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Anticancer activity of isolated beta-glucan from *Chrocccoccus turgidus* (Kützing) Nägeli

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Abstract

Purpose: To evaluate the anti-cancer properties of the isolated beta glucan extract from *Chroococcus turgidus*
Methods: The extract was tested in Human breast cancer and Vero cell lines and examined for its effect on cell viability, nuclear morphology and sub-G1 formation. Cell viability was determined by micro culture tetrazolium technique (MTT), apoptosis using DNA fragmentation and capsase-3 assay
Results: The results showed isolated beta glucan had decreasing cell viability in a concentration-dependent manner. Altered cell morphology after treatment with the extract demonstrated that cells experienced apoptosis.
Conclusion: The data demonstrate that isolated beta glucan induced apoptosis in Human breast cancer MCF7 cells, and therefore, has a potential as an anti-cancer agent.

Keywords: MTT, Human lung cancer cells, *Chroococcus turgidus*, DNA fragmentation, MTT, Tryphan blue assay, Apoptosis, capase 3 assay, cell adhesion studies, wound healing

Introduction

Cyanobacteria have a long history of existence and use and are the oldest known oxygenic organisms, which by their photosynthetic activity probably made a fundamental contribution to the development of the present oxygen-rich atmosphere. However, the commercialization has been triggered off only recently. Cyanobacteria constitute a vast potential resource, but only a few species have been studied intensively and used commercially. They are cultivated for use as a health food in the form of tablets and capsules and mostly species of Spirulina are mass cultivated globally (Lee, 1997). It is a non-toxic protein-rich product with various pharmacological properties (Belay *et al*, 1993; Fox 1996). **β-Glucans** (**beta-glucans**) are polysaccharides of D-glucose monomers linked by β-glycosidic bonds. β-glucans are a diverse group of molecules that can vary with respect to molecular mass, solubility, viscosity, and three-dimensional configuration. It is becoming clear that β-glucans themselves have no direct cytotoxic effects. Studies implicating the cytotoxic effects of β-glucans were either from studies using crude extracts of β-glucan containing herbs or the use of β-glucan primed monocytes.

For β-glucan containing herbs like *Ganoderma lucidum* (Lingzhi), there are other active components such as ganoderic acid from its mycelium (Tang *etal*. 2006)
and triterpenes from its spore [(Lin ,2003, Min, 2000 and Muller,2006), which have all been shown to have direct anti-cancer effects independently. Intensive research is thus warranted to understand many of the basic aspects pertaining to the production of a metabolite with the concurrent evolution of applied research towards the large-scale production of the product.Therefore, the aim of this study was to isolate the beta glucan from *chroccocus turgidus* and studies its anticancer activity in cell lines.

Materials and Methods

Cyanobacterial culture

Chroococcus turgidus, a cyanobacterium was obtained from the culture collection of Vivekananda Institute of Algal technology (VIAT) Chennai. Biomass was obtained by growing algal cultures in 20L of water and 0.25g / L of NPK fertilizer was added with a facility to pump the culture with aeration pump. The algae was grown for 20 days and harvested.

Extraction and Estimation of Beta-glucan

Extraction and Drying

The *Chroococcus turgidus* were air-dried at room temperature (30°C) for two weeks, after which it was ground to a uniform powder. The extracts of the dried samples were prepared in a sequential procedure by soaking 20 g of dried powder in 60 ml of 80%methanol for 48 h. procedure was repeated. At the end of each respective extraction,

the extracts were filtered using Whatman no.1 filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator (Super fit-rotavap, India). The percentage yield of extracts was calculated

Anticancer in cell lines

MCF7 Cell Line

Cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents. MEM, Fetal bovine serum (FBS), Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and **MCF7** Dimethyl sulfoxide (DMSO) were purchased from Hi media & Sigma Aldrich Mumbai.

In vitro assay for Cytotoxicity activity (MTT assay).

The anticancer activity of samples on **MCF7** was determined by the MTT assay (Mosmann *et al*.,1983). Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO_2 incubator for 72 hours. Then, add various concentrations of the samples in 0.1% DMSO for 24hrs at 5 % CO_2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm.

Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the samples on the proliferation of **MCF7** cells was expressed as the % cell viability, using the following formula:

Calculation

% cell viability = A540 of treated cells / A540 of control cells × 100%

Data analysis

The IC50 values (concentration at which 50% of cells were death) are reported as mean± standard deviation of three independent experiments. The IC50 values against the human cancer cell lines were calculated for the solvent extracts inhibiting at least 50% inhibition when tested at a concentration. One-way analysis of variance (ANOVA) and Student t-tests were used to compare data using Statistical version 5.0 software at a 95% confidence limit.

DNA Fragmentation Analysis

A semi quantitative method for measuring apoptosis was described by Bortner, *et. al* (1995).Fragmentation of chromatin to units of single or multiple nucleosomes that form the nucleosomal DNA ladder in agarose gel is an established hallmark of programmed cell death or apoptosis. Briefly, the cells were cultured in 100 mm dishes, treated with sardine oil for 48 h. Following this treatment, the cells were washed with PBS (pH 7.5), harvested and pelleted by centrifugation (12000 rpm) at 4 ˚C. The pellet was incubated with DNA lysis buffer (10 nMTris pH (7.5), 400 mM NaCl, 1mM EDTA and1%Triton X-100) for 30 min on ice and then centrifuged at 12000 rpm. The supernatant that was obtained was incubated overnight with RNase (0.2mg/ml) at room temperature and then with proteinase K (0.1mg/ml) for 2 h at 37 ˚C. DNA was extracted using Phenol: choloroform: isoamylalcohol (25:24:1) mixture and precipitated with 0.1M of sodium acetate and 2 volume of absolute ethanol. Equal amount of DNA samples (20 µg) were electrophoresed on a 1.5% agarose gel in Tris-borate EDTA buffer and visualized by ethidium bromide staining.

