

## Original Article

# Boeravinone B a natural rotenoid exerts anticancer activity via inducing internalization and degradation of inactivated EGFR and ErbB2 in human colon cancer cells

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**Abstract:** Background: Epidermal growth factor receptors (EGFR) are identified to be favorable targets for cancer treatment. In present work, we showed that Boeravinone B, a Rotenoid from natural origin has significant anticancer activity via internalization of ErbB2 and EGFR, and thereby resulting in destruction of the receptors. Methods: For cell viability and apoptosis were done by MTT assay. Annexin V-FITC staining was done for determining the extent of apoptosis. Immunoblotting for expression of proteins in HT-29 cell lysates after exposing them to Boeravinone G. Immunofluorescence and Confocal microscopic analysis was done for HT-29 cells incubated with anti-EGFR or anti-ErbB2 antibodies. Surface biotinylation assay was done followed by western blot analysis for expression of proteins using antibodies against transferrin receptor, ErbB2 and EGFR. Results: Exposure of HT-29 cells with Boeravinone B suppressed constitutive as well as ligand mediated phosphorylation of ErbB2, ErbB3 and EGFR. The treatment also inhibited the activation of mitogen-activated protein kinase (MAPK), Akt and Erk1/2 which are downstream signaling molecules. The treatment also brought about internalization of ErbB2 and EGFR causing destruction of receptors, Boeravinone B also caused apoptosis in HT-29 cells. Boeravinone B mediated degradation was halted by Chloroquine (lysosomal inhibitor). Boeravinone B caused nuclear translocation of apoptosis-inducing factor (AIF) and caused proteolytic processing of PARP along with caspase-3, confirming Boeravinone B may induce caspase-independent apoptosis in HT-29 cells. Conclusion: The findings of present study provide first ever evidences for Boeravinone B suggesting anticancer activity via internalization and destruction of EGFR family receptors i.e. ErbB2 and EGFR in HT-29 cell lines.

**Keywords:** Boeravinone, HT-29 cells, EGFR, internalization

## Introduction

Growth factors are discovered to be vital in the development and homeostasis of multi-cellular organisms. The epidermal growth factor family of receptor (EGFR) tyrosine kinases (ErbBs) plays a crucial role in cell regulation, survival, migration, proliferation and differentiation. There are about four members of the EGFR class named as ErbB4, ErbB2/Neu/HER2, EGFR/ErbB1 and ErbB3. All the four members have domain containing cytoplasmic tyrosine-kinase, a membrane-spanning region and also have an extracellular ligand-binding site. Binding of ligand to the site results in formation of receptor heterodimers and homodimers, and stimu-

lation of intrinsic kinase domain which further causes phosphorylation on specific tyrosine residues. These residues further act as docking sites for numerous proteins which trigger cellular signaling pathways including Ras/MAPK and phosphatidylinositol-3 kinase/Akt pathways [1-3].

The expression of EGFR receptors is found in epithelial, neuronal and mesenchymal tissues. The activation of EGFR is commanded by ligands belonging to EGF family growth factors [2, 3]. Among the EGFR receptors, EGFR and ErbB2 are over-expressed most commonly in human cancers and play a important role in there progression and malignancy [4-6]. The

## Boeravinone B exerts anticancer effect in human colon cancer cells

Over expression of EGFR and ErbB2 is found to be associated with shorter survival rates [7-10]. Overall the literature suggest activation of EGFR family receptors and associated pathways as potential targets for novel and selective anticancer therapies [3, 9]. Presently, therapies that target EGFR receptor family proteins are being used to encounter various types of cancer. Gefitinib, Erlotinib are EGFR kinase inhibitors, presently used to treat non-small cell lung cancer mediated EGFR gene mutations [11]. The Federal Drug Administration had approved monoclonal antibodies to EGFR and ErbB2 for treating tumors with high levels of EGFR and ErbB2.

Rotenoids, are class of compounds belonging to flavonoid family and are reported to possess anticancer properties [12, 13]. Literature evidence Rotenoids such as Tephrosin, Rotenone and Degulin to possess inhibitory effect on phorbol ester-induced ornithine decarboxylase activity in cancer [12]. Among the Rotenoids, Degulin has been found to exert anticancer effect by inducing apoptosis via cell cycle arrest in colon cancer [14]. In addition to this Degulin exerts anticancer effect via mitochondrial bioenergetics inhibition [15], down-regulation of phosphatidylinositol 3-kinase/Akt [17] and suppression of COX-2 [16].

Boeravinone B is natural Rotenoid isolated from *Boerhaavia diffusa* [18]. The herb has been used from ancient times to treat gastric alignments (abdominal pain and dyspepsia) [19]. Among the Rotenoid family, Boeravinone C and B are reported to show *P*-gp inhibitory activity [20], Boeravinone G has been found to exhibit geno-protective and antioxidant effect [21]. Till date none of the Rotenoid among Boeravinone family has been explored for their anticancer effect. Boeravinone B being a member of Rotenoid family, we postulated that it could exert anticancer effect on human colon cancer cells.

