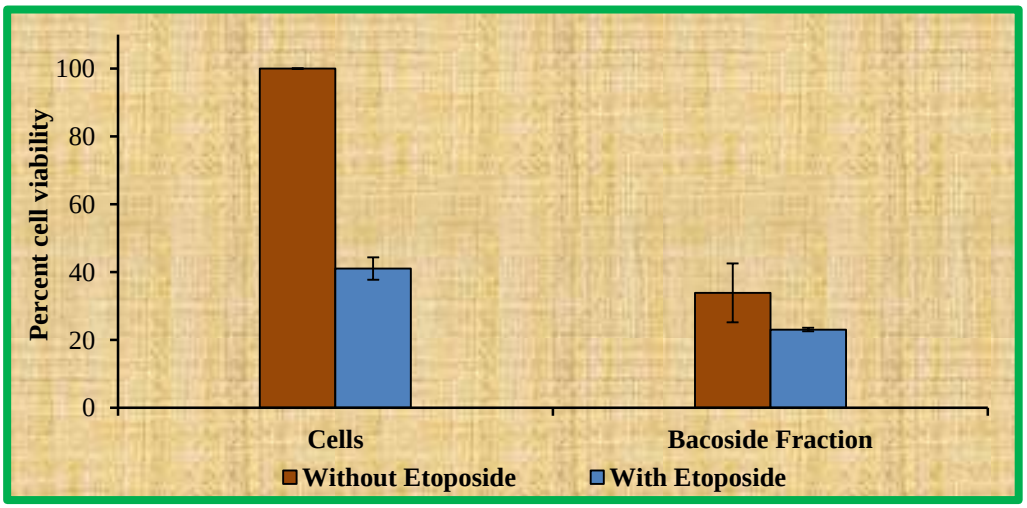


The values are mean ± SD of triplicates.

The viability of the untreated (negative) control group was fixed as 100% and the per cent viabilities in the other groups were calculated relative to this.

Figure 4

Effect of the bacoside fraction on the viability of KB cells subjected to oxidative stress as determined by SRB assay



The values are mean ± SD of triplicates.

The viability of the untreated (negative) control group was fixed as 100% and the per cent viabilities in the other groups were calculated relative to this.

Table 1: Effect of the bacoside fraction in buccal and KB cells subjected to oxidative stress

Staining	Treatment Groups	Number of Apoptotic cells / 100 cells			
		Buccal cells		KB cells	
		Without Etoposide	With Etoposide	Without Etoposide	With Etoposide
Giemsa	Without bacoside fraction	7 ± 2	46 ± 1 [a]	11 ± 2	81 ± 1 [a]
	With bacoside fraction	12 ± 1 [a]	29 ± 2 [a,b,c]	71 ± 3 [a]	85 ± 2 [a,b,c]
EtBr	Without bacoside fraction	6 ± 1	43 ± 3 [a]	10 ± 1	76 ± 3 [a]
	With bacoside fraction	13 ± 2 [a]	27 ± 2 [a,b,c]	69 ± 2 [a]	81 ± 2 [a,b,c]
PI	Without bacoside fraction	5 ± 3	45 ± 2 [a]	5 ± 2	75 ± 2 [a]
	With bacoside fraction	14 ± 1 [a]	30 ± 1 [a,b,c]	67 ± 1 [a]	79 ± 3 [a,b,c]
DAPI	Without bacoside fraction	4 ± 3	48 ± 1 [a]	5 ± 4	78 ± 1 [a]
	With bacoside fraction	12 ± 1 [a]	29 ± 3 [a,b,c]	65 ± 2 [a]	84 ± 2 [a,b,c]
AO/EtBr	Without bacoside fraction	7±1	45±3 [a]	7±3	79±1 [a]
	With bacoside fraction	15±2 [a]	30±1 [a,b,c]	63±1 [a]	84±2 [a,b,c]

Figure 5: Effect of bacoside fraction on the morphological and nuclear changes in buccal cell culture