Tryphan blue exclusion test of cell viability

The inhibition of cell proliferation by the crude aqueous extract was also checked by using the vital dye Trypan Blue having a negative chromophore interacts only with damaged cell membranes of dead cells. Viable cells exclude this dye. MCF7 cells were plated at an initial density of $1x10^5$ cells per well in a 6 well plate (HiMedia) and incubated in 2 ml MEM Eagle medium for 24 hrs at 37ºC with 5% CO_2. After incubation the old medium was discarded and fresh medium was poured in each well having 0 and 250 µl/ml each AAE treatments in duplicate and incubated for 24 hrs keeping earlier culture conditions similar. After incubation the medium of each well containing detached dead cells was collected in separate micro centrifuge tubes along with attached cells that were scraped using sterile cell scraper (Corning).

The cell suspensions were centrifuged at 6000 x g, 4ºC to pellet down the cell and rewashed with Phosphate Buffered Saline (PBS) pH 7.4 and centrifuged again, finally dissolved in 0.5 ml PBS in sterile vials. Then 0.1 ml of 0.4 % (w/v) Trypan Blue (Sigma) solution was added to each vial and allowed to stand at room temperature for 5 min, filled in haemocytometer (Marienfeld-Neubauer) and cell count was performed under 10X magnification of a phase contrast microscope (Olympus). The percent inhibition of cell proliferation was calculated as follows**;**

% inhibition = (Number of dead cells/ Number of total cells) x 100

To calculate the number of viable cells per ml of culture, use the formula below. Remember to correct for the dilution factor

$$\textbf{Number of viable cells} \times 10^4 \times 1.1 = \textbf{cells/ml culture}$$

Caspase assay

Caspases activities were determined by chromogenic assays using caspase-3and caspase-9 activation kits according to the manufacturer's protocol (Calbiochem, Merck). After treated with designated concentrations of active protein fraction (0 µg/ml (control), 50, 75 and 100µl), cell lysates were prepared by incubating 2×10^6 cells/ml in cell lysis buffer for 10 min on ice. Lysates were centrifuged at 10,000×*g* for 1 min. The supernatants (cytosolic extract) were collected and protein concentration was determined by the Lowry's method using BSA as a standard. Cellular protein was diluted in 50 µl cell lysis buffer for each assay. Cellular protein was then incubated in 96-well microtiter plates with 5 µl of the 4mM p-nitroanilide (pNA) substrates, DEVD--pNA (caspase-3 activity) for 2 h at 37°C. Caspase activity was measured by cleavage of the above substrates to free pNA. Free pNA (cleaved substrates) was measured by absorbance at 405nm in a microtiter plate reader. Relative caspase-3 activity was calculated using the following formula

$$\text{Activity, µmol pNA/min/ml} = \frac{\text{OD x d}}{\varepsilon\text{mM x t x v}}$$

Where: εmM = 10.5

v - volume of sample in ml; d - dilution factor; t - reaction time in minutes

Cytotoxicity effect of *Chroococcus turgidus* in VERO Cell Line

Cell line

Cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents. MEM, Fetal bovine serum (FBS), Trypsin, Dimethyl sulfoxide (DMSO) was purchased from Hi media & Sigma Aldrich Mumbai.

Trypsin, PBS, Versene, Glucose solution: (TPVG)

2% Trypsin: 100 mL

2 g of trypsin was weighed accurately and dissolved in 100 mL sterile Millipore distilled water with magnetic stirrer for ½ hr.it is then filtered through membrane filter. Store at -20 °C.

0.2% EDTA (Versene)

200 mg of EDTA was weighed 200 mg accurately and dissolved in 100 mL of sterile Millipore distilled water. The solution was kept in autoclave at 15 lbs / 15 min.

10% Glucose A.R. - 10mL

Weigh 1g of Glucose accurately, dissolve in 10 mL of sterile Millipore distilled water and filter through Whatman filter paper and autoclave at 15 lbs / 15 min.

TPVG – 1000mL

PBS	- 840 mL
2% Trypsin	- 50 mL
0.2% EDTA	- 100 mL
10% Glucose	- 5 mL
Penicillin & Streptomycin	- 5 mL

Media preparation

MEM 9.5g – 900ml D.Dwater
Sodium bicarbonate 3.75g – 50ml
L-glutamine -0.3g/10 ml – sterile it. Cool it.
Mix with 900ml mem (yellow) + 30 ml sodium bicarbonate+ 10 ml L glutamine + 1ml of 1mg/ml streptomycin+5mg/ml 20µl of Amphotericin B. change colour red adjust the ph 7.2-7.4 change pale colour orange colour. Filter it syringe filter 0.45µm. store it 4C.Serum media 10% FBS (10ml serum +90 ml MEM)

Sub culturing of cells

1. Bring the medium and TPVG to room temperature.
2. Observe the tissue culture bottles for growth, cell degeneration, pH & turbidity. Select the bottles for splitting.
1. Remove the medium using a 10ml pipette.
2. Gently rinse the cells with PBS.
3. Add 4ml of TVPG (prewarmed to 37°C) over the cells.
4. Allow TPVG to act for 1-2 minutes
5. Discard the TPVG and add 5ml of 5% MEM
6. Break off the cell clusters by gently pipetting back and forth with pipette.
7. Add 1ml of cells /wells incubate at 5% CO_2 incubator.