In the present research, we found that upon exposing H-29 (human colon cancer cells) to Boeravinone B caused in suppression of EGFR and ErbB2 along with Akt and Erk1/2. We also found that Boeravinone B caused apoptosis in human colon cancer cells independent of caspase.

## Materials and methods

### Cell culture and reagents

For the study human colon cancer cell lines HT-29, HCT-116 and SW-620 were selected and were obtained from American Type Culture Collection. The cells were maintained in The cell lines were cultured in Roswell Park Memorial Institute medium (RPMI medium) along with penicillin-streptomycin, 10% fetal bovine serum (FBS) previously inactivated by heat, 2 mM glutamine and 0.6% Pen-Strep in a humidified condition with 5% CO<sub>2</sub> maintained at 37°C. Chloroquine was procured from procured from Shanghai Xudong Haipu Pharmaceutical Co., China, Epidermal growth factor (EGF) from (Rocky Hill, NJ, USA). Boeravinone B and heregulin-b1 was procured from Sigma Aldrich, USA. BG was dissolved in dimethyl sulfoxide (DMSO) and finally diluted with culture medium so that concentration of DMSO was not more than 0.1%, for animal studies Boeravinone B was dissolved in corn oil [20] at dose of 50 mg/kg just prior to administration and was given by gavage route (intragastrically) throughout the study [21].

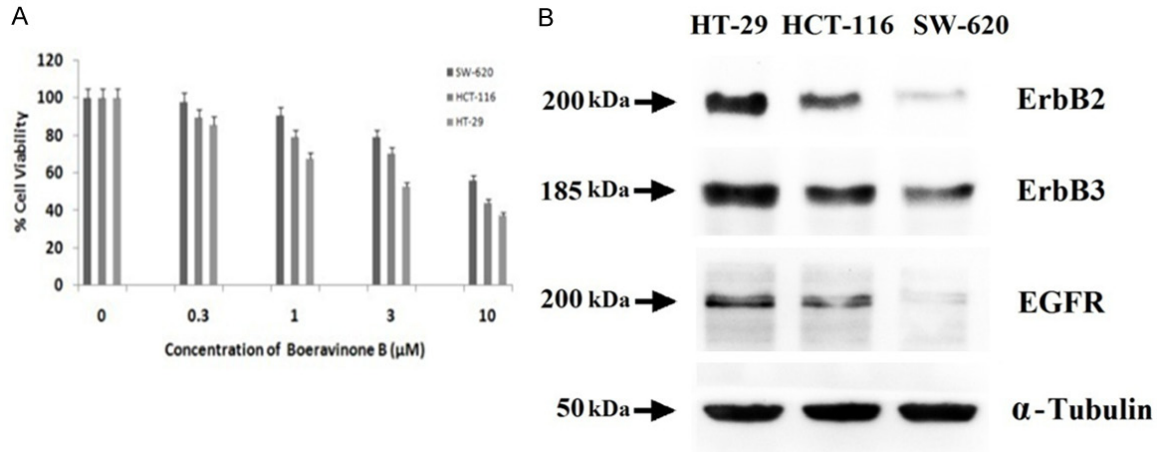
### Antibodies

Antibodies for AIF, PARP, Erk1/2, caspase-3, phospho-EGFR (Tyr1068), phospho-ErbB3 (Tyr1289), phospho-AKT (Ser473), phospho-Erk1/2 (Thr202/Tyr204), phospho-ErbB2 (Tyr877), Erk1/2, EGFR, GAPDH,  $\alpha$ -tubulin, ErbB2, transferring and ErbB3 were obtained from Cell Signaling Tech. USA.

### Cell viability and apoptosis assays

For evaluating cytotoxicity, the cells were subjected to seeding in 96-well microtiter plate having flat bottom at a density of  $1 \times 10^5$ /well. The cells were treated with Boeravinone G (0.1, 0.3 and 1 ng/ml) and vehicle comprising 0.1% DMSO for 24 followed by incubation with MTT solution (250  $\mu$ g/ml) for 60 min at 37°C. The formazan crystals were separated from supernatant and were solubilized in DMSO at room temperature for 15 min. The absorbance was recorded at 490 nm using a plate reader (Bio-Rad). The mean  $\pm$  SD for absorbance was calculated after three independent experiments. Annexin V-FITC apoptosis detection kit (Abcam, USA) was used for determining the extent of

## Boeravinone B exerts anticancer effect in human colon cancer cells



**Figure 1.** Effect of Boeravinone B on human colon cancer cell viability. A. The human colon cancer cells were treated with Boeravinone B for 48 h followed by MTT assay for cell viability, results are percentage mean  $\pm$  SD of the number cell of control (n = 2 experiments). B. Immunoblotting studies shows expression of ErbB2, ErbB3 and EGFR in selected three cell lines (SW-620, HCT-116 and HT-29),  $\alpha$ -tubulin was used as loading control.

apoptosis. The Annexin V staining was done in accordance to procedure given by supplier. Briefly, after incubating the cells were washed with 7.4 pH Phosphate buffer saline (PBS) followed by centrifugation at 5000 rpm, staining with annexin V-FITC and propidium iodide (2  $\mu$ g/ml) in a binding buffer (Thermo-Fisher USA). The samples were subjected to flow cytometry analysis (FACScan flow cytometer, BD Biosciences) using CellQuest software for data analysis (BD Biosciences).