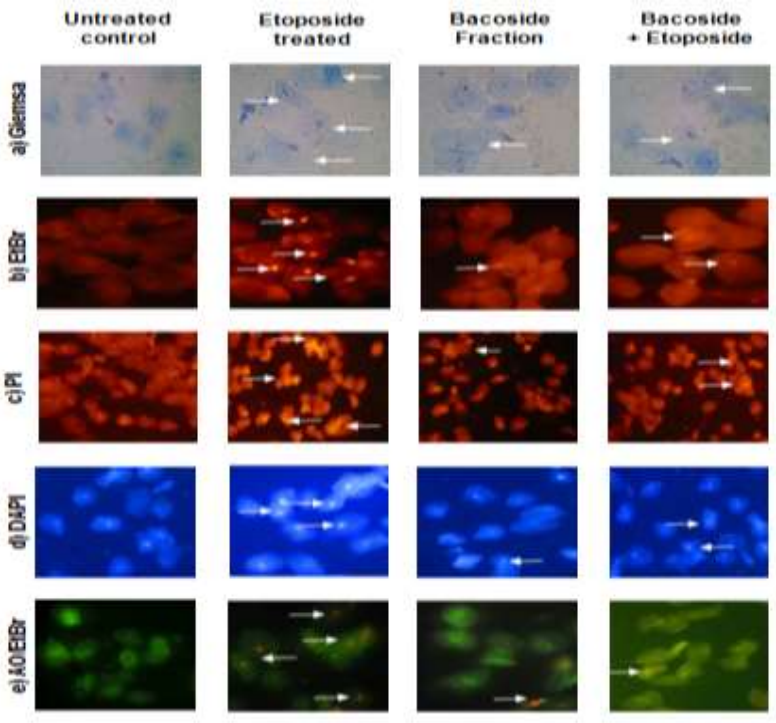
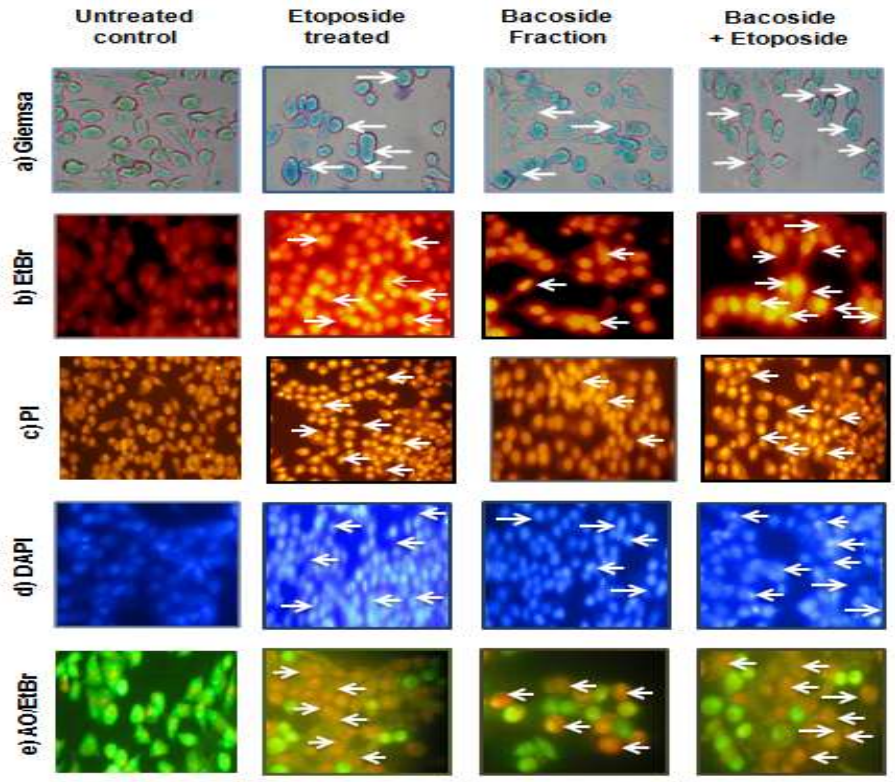


Figure 6

Effect of bacoside fraction on the morphological and nuclear changes in KB cells



It is clear from the results obtained with the viability assays and the staining techniques that the bacoside fraction exhibits an anticancer effect on KB oral carcinoma cells, but was non-toxic in normal buccal cells at the same dose.