Wound healing activity of *Chroococcus turgidus*

Cells were plated in 1 ml of medium/well in 6-well plates. Incubate at 5 % CO_2 incubator for 72 hours. Draw a line with a marker on the bottom of the dish. Using a sterile 200 µl pipet tip, scratch one separate wound through the cells moving perpendicular to the line drawn in the step above. Rinse the cells (very gently as sheets of the cells may lift off if you are not careful) with PBS. Then, add 1 ml of sample for 24hrs at 5 % CO_2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4).and replace with 1.5 ml of media. Take the photo 10X & 20X Inverted microscope (Lobomed).Incubate at 24hrs. Check it wound is healing or not.

Cell Adhesion Analysis of *Chroococcus turgidus*

Procedure:

Cover slips were placed in 6 well plates and added 2 ml of Cells each wells. Incubate at 37°C in 5% CO2 incubator for 72 hrs. Add 500µl of sample and incubate at37°C in 5% CO2 incubator for 24 hrs. Wash the wells with sterile 1x PBS buffer (Phosphate buffered saline). Add 0.5 ml of Lactobacillus broth cultures in each well. Incubate at 37°C in 5% CO2 incubator for 2-3 hrs. After incubation wash the wells with 1x sterile PBS for 4-5 times. The cells were fixed with methanol, Gram-stained, and examined microscopically under oil immersion.

Statistical Analysis

A.Student's t- test was used for the analysis of paired samples during the effluent treatment process. This statistical analysis was done to determine whether the difference is significant or not. The t-test based on paired observations is defined by the following formula:

$$t = \frac{\bar{d}}{SE_d}$$ Where, $\bar{d}$ = mean of the difference between paired values and SE_d = standard error of the difference.

B. Statistical analysis by Online software method (http://www.easycalculation.com/statistics/standard-deviation.php).

Results

Extraction of Beta-glucan from *Chroococcus turgidus*

Using the above mentioned procedure, the cyanobacterium chrococcus turgidus dried biomass from was analyzed. The extract was used for further analysis. The percentage yield of extracts was calculated. The yield beta-glucan obtained (0.0344g/10g) of 80% methanol extract from the *Chroococcus turgidus* algal dried biomass.

Studies on anticancer activity of *Chroococus turgidus* in MCF7 Cell Line

Anticancer properties were studied from isolated beta-glucan extract of *Chroococus turgidus* in MCF7 Breast cancer cells. The assays consisted of MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide)) assay, viable cell count, DNA fragmentation, and capase -3-assay . The extract from *Chroococus turgidus* was also tested for their proliferation inhibition ability of MCF7 cancer cells. The results obtained from these studies are discussed below.

MTT assay

Data on the cytotoxic effects of *Chroococus turgidus* extract using MCF7 cancer cell lines in vitro are showed in Figure 1. *Chroococus turgidus* extract has cytotoxic effects in vitro at clinical acceptable concentrations (IC_{50} values ≤ 50 mg L-1) by MTT method. The cytotoxic effect of *Chroococus turgidus* extract was determined using concentrations ranging 0 to 1000 µg/ml for 48 hrs. After 48 hrs exposure, *Chroococus turgidus* extract induced concentration-dependent cytotoxic effects in MCF7 Cell Lines with 1000µg/ml of cell viability 8.4% in MCF7 cells using MTT method. Differential inhibitions of compounds with incubation time could be due to their solubility, which determines their bio-accessibility (Plate 1).