### Immunoblotting studies

The proteins were extracted using ice-cold cell lysis buffer (Thermo Fisher USA). Nuclear extracts were prepared using nuclear extraction kit (Abcam USA) for analyzing nuclear translocation of AIF. Proteins were separated using SDS-PAGE by transferring them on PVDF membranes (0.45  $\mu$ m, Thermo Fisher, USA). The membranes before incubating them with corresponding antibodies were blocked with 5% skim milk. The membranes were again bind with secondary antibodies and were then coupled with horseradish peroxidase and then subjected to enhanced chemiluminescence for visualizing proteins.

### Immunofluorescence and confocal microscopy

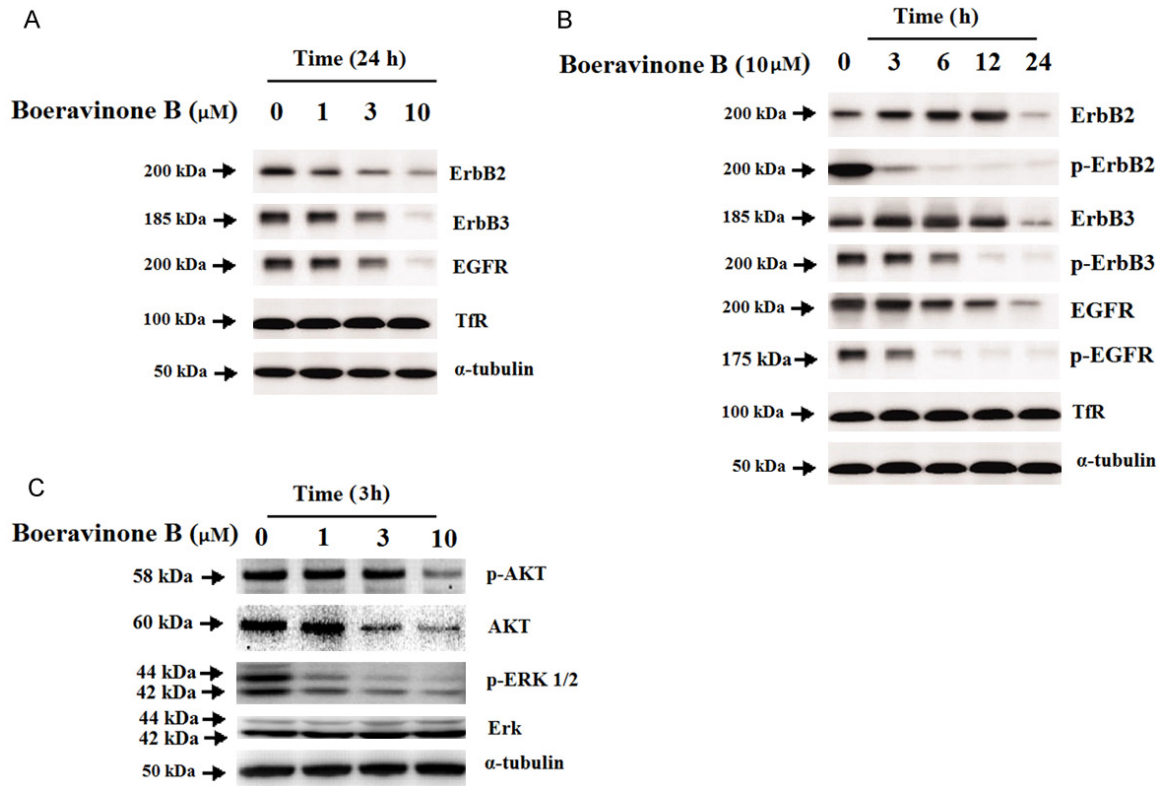
The cells were harvested and washed with PBS and then fixed in fresh para-formaldehyde (4%) for 20 min at 37°C, the cells were rinsed one

time using glycine (0.1 M) followed by washing with PBS. The cells were treated with Triton X-100 (0.5%) to increase permeability followed by washing with PBS. The cells were incubated with anti-EGFR or anti-ErbB2 antibody for 3 h at room temperature but prior to this the nonspecific sites in cell were blocked using goat serum (1% in PBS). The cells were washed using PBS followed by incubation with goat anti-rabbit IgG antibody for 60 min at room temperature in dark. The cells were then mounted using Vectashield mounting medium and subjected to confocal microscope (Zeiss) for capturing images.