DISCUSSION

Programmed cell death via apoptosis is characteristically disturbed in human cancers. This not only facilitates tumour formation and progression, but also treatment resistance. In order to bypass the resistance, a better understanding of the regulatory mechanisms that control cell death signaling pathways is needed. Many anticancer treatment strategies rely on intact cell death signaling pathways for their therapeutic efficacy. Thus, reactivation of cell death programs in cancer cells may open up new perspectives for more effective and more tumour-selective, yet less toxic anticancer therapies [6].

The MTT reduction assay was the first homogeneous cell viability assay developed for a 96-well format that was suitable for high throughput screening. The MTT tetrazolium assay has been widely adopted and remains popular in academic laboratories as evidenced by thousands of

published articles[24]. SRB cell viability assays were used to quantify growth inhibition after exposure to single drug and in combinations with other chemotherapeutic agents using the median effect principle[25].

The results of MTT and SRB assays demonstrated that the etoposide treatment caused a significant reduction in t he viability of the buccal cells. This was reverted back in the presence of the bacoside fraction indicating the anti-apoptotic effect in the primary buccal cell culture. In the case of KB cells, it caused an steep increase in the proportion of apoptotic cells in bacoside fraction treated group, which indicates the anticancer activity of the bacoside fraction.

These observations clearly indicate that, not only does the bacoside fraction possess anticancer activity against KB cells, but it also enhances specific cytotoxicity in the cancer cells alone. This differential pattern of action suggests that the bacoside fraction can be effectively used as a combinational therapy during chemotherapy to decrease the undesirable side effects.

Cell viability in a prostate cancer cell line was significantly lower in the micro-ribonucleic acid 29b plus cisplatin group, compared to that of the cisplastin control group[26]. A dose-dependent inhibiton of cell viability was observed in human urinary bladder cancer cells (TSGH-8301) when treated with various concentrations of apple polyphenols[27].

In the present study, Giemsa and the EtBr, PI, DAPI and AO/EtBr fluorescent stains were effectively used to characterize the extent of cell death in buccal and KB cells, in the presence and the absence of etoposide and / or bacoside fraction. The effect of bacoside fraction on both morphological and nuclear changes in both primary and cancer cells also proved the differential effect exhibited by the bacoside fraction in selectively killing the cancer cells and protecting the normal cells.

Light microscopic observation of breast cancer MCF-7 cells and normal human skin fibroblasts treated with the aqueous extract of *Lepidium sativum* showed typical apoptotic features in the cancer cells, but exhibited protective effect in the normal cells[28]. DAPI staining showed significant increase in the apoptotic cells with nuclear changes in IK and cisplatin-treated metastatic oral cancer cells HN4 and HN12 cells compared with the normal cells, which also confirmed the differential effect[29]. Various staining procedures, such as AO/EtBr, Giemsa, EtBr, propidium iodide and Hoechst 33342, showed that the methanolic extract of both the leaves and the rhizomes of *Curcuma amada* induced cell death in cancer cells through apoptotic pathway in breast cancer cell lines MCF-7 and MDA MB 231, at the same time protecting the non-cancerous HBL-100 breast cells, which confirmed the differential effect of the extract in killing the cancer cells along with normal cells[30]. Licochalcone A, a natural phenol licorice compound, induced the apoptotic effects in KB human oral cancer cells, but did not have any effect on primary normal human oral keratinocytes[31].

Thus, the present study showed the differential effect of the bacoside fraction, which selectively kills the cancer cells and protect the normal cells.

CONCLUSION

This is a very significant observation, as the bacoside fraction discriminates between the non-cancerous and cancerous cells, and targets its action on the cancer cells. It is inferable that the fraction is able to selectively recognize a specific component expressed by the cancer cells alone. More in-depth mechanistic studies are needed to confirm this influence and to identify the signal process involved.