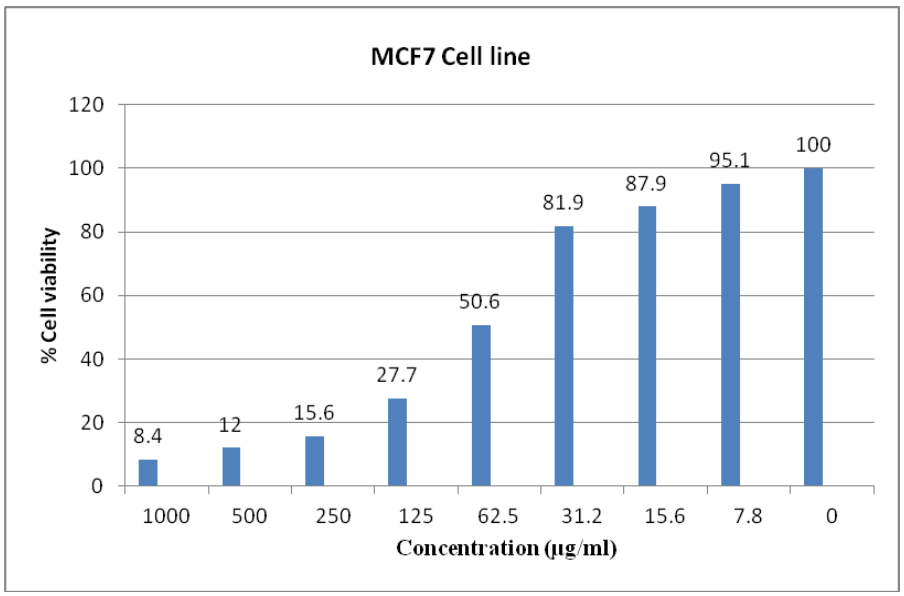

Figure 1: Cytotoxicity effect of *Chroococcus turgidus* on MCF7 Cell Line

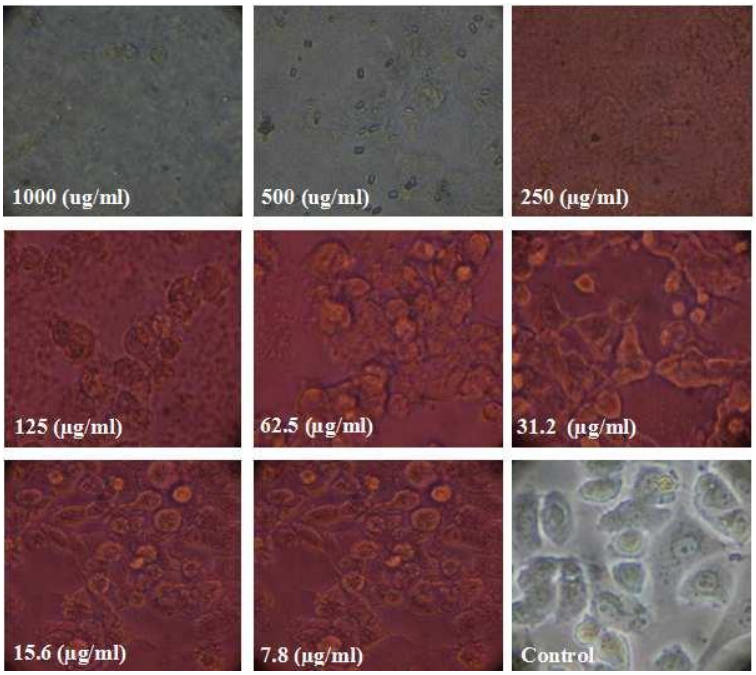

Plate 1: Cytotoxicity Effect of *Chroococcus turgidus* on MCF7 Cell Line

DNA Fragmentation of *Chroococus turgidus*

Validation of apoptosis measurement by DNA laddering

In the results, the MCF7 cells were treated with cyanobacterial extract, and the DNA was directly extracted and run on agarose gel. DNA fragmentation, if presented, was seen as a stepwise ladder of DNA fragments. The data (Plate 2) shows that DNA laddering is pronounced for *Chroococus turgidus* extract (31.25µg/ml) in MCF7 cells. These results confirm that cyanobacterial extract can induce apoptosis of MCF7 cells.

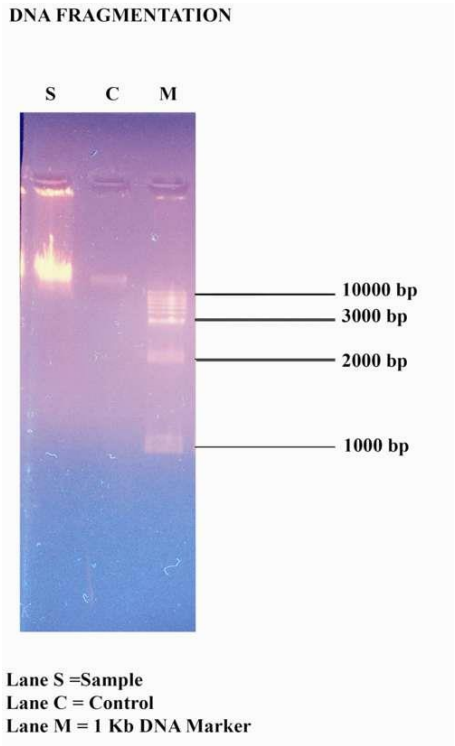

Plate 2: DNA Fragmentation Analysis of *Chroococcus turgidus*

Apoptosis DNA Fragmentation is a key feature of programmed cell death and also occurs in certain stages of necrosis. Apoptosis is characterized by the activation of endogenous endonucleases with subsequent cleavage of chromatin DNA into internucleosomal fragments of 180 BP and multiples there of DNA cleavage during apoptosis occurs at sites between nucleosomes, protein-containing structures that occur in chromatin at -200-BP intervals. This DNA fragmentation is often analyzed using agarose gel electrophoresis to demonstrate a "ladder" pattern at ~200-BP intervals.

Tryphan blue exclusion test of cell viability

Trypan blue is a diazo dye that has been widely used to colour dead tissues or cells selectively. The mechanism of trypan blue staining is based on it being negatively charged and not interacting with cells unless the membrane is damaged. Indeed, undamaged cells are very selective concerning the compounds that pass through their membrane, and thus should not take up trypan blue. Therefore, all the cells that exclude the dye are considered viable. By contrast, cells with damaged membranes are stained in a distinctive blue colour readily observed under a microscope. Thus trypan blue dye is described as being a vital stain allowing discrimination between viable cells and cells with damaged membranes that are usually considered to be dead cells. In vitro cytotoxicity studies by tryphan blue assay was employed to assess the cytotoxic potential of *Chroococcous turgidus*. The principle is based on the fact that the live cells with rigid cell membrane would not uptake the tryphan blue dye whereas the dead cells with disrupted cell membrane would uptake tryphan blue. When different concentrations of beta-glucan extract of *Chroococcous turgidus* were assayed, the dead cells were found to increase with increase in concentration of the extracts. The % cytotoxicity was found to be increasing with increase in concentration of extract that shows the anticancer property of *Chroococcous turgidus* that is indicated in Figure 3. In vitro cytotoxicity studies by tryphan blue assay was employed to assess the cytotoxic potential of the *Chroococcous turgidus*. The principle is based on the fact that the live cells with rigid cell membrane would not uptake the tryphan blue dye whereas the dead cells with disrupted cell membrane would uptake tryphan blue. When different concentrations of beta-glucan extract of *Chroococcous turgidus* were assayed, the dead cells were found to increase with increase in concentration of the extracts. The results showed that Total Cell counted (248 x10^5 cells/ml), Viable cell counted (197 x10^5 cells/ml) and Dead Cell counted (51x10^5cells/ml) respectively.