### Surface biotinylation assay

For surface biotinylation assay the selected cancer cell lines were labeled using cell surface biotinylation kit (Thermo Fisher USA) as per manufacturer's protocol. The cells were then washed using Tris buffer saline (SigmaAldrich USA) followed by incubation in medium loaded with various concentrations (0.1, 0.3 and 1 ng/ml) of Boeravinone G for 24 h at room temperature, the cells were then cooled rapidly at 4°C to halt the trafficking. The Biotinylated proteins present on surface of cells were leached by a glutathione reagent (NaCl and glutathione 150 mM each, pH 8.75). The cells were then centrifuged at 10,000 g and supernatants were collected and incubated along with streptavidin beads for capturing biotinylated proteins. The incubated supernatants were washed using

## Boeravinone B exerts anticancer effect in human colon cancer cells



**Figure 2.** Effect of Boeravinone B on levels of p-ErbB2, p-ErbB3 and EGFR. A. The human colon cancer HT-29 cells were treated with 0, 1, 3 and 10  $\mu\text{M}$  concentration of Boeravinone B for 24 h followed by western blot analysis for expression of ErbB2, ErbB3, EGFR and transferrin (Tfr). B. The HT-29 cells were treated with Boeravinone B (10  $\mu\text{M}$ ) for 1, 3, 6, 12 and 24 h. The cell lysates were subjected to western blot analysis along with antibodies specific for ErbB2, p-ErbB2, ErbB3, p-ErbB3, EGFR, p-EGFR and Tfr. C. The HT-29 cells were treated with predefined concentrations of Boeravinone B for 3 h. The cell lysates were subjected to western blot analysis using antibodies specific for AKT, pAKT, Erk1/2 and p-Erk1/2. In all the three experiments the blots were compared against loading control  $\alpha$ -tubulin.

extraction buffer, the streptavidin beads were boiled in sample buffer for eluting biotinylated proteins. Western blotting was done for expressing proteins using antibodies against transferrin receptor, ErbB2 and EGFR.

### Statistical analysis

All the results were mean  $\pm$  Standard deviation. Paired students t-test was done to establish statistical significance, values of  $P < 0.05$  were regarded as significant.

### Results

#### Boeravinone B causes cell death in human colon cancer cells

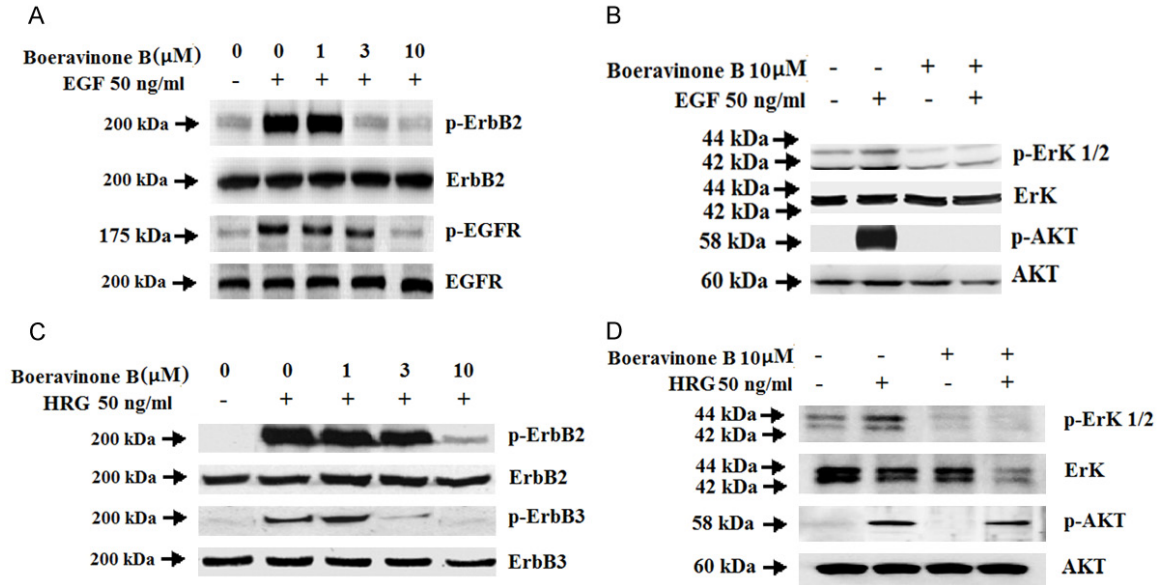
MTT assay was done to evaluate cytotoxic activity of Boeravinone B in the human colon cancer cell lines SW-620, H-29 and HCT-116 (Figure 1A). It was evidenced that concentra-

tion of 0.3-10  $\mu\text{M}$  of Boeravinone B resulted in a gradual decrease in cell proliferation in all the three human colon cancer cell lines in a dose dependent manner. The IC<sub>50</sub> values were found to be  $5.7 \pm 0.24$ ,  $8.4 \pm 0.37$ , and  $3.7 \pm 0.14$  for HCT-116, SW-620 and HT-29 respectively, indicating HT-29 as most sensitive cell lines among the three and was hence selected for the study. Further, in order to establish the expression of ErbB3, ErbB2 and EGFR in all the three human colon cancer cell lines, Immunoblotting studies (Figure 1B) were carried out, the outcomes suggested higher expression of ErbB3, ErbB2 and EGFR in HT-29 compared to HCT-116 and SW-620 cells.