REFERENCES

1. Iweala, E.E.J., Liu, F.F., Cheng, R.R., Li, Y., Omomhinmin, C.A. and Zhang, Y.J. (2015) Anti-cancer and free radical scavenging activity of some Nigerian food plants in vitro, Int. J. Cancer Res., 11, 41-51.

2. Entezari, M., Dabaghian, F.H. and Hashemi, M. (2014) The comparison of antimutagenicity and anticancer activities of Echinophora platyloba DC on acute promyelocytic leukemia cancer cells, J. Cancer Res. Ther., 10, 1004-1007.

3. Solowey, E., Lichtenstein, E., Sallon, S., Paavilainen, H., Solowey, E. and Galski, H.L. (2014) Evaluating medicinal plants for anticancer activity, Sci. World J., 2014, doi:10.1155/2014/721402.

4. Prasad, L.K. (2014) Burden of oral cancer: An Indian scenario, J. Orofac. Sci., 6, 77-83.

5. Lopez, J. and Tait, S.W.G. (2015) Mitochondrial apoptosis: killing cancer using the enemy within, Br. J. Cancer, 112, 957-962.

6. Fulda, S. (2015) Targeting apoptosis for anticancer therapy, Sem. Cancer Biol., 31, 84-88.

7. Ma, Y., Kanakousaki, K. and Buttitta, L. (2015) How the cell cycle impacts chromatin architecture and influences cell fate, Front. Genet., 6, doi:10.3389/fgene.2015. 00019.

8. Samavati, S.F., Motlagh, H.R.M. and Mostafaie, A. (2014) A highly pure sub-fraction of shallot extract with potent in vitro anti-angiogenic activity, Int. J. Mol. Cell. Med., 3, 237-245.

9. Akinboro, A., Mohamed, K.B., Asmawi, M.Z. and Yekeen, T.A. (2014) Antimutagenic effects of aqueous fraction of Myristica fragrans (Houtt.) leaves on Salmonella typhimurium and Mus musculus, Acta. Biochim. Pol., 61, 779-785.

10. Elumalai, P. and Arunakaran, J. (2014) Review on molecular and chemopreventive potential of nimbolide in cancer, Genomics Inform., 12, 156-164.

11. Verma, P., Singh, P. and Gandhi, B.S. (2014) Neuromodulatory role of Bacopa monnieri on oxidative stress induced by postnatal exposure to decabromodiphenyl ether (PBDE -209) in neonate and young female mice, Iran. J. Basic Med. Sci., 17, 307-311.

12. Gupta, P., Khatoon, S., Tandon, P.K. and Rai, V. (2014) Effect of cadmium on growth, bacoside A, and bacopaside I of Bacopa monnieri (L.), a memory enhancing herb, Sci. World J., 2014, doi: 10.1155/2014/824586.

13. Radha, P. (2010) Antioxidant responses evoked in vitro by Bacopa monnieri leaf extracts, A thesis submitted to Avinashilingam University for Women, Coimbatore in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Biochemistry.

14. Kahol, A.P., Singh, T., Tandon, S., Gupta, M.M. and Khanuja, S.P.S. (2004) Process for the preparation of the extract rich in bacosides from the herb Bacopa monnieri, US Patent, US 6833143B1.

15. Pradeep, M.R., Guruprasad, Y., Jose, M., Saxena, K., Deepa, K. and Prabhu, V. (2014) Comparative study of genotoxicity in different tobacco related habits using micronucleus assay in exfoliated buccal epithelial cells, J. Clin. Diagn. Res., 8, ZC21-ZC24.

16. Michalczyk, A., Varigos, G., Smith, L. and Ackland, M.L. (2004) Fresh and cultured buccal cells as a source of mRNA and protein for molecular analysis, Biotechniques, 37, 262-269.

17. Igarashi, M. and Miyazawa, T. (2001) The growth inhibiting effect of conjugated linoleic acid on a human hepatoma cell line Hep G2, is induced by a change in fatty acid metabolism but not the facilitation of lipid peroxidation in cells, Biochem. Biophys. Acta/Mol. Cell Biol. Lipids, 1530, 162-171.

18. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S. and Boyd, M.R. (1990) New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst., 82, 1107-1112.

19. Chih, H.W., Chill, H.F., Tang, K.S., Chang, F.R. and Wu, Y.C. (2001) Bullatacin a potent antitumour annonaceous acetogenin, inhibits proliferation of human hepatocarcinoma cell line by apoptosis induction, Life Sci., 69, 1321-1331.

20. Sarker, K.P., Obara, S., Nakata, M., Kitajima, I. and Maruyama, I. (2000) Anandamide induces apoptosis of PC-2 cells: Involvement of superoxide and caspases-3, FEBS Lett., 472, 39-44.

21. Mercille, S. and Massie, B. (1994) Induction of apoptosis in nutrient-deprived cultures of hybridoma and myeloma cells, Biotech. Bioengin., 44, 1140-1154.

22. Rashmi, R., Santhosh Kumar, T.R. and Karunagaran, D. (2003) Human colon cancer cells differ in their sensitivity to curcumin-induced apoptosis and heat shock protects them by inhibiting the release of apoptosis-inducing caspases, FEBS Lett., 538, 19-24.

23. Parks, D.R., Bryan, V.M., Oi, V.T. and Herzenberg, L.A. (1979) Antigen-specific identification and cloning of hybridomas with fluorescence-activated cell sorter, Proc. Nat. Acad. Sci. USA., 76, 1962-1966.

24. Riss, T.L., Moravec, R.A., Niles, A.L., Benink, H.A., Worzella, T.J. and Minor, L. (2013) Cell viability assays, assay guidance manual, Bookshelf ID: NBK144065.

25. Wu, J.Y., Yu, Z.L., Fong, W.F. and Shi, Y.Q. (2013) Chemotherapeutic activities of Carthami flos and its reversal effect on multidrug resistance in cancer cells, Afr. J. Tradit. Complement. Altern. Med., 10, 36-40.

26. Yan, B., Guo, Q., Nan, X.X., Wang, Z., Yin, Z., Yi, L., Wei, Y.B., Gao, Y.L., Zhou, K.Q. and Yang, K.R. (2015) Micro-ribonucleic acid 29b inhibits cell proliferation and invasion and enhances cell apoptosis and chemotherapy effects of cisplatin via targeting of DNMT3b and AKT3 in prostate cancer, Onco. Targets Ther., 8, 557-565.

27. Kao, Y.L., Kuo, Y.M., Lee, Y.R., Yang, S.F., Chen, W.R. and Lee, H.J. (2015) Apple polyphenol induces cell apoptosis, cell cycle arrest at G2/M phase, and mitotic catastrophe in human bladder transitional carcinoma cells, J. Funct. Foods, 14, 384-394.

28. Mahassni, S.H. and Al-Reemi, R.M. (2013) Apoptosis and necrosis of human breast cancer cells by an aqueous extract of garden cress (Lepidium sativum) seeds, Saudi J. Biol. Sci., 20, 131-139.

29. Shin, M.R., Lee, H.J., Kang, S.K., Auh, Q.S., Lee, Y.M., Kim, Y.C. and Kim, E.C. (2014) Isocudraxanthone K induces growth inhibition and apoptosis in oral cancer cells via hypoxia inducible factor-1, Biomed. Res. Int., 2014, doi: 10.1155/2014/934691.

30. Jambunathan, S., Bangarusamy, D., Padma, P.R. and Sundaravadivelu, S. (2014) Cytotoxic activity of the methanolic extract of leaves and rhizomes of Curcuma amada Roxb. against breast cancer cell lines, Asian Pac. J. Trop. Med., 7, 405-409.

31. Kim, J.S., Park, M.R., Lee, S.Y., Kim, D.K., Moon, S.M., Kim, C.S., Cho, S.S., Yoon, G., Im, H.J., You, J.S., Oh, J.S. and Kim, S.G. (2014b) Licochalcone A induces apoptosis in KB human oral cancer cells via a caspase-dependent FasL signaling pathway, Oncol. Rep., 31, 755-762.