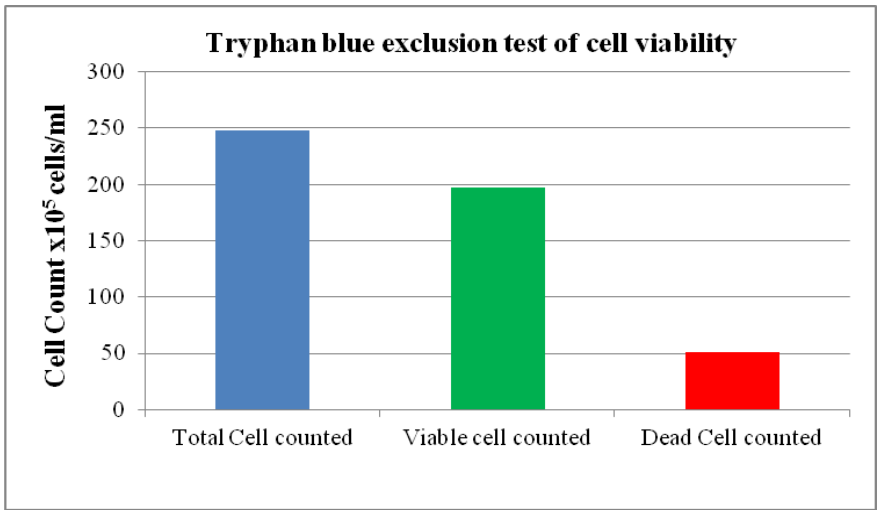

Figure 3: Tryphan blue exclusion test of cell viability

Caspase assay

Caspase activities of *Chroococus turgidus* were determined by chromogenic assays using caspase-3and caspase-9 activation kits according to the manufacturer's protocol (Calbiochem, Merck). After treated with designated concentrations of active protein fraction (0 µg/ml (control), 50, 75 and 100µl), cell lysates were prepared by incubating 2×10^6 cells/ml in cell lysis buffer for 10 min on ice. Lysates were centrifuged at 10,000×*g* for 1 min. The supernatants (cytosolic extract) were collected and protein concentration was determined by the Lowry's method using BSA as a standard. Cellular protein was diluted in 50 µl cell lysis buffer for each assay.

Cellular proteins were then incubated in 96-well microtiter plates with 5 µl of the 4mM p-nitroanilide (pNA) substrates, DEVD--pNA (caspase-3 activity) for 2 h at 37°C. Caspase activity was measured by cleavage of the above substrates to free pNA. Free pNA (cleaved substrates) was measured by absorbance at 405nm in a microtiter plate reader. Relative caspase-3 activity was calculated using the standard formula. The Caspase activity of *Chroococus turgidus* was estimated the results indicated that, activity of Caspase assay levels were found to high 0.159 Caspase 3 Activity in (µmol pNA/min/ml)/ 75µg concentration of algal sample (Figure 4).

Cytotoxicity effect of *Chroococcus turgidus* in VERO Cell line

Cytotoxic effects of *Chroococus turgidus* extract using VERO cell lines in vitro are showed in Figure 5. *Chroococus turgidus* extract has cytotoxic effects in vitro at clinical acceptable concentrations (IC_{50} values ≤ 50 mg L-1) by MTT method. The cytotoxic effect of *Chroococusturgidus* extract was determined using concentrations ranging 0 to 1000 µg/ml for 48 hrs. After 48 hrs exposure, *Chroococus turgidus's* beta-glucan extract induced concentration-dependent cytotoxic effects in VERO Cell Lines with 1000µg/ml of cell viability 24.7% in VERO cells using MTT method. Differential inhibitions of compounds with incubation time could be due to their solubility, which determines their bio-accessibility (Plate 3).