#### Boeravinone B inhibits ErbB3, ErbB2 and EGFR phosphorylation

The outcomes of immunoblotting studies suggested HT-29 cell lines with higher expression of ErbB3, ErbB2 and EGFR and also were more

## Boeravinone B exerts anticancer effect in human colon cancer cells



**Figure 3.** Effect of Boeravinone B on activation of ErbB2, ErbB3 and EGFR mediated by EGF and HRG. **A.** The HT-29 cells were pre-treated with Boeravinone B at defined concentrations for 3 h followed by treatment with EGF (50 ng/ml) for 15 min. The whole cell lysates were incubated with antibodies specific for ErbB2, and phospho-ErbB2 and EGFR, phospho-EGFR. **B.** The human colon cancer HT-29 cells were pre-exposed with 10 μM concentration of Boeravinone B for 3 h followed by treatment of 15 min with EGF (50 ng/ml). The lysates were subjected to western blot analysis with antibodies specific for Erk1/2, pErk1/2, AKT and pAKT. **C.** The HT-29 cells after exposure to Boeravinone B for 3 h followed by treatment with HRG (50 ng/ml) for 15 min followed by stimulation with HRG (50 ng/ml). Western blot analysis was done for lysate along with antibodies specific for ErbB2, p-ErbB2, ErbB3 and p-ErbB3. **D.** The HT-29 cells were pre-exposed with 10 μM concentration of Boeravinone B for 3 h followed by treatment with HRG (50 ng/ml) for 15 min. The lysates were subjected to western blot analysis with antibodies specific for Erk1/2, pErk1/2, AKT and p-AKT.

sensitive to Boeravinone B mediated death, we postulated potential role of EGFR receptors in Boeravinone B mediated cell death. In order to establish this hypothesis, we evaluated effect of Boeravinone B on expression levels of these three EGFR family receptor proteins (**Figure 2A**). In the process, we treated HT-29 cells with gradually increasing concentrations of Boeravinone B for 24 h followed by evaluating expression of EGFR family proteins and transferrin using western blot. We found that exposure of Boeravinone B suppressed the levels of all the three EGFR family proteins in concentration dependent pattern, whereas Boeravinone B was not able to affect the levels of transferrin, proposing specific degradation activity of Boeravinone B against ErbB3, ErbB2 and EGFR proteins. Further, in a time dependent protocol involving exposing HT-29 cells to Boeravinone B, a non significant decrease in levels of ErbB3, ErbB2 and EGFR was observed until more than 12 h of exposing time, while the level of transferrin receptor was found to be stable until 24 h of treatment (**Figure 2B**). The results of cell

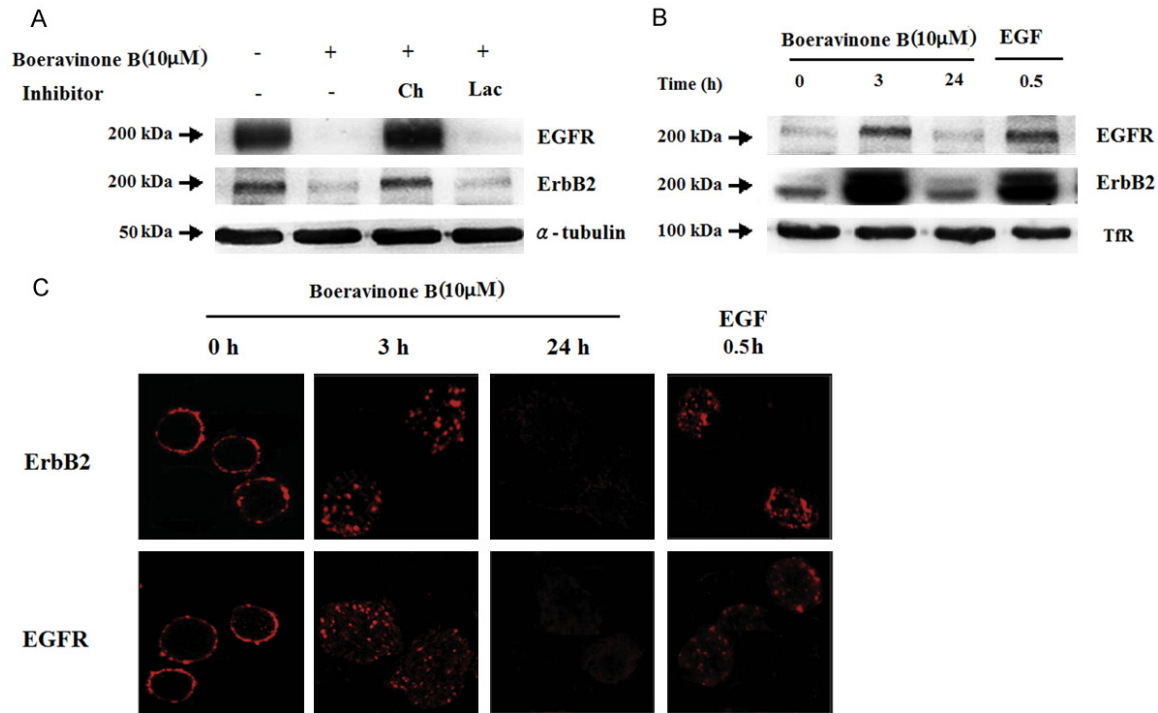
viability suggested about  $20 \pm 2.4\%$  reductions in cell viability count when the HT-29 cells were treated with 10 μM of Boeravinone B for 24 h.

On evaluating the effect of Boeravinone B on phosphorylation of ErbB3, ErbB2, EGFR, Akt and Erk1/2 MAPK we found that after treatment of 3h, a significant suppression in expression of p-ErbB3, p-ErbB2, p-EGFR, p-Akt and p-Erk1/2 was observed (**Figure 2B, 2C**) all these are regarded to be important markers among the EGFR family proteins [3]. In total, the results indicated ErbB3, ErbB2, EGFR inhibitory activity of Boeravinone B prior to their degradation.

### *Boeravinone B suppresses ligand mediated activation of EGFR family receptors*

To, confirm the Boeravinone B induced suppression of EGFR family receptors, we evaluated effect of Boeravinone B on levels of EGFR and ErbB3 induced by their respective ligands named EGF and HRG. The HT-29 human colon

## Boeravinone B exerts anticancer effect in human colon cancer cells



**Figure 4.** Boeravinone B leads to internalization of ErbB2 and EGFR in human colon cancer HT-29 cells. A. The HT-29 cells were treated with 10  $\mu$ M concentration of Boeravinone B for 24 h along with Lactacystin (5  $\mu$ M) and Chloroquine (30  $\mu$ M) followed by western blot analysis of lysates along with ErbB2 and EGFR antibodies. B. Western blot analysis for confirming internalization of ErbB2, EGFR and TIR by surface biotinylation assay in HT-29 cells treated with Boeravinone B, control and positive control (EGF treated for 0.5 h). C. The colon cancer HT-29 cells were treated with 10  $\mu$ M of Boeravinone B for 0, 3 and 24 h, after fixation and incubating cells with anti-EGFR or anti-ErbB2 antibodies followed by subsequent exposure to Alexa 546-conjugated anti-rabbit secondary followed by microscopic analysis.

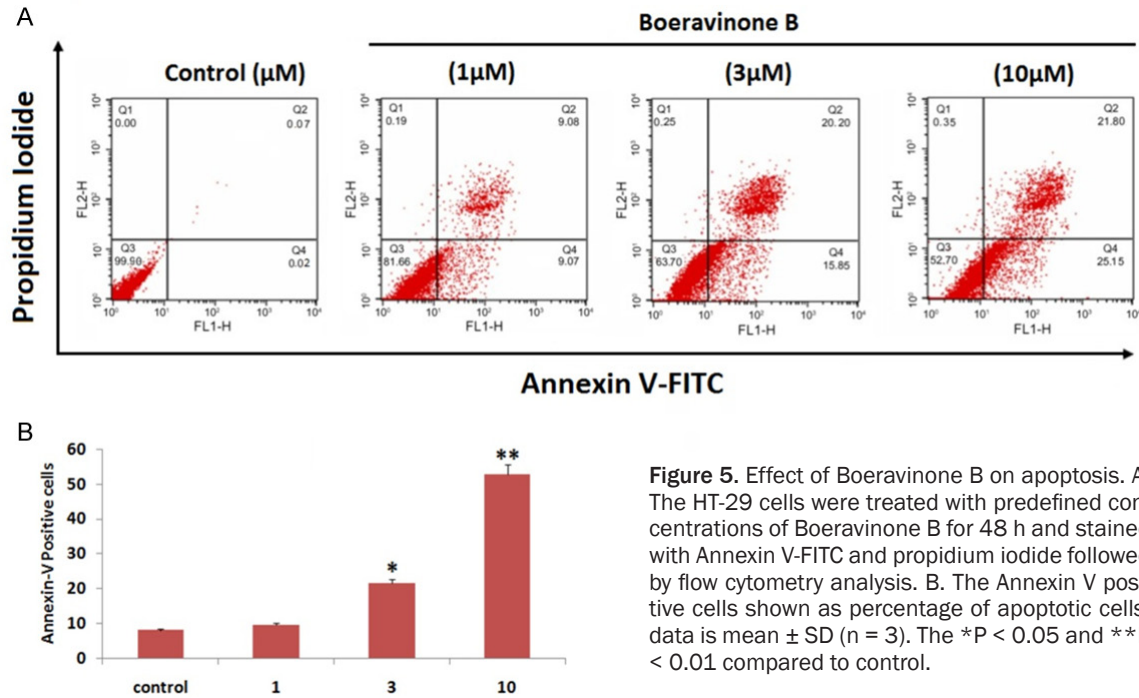
cancer cells were pretreated with Boeravinone B for 3 h were treated with EGF and HRG for 15 min followed by evaluation of p-EGFR and p-ErbB3 by western blot (**Figure 3A**). The experiment concluded that Boeravinone B suppressed EGF mediated phosphorylation of ErbB2 at Tyr877 and EGFR at Tyr1068 with increasing dose. At the same time, pre-exposure of Boeravinone B given to HT-29 cells for 3 h resulted in suppression of EGF mediated Akt and Erk1/2 activation (**Figure 3B**). We also found that, Boeravinone B suppressed HRG mediated phosphorylation of ErbB3 and ErbB2 also activation of Akt and Erk1/2 with increasing doses (**Figure 3C**). The outcome of the experiment confirmed that Boeravinone B could inhibit ligand-mediated activation of EGFR and its family receptors.