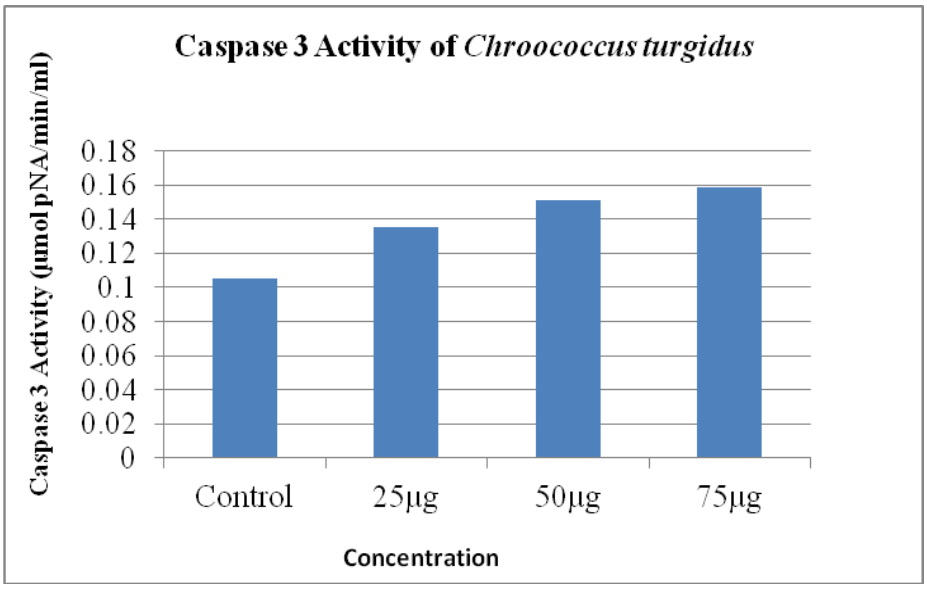

Figure 4: Caspase assay of *Chroococcus turgidus*

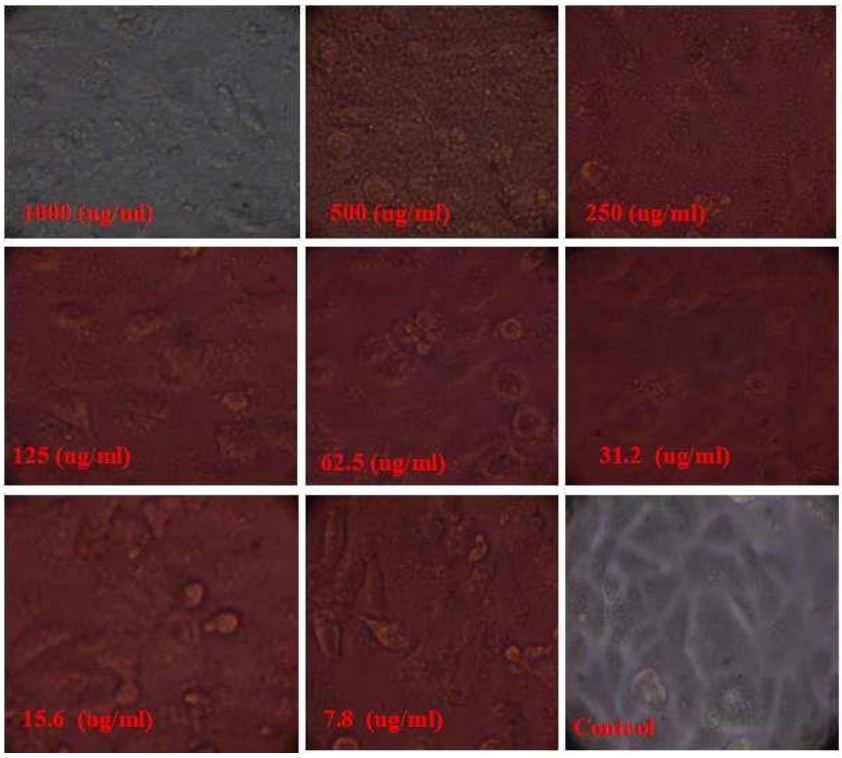

Plate 3: Cytotoxicity Effect of *Chroococcus turgidus* on VERO Cell Line

Wound healing activity of *Chroococcus turgidus*

The wound healing activity of *Chroococus turgidus* was estimated by using VERO cell lines method. The Cells were plated in 1 ml of medium/well in 6-well plates. Incubate at 5 % CO_2 incubator for 72 hours. Draw a line with a marker on the bottom of the dish. Using a sterile 200 µl pipet tip, scratch one separate wound through the cells moving perpendicular to the line drawn in the step above. Rinse the cells (very gently as sheets of the cells may lift off if you are not careful) with PBS. Then, add 1 ml of sample for 24hrs at 5 % CO_2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4).and replace with 1.5 ml of media. Take the photo 10X & 20X Inverted microscope (Lobomed) Incubate at 24hrs. Check it wound is healing or not. The result showed that (Plate 4) the significant level of wound healing activity was found when used *Chroococus turgidus* algal biomass extract against to VERO cell lines.