### *Boeravinone B causes degradation and internalization of EGFR and ErbB2*

In direction to identify the mechanism by which Boeravinone B inhibits activation of ErbB2 and

EGFR, we evaluated the effect of both lysosome inhibitor (Chloroquine) and proteasome inhibitor (lactacystin) on Boeravinone B mediated degradation of EGFR family receptors (**Figure 4A**). The outcome of experiment found that Chloroquine provided a significant protection to EGFR and ErbB2 against Boeravinone B mediated degradation of receptors, whereas Lactacystin failed to protect Boeravinone B mediated degradation of both the EGFR family receptors. The outcomes hence suggested involvement of lysosomal pathway in degradation of both ErbB2 and EGFR receptors. To confirm this outcome further, we had done immunofluorescence study for evaluating Boeravinone B mediated changes on cellular localization of both ErbB2 and EGFR. Upon exposing HT-29 cells to Boeravinone B (10  $\mu$ M) for 3 h resulted in significant internalization of ErbB2 and EGFR and the receptors degraded after 24 h, whereas on not treating the cells with Boeravinone B resulted in fluorescence staining to be localized on the cell surface (**Figure**

## Boeravinone B exerts anticancer effect in human colon cancer cells



**Figure 5.** Effect of Boeravinone B on apoptosis. A. The HT-29 cells were treated with predefined concentrations of Boeravinone B for 48 h and stained with Annexin V-FITC and propidium iodide followed by flow cytometry analysis. B. The Annexin V positive cells shown as percentage of apoptotic cells, data is mean  $\pm$  SD (n = 3). The \*P < 0.05 and \*\*P < 0.01 compared to control.

4C), in the study EGF was selected as positive control. The results also showed that EGF mediated stimulation to HT-29 cells for 30 min resulted in internalization of ErbB2 and EGFR (Figure 4C). Surface biotinylation assay was done to confirm the results (Figure 4B). Before exposing cells to Boeravinone B for 3 h/24 h HT-29 cells were biotinylated, after which proteins on cell surface were striped and cells were lysed. The obtained lysates were incubated with streptavidin beads and were subjected to SDS-PAGE followed by western blot studies. The western blots hardly showed the presence of internalized ErbB2 and EGFR proteins in untreated cells, on contrary significant presence was noticed in blots of cells treated with Boeravinone B for 3 h, whereas a complete absence was seen upon exposure time of 24 h, indicating Boeravinone B mediated degradation after 24 h. all together the outcome of experiment suggested that exposure of Boeravinone B in HT-29 cells could lead to internalization of ErbB2 and EGFR and thereby cause degradation of EGFR family receptors through lysosomal cascade.

### *Boeravinone B causes apoptosis independent of caspase*

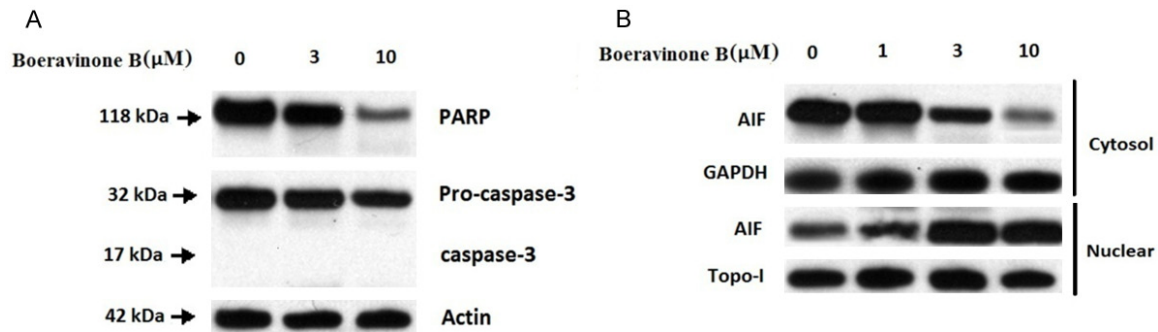
Apoptosis assay done by annexin V/PI staining suggested a dose-dependent correlation of

Boeravinone B (Figure 5). In direction to establish the possible mechanism responsible for apoptosis, we evaluated proteolytic processing of PARP which is caspase-3 substrate and caspase-3 using western blot analysis. Upon exposing colon cancer cells with Boeravinone B do not caused proteolytic processing of both PARP and caspase-3 (Figure 6A), a significant rise in the nuclear translocation of AIF was observed upon Boeravinone B treatment with increasing dose. These outcomes supported Boeravinone B may cause apoptosis in HT-29 cells independent of caspase.

### Discussion

The molecules of the family Rotenoids are discovered to possess anticancer activity against number of cancers. Deguelin a Rotenoid is found to possess anticancer activity against many types of cancers which include breast, colon and lung cancer [23-25]. Tephrosin Is found to exhibit anticancer activity against [26, 27]. Boeravinone B is a Rotenoid obtained from *Boerhaavia diffusa* [18], however no reports are present currently confirming anticancer effect and the mechanism involved of this Rotenoidal compounds. In present work we evaluated the anticancer effect of Boeravinone B on colon cancer HT-29 cell lines for the first time. In the study we established that

## Boeravinone B exerts anticancer effect in human colon cancer cells



**Figure 6.** A. Boeravinone B inhibited proteolytic processing of PARP and caspase-3. The HT-29 cells were treated with Boeravinone B for 48 h and subjected lysates for immunoblotting studies with anti-PARP and anti-caspase-3. B. Boeravinone B caused significant rise in nuclear translocation of AIF. The HT-29 cells were treated with various concentrations of Boeravinone B for 48 h, the fraction Cytosol and nuclear were immunoblotted with AIF antibody. The Topoisomerase-I was employed as nuclear protein marker, whereas GAPDH as cytosol marker.

Boeravinone B leads to internalization of EGFR family receptors i.e ErbB2 and EGFR without altering transferrin and thereby causing degradation in selected HT-29 cells. We found that Boeravinone B brings apoptosis independent of caspase in HT-29 cells, this finding can provide an important lead for its anticancer potential. Our experiments found that Boeravinone B elevated the internalization of both ErbB2 and EGFR bringing about destruction of receptors via lysosomal cascade. We also found that exposure of Boeravinone B to HT-29 cells brought about inhibition of EGFR family receptors both ligand-mediated as well as constitutive activated; hinting Boeravinone B induced internalization of both ErbB2 and EGFR as mechanism behind inhibition of these receptors as well as Erk1/2 MAPK and Akt pathways. Previously in study involving Rotenoid Tephrosin against human breast cancer cell line suggested involvement of similar pathway in their proliferation and progression, the results of present study confined to this study and suggested that the activity is not limited to a single cell line. The inhibitory activity of Boeravinone B on EGFR family receptors is accounted due to its ability to cause internalization of ErbB2 and EGFR mediated by endocytosis leading to unavailability of these receptors for ligands on the cell surface. This findings finally establishes here that the Boeravinone B internalized receptors degrade through lysosomal cascade. HRG has been reported to be a ligand specifically for ErbB3, which has been found to have vitiated activity for tyrosine kinase [28] and requires a dimerization collaborator like ErbB2 to get phosphorylated and gain signaling po-

tential [29]. The experiment confirmed that Boeravinone B mediated internalization of ErbB2 leads to a significant suppression of HRG mediated activation of ErbB3.

Previously studies have confirmed that exposure of EGF to cancerous cells leads to a speedy internalization of EGFR [30]. The binding of ligand causes down regulation of tyrosine kinases receptors as well as G-protein coupled receptors [31]. In present study we demonstrated that upon exposure of HT-29 cells with Boeravinone B for time period of 24 h caused in degradation of EGFR family receptors (ErbB2 and EGFR) and were protected when the cells were co-treated with Chloroquine (a lysosomal inhibitor). The results hence made a possible confirmation that pathways involved in degradation of ErbB2 and EGFR caused by Boeravinone B could be favorable targets to late lysosomes and endosome for proteolytic degradation. In the present study, we showed that exposure of Boeravinone B resulted in internalization of ErbB2 and EGFR confirmed by surface biotinylation assay and immunofluorescence staining. Still the detailed mechanism for Boeravinone B responsible for the internalization of ErbB2 and EGFR causing their destruction needs to be explored.

Reports published till now have confirmed role of ErbB2 and EGFR in colon cancer, inhibition of these receptors causes apoptosis [32]. In the current context, we demonstrated that exposure of Boeravinone B caused apoptosis in human colon cancer HT-29 cell lines. We made a confirmation that interfering in the activation



## Boeravinone B exerts anticancer effect in human colon cancer cells

of EGFR family receptors could be a potential strategy in designing therapies against number of cancers of colon exhibiting higher expression of these receptors.

Prevalence of ErbB2 and EGFR has been a prominent feature of number of tumors which in turn has lead in development of new molecules targeting these pathways. Boeravinone B could be a potential molecule in targeting these receptors (ErbB2 and EGFR) and could be used in combating cancers specifically exhibiting different expression levels of EGFR family receptors.

In conclusion, all the outcomes of the study confirm exposure of Boeravinone B resulted in internalization of ErbB2 and EGFR causing there degradation, inhibiting there activity and finally causing apoptosis in HT-29 cells. Our study explicates the anticancer potential of Boeravinone B via inhabiting ErbB2 and EGFR receptors.

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### Disclosure of conflict of interest

None.

### Abbreviations

EGFR, Epidermal growth factor receptors; MAPK, mitogen-activated protein kinase; AIF, apoptosis-inducing factor.

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## Boeravinone B exerts anticancer effect in human colon cancer cells

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