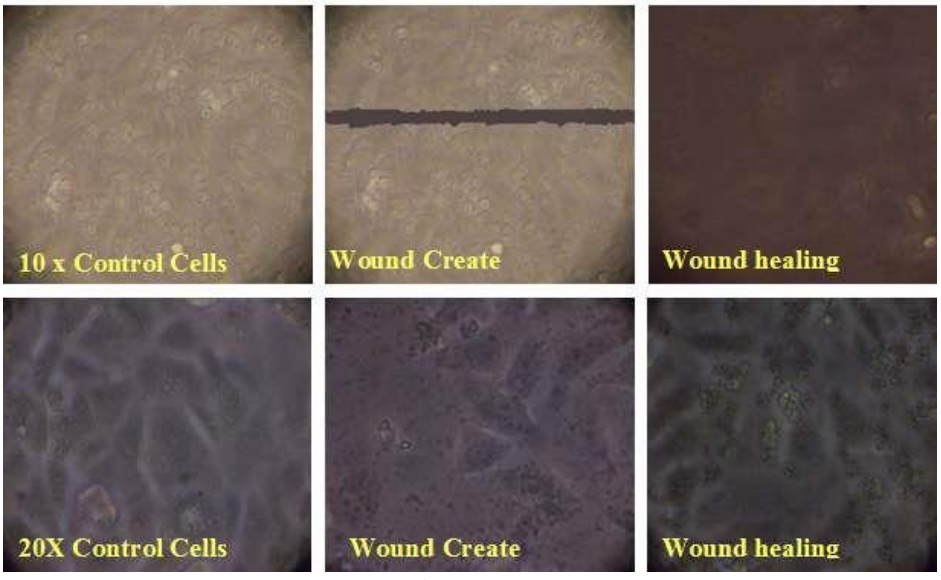

Plate 4: Wound Healing Activity of *Chroococcus turgidus* on VERO Cell Line

Cell Adhesion effect of *Chroococus turgidus*

The cell adhesion activity of *Chroococus turgidus* was estimated by using bacteria *Lactobacillus* sp. method. Cover slips were placed in 6 well plates and added 2 ml of Cells each wells. Incubate at 37°C in 5% CO_2 incubator for 72 hrs. Add 500µl of sample and incubate at37°C in 5% CO_2 incubator for 24 hrs. Wash the wells with sterile 1x PBS buffer (Phosphate buffered saline). Add 0.5 ml of *Lactobacillus* sp. broth cultures in each well. Incubate at 37°C in 5% CO_2 incubator for 2-3 hrs. After incubation wash the wells with 1x sterile PBS for 4-5 times. The cells were fixed with methanol, Gram-stained, and examined microscopically under oil immersion. The result showed that (Plate 5) the significant level of cell adhesion activity was found when used *Chroococus turgidus* algal biomass extract against to *Lactobacillus* sp.

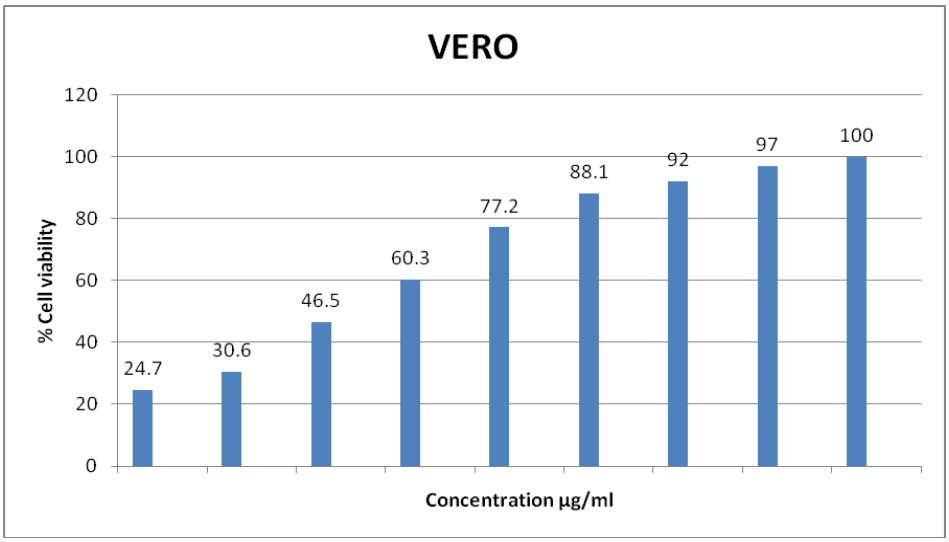

Figure 5: Cytotoxicity effect of *Chroococcusturgidus* in VERO Cell line

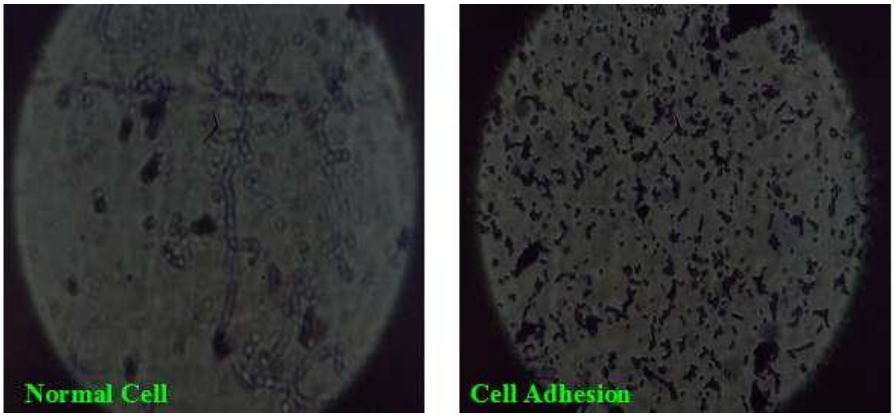

Plate 5: Cell Adhesion Effect of *Chroococcus turgidus*

Discussion

The extract from *Chroococus turgidus* exhibited significant inhibition of MCF7 cells with IC50 values in the range of (1.953- 1000) µM in MTT assay. Based on the MTT assay results, was conducted to investigate whether *Chroococus turgidus* extract induced apoptosis. Higher concentrations of *Chroococus turgidus* extract appeared to cause more morphological changes, indicating that apoptosis was present based on the concentration of extract. DNA fragmentations were studied in cells treated with extract in order to understand the mechanism.

MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide)) assay

In order to know the effect of isolated beta glucan extracted from Chroococus *turgidus* on breast cancer cells, experiments were conducted using cultured human breast carcinoma cells. Streptomycin was used as positive

control for the comparison of inhibitory potentials. The cytotoxic effect of *Chroococus turgidus* extract was determined using concentrations ranging 0 to 1000 µg/ml for 48 hrs. After 48 hrs exposure, *Chroococus turgidus* extract induced concentration-dependent cytotoxic effects in VERO Cell Lines with 1000µg/ml of cell viability 24.7% in VERO cells using MTT method. Dose dependent inhibition of MCF7 cells was observed at different concentrations, the cell viability showed (8.4%/1000 µg/ml) of *Chroococus turgidus* extract. Cell proliferation was highly significant at concentrations above 50 µg/ml ($P<0.001$) and dose dependent inhibition of VERO cells was observed at different concentrations, the cell viability showed (0.25%/1000 µg/ml) of *Chroococus turgidus* extract. Cell proliferation was highly significant at concentrations above 50 µg/ml $P<0.001$).Recent results have demonstrated the inhibition of proliferation of number of cancer cells by *Chroococus turgidus* extract.

DNA Fragmentation of MFC7 cells

Most of the anticancer drugs in current use have been shown to induce apoptosis insusceptible cells (**John *etal*., 1992**). German scientist Carl Vogt was first to describe the principle of apoptosis in 1842, which is a programmed cell death that may occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include loss of cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. One of the later steps in apoptosis is DNA fragmentation, a process which results from the activation of calcium and magnesium dependent nucleases which degrade DNA endonucleases during the apoptotic program. These nucleases degrade the higher order chromatin structure into fragments of ~300 kb and subsequently into smaller DNA pieces of about 50 bp in length. Excessive apoptosis causes atrophy, such as in ischemic damage, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer (**Kerr,1972 and Gavrieli.,1992).**

Tryphan Blue Assay

Trypan blue is a diazo dye that has been widely used to colour dead tissues or cells selectively. The mechanism of trypan blue staining is based on it being negatively charged and not interacting with cells unless the membrane is damaged. Indeed, undamaged cells are very selective concerning the compounds that pass through their membrane, and thus should not take up trypan blue. Therefore, all the cells that exclude the dye are considered viable. By contrast, cells with damaged membranes are stained in a distinctive blue color readily observed under a microscope. Thus trypan blue dye is described as being a vital stain allowing discrimination between viable cells and cells with damaged membranes that are usually considered to be dead cells. *In vitro* cytotoxicity studies by tryphan blue assay was employed to assess the cytotoxic potential of the *Chroococus turgidus*. The principle is based on the fact that the live cells with rigid cell membrane would not uptake the tryphan blue dye whereas the dead cells with disrupted cell membrane would uptake tryphan blue. When different concentrations of *Chroococus turgidus* extract were assayed, the dead cells were found to increase with increase in concentration of the extracts. The % cytotoxicity was found to be increasing with increase in concentration of extract that shows the anticancer property of *Chroococus turgidus* that is indicated. The results showed that Total Cell counted (248 $x10^5$ cells/ml), Viable cell counted (197 $x10^5$ cells/ml) and Dead Cell counted ($51x10^5$cells/ml) respectively.

Caspase -3-assay

Caspases (cysteinyl aspartate-specific proteases) are a family of important signaling molecules with various tasks depending on the subtype and organ involved. The activation of caspases also is a marker for cellular damage in diseases such as stroke and myocardial infarction. Although the precise role in the initiation and progression of apoptosis is not known for all caspases, their involvement as an indicator alone and as a potential leverage point for drug research makes them widely researched molecules (Lavrik *etal.* 2005). Activation of the caspase-3 pathway is a hallmark of apoptosis and can be used in cellular assays to quantify activators and inhibitors of the "death cascade." The response is both time and concentration dependent, suggesting that multiple pathways play a role in triggering the caspase-3 activation. One could hypothesize that cells are most susceptible to staurosporine in a specific phase of the cell cycle and therefore, over time, most cells will die, similar to previous findings (Vermeulen *et al*., 2004).The Caspase activity of *Chroococus turgidus* was estimated the results indicated that, activity of Caspase assay levels were found to high 0.159 Caspase 3 Activity in (µmol pNA/min/ml)/ 75µg concentration of algal sample.

Wound healing activity of *Chroococcus turgidus*

Wound healing is a process by which damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound. It is mainly dependent upon the type

and extent of damage, the general state of health and the ability of the tissue to repair. In our study the extract of *Chroococus turgidus* significantly increased the rate of wound contraction and
collagen turnover. Collagen, the major component which strengthens and supports extracellular tissue, is composed of the amino acid, hydroxyproline,which has been used as a biochemical marker for tissue collagen The wound healing activity of *Chroococus turgidus* was estimated by using VERO cell lines method. The result showed that the significant level of wound healing activity was found when used *Chroococus turgidus* algal biomass extract against to VERO cell lines.

Cell Adhesion effect of *Chroococus turgidus*

Platelets play a key role in the adhesion of cancer cells to the endothelial layer, and as plasma alone shows no enhanced cancer cell attachment. Data in the present study show that cancer cell adhesion to endothelial cells is accelerated in the presence of human platelets relative to its absence. The primary algae samples used were consistent throughout this study; however, because the constituents are largely unknown, we expect considerable variability between batches and manufacturers within the health food industry. This lack of uniformity compounded with several unknown bioactive components is a significant concern for healthcare providers, where patients take various herbal or algal supplements. To address this aspect of herbal pharmacology, we have used algae readily available to the general public, sold by the health food industry. For example, secondary metabolites of various algae have been found to affect cell adhesion (Takamatsu *etal*. 2004)].In other cases, polysaccharide or deacetylated components have been responsible for activities reported (Ishikawa *etal*. 2008).The cell adhesion activity of *Chroococus turgidus* was estimated by using bacteria *Lactobacillus* sp. method. The result showed that the significant level of cell adhesion activity was found when used *Chroococus turgidus* algal biomass extract against to *Lactobacillus* sp.

Conclusion

Isolated Beta glucan of *Chroococcus turgidus* had the antiproliferative activity on MCF7 cell line, these extracts demonstrated higher DNA damage. Cell growth inhibition was mainly due to apoptosis, proved by DNA fragmentation analysis. Present findings encourage for further studies for isolated beta glucan and identifying of active components in *Chroococcus turgidus* and also in vivo studies for their mechanism of action.

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