ABSTRACT

About (20) Pseudomonas rogenosa isolate was experienced for their ability of protease production by calculating the diameter of lysis area after developing on skim milk agar medium (qualitatively). The results exhibited that only isolate no 5 was higher isolate for protease making of (26mm) of lysis area.

Then, the protein concentration was also identified by the Bradford method and found of 0.16 mg/mL; then purification was done by using an ion-exchange chromatography with DEAE sephadex G-100 column. The results showed the presence of 1-peak of the enzyme with 50 Kd of molecular weight two peaks of other proteins. We tried to investigate the cytotoxic invitro effect of the purified enzyme against two human cancer lines, HeP2 (human larynx epidermed carcinoma), RD (Rabdo- Sarcoma), and one normal cell line Ref ( Rat embryonic fibroblast) . The cancer and normal cells were treated with different concentrations of protease enzyme ranging from (0.05, 0.1, 0.2, 0.4,0.8 and 0.16 mg/mL) then incubated for additional 48 hours at 370°C and the results showed highest toxicity (80.28%) of protease enzyme on RD , moderate cytotoxicity (45.52%) on Hep and slight toxicity (37.12%) on normal cell line (Ref) in a concentration (0.8mg/mL).

Keywords: Protease, Pseudomonas rogenosa, Cancer cells.

INTRODUCTION

Pseudomonas aeriginosa is the most Common gram-negative organism causing nosocomial infections and is a noteworthy pathogen in immune and myelosuppressed patients. It is non-fermentative and typically aerobic however will develop under an aerobic conditions in the presence of an appropriate nitrogen source. So, it very well may be isolated from soil, water, wounds, dental tissues.1 The living being likewise can expound an expansive number of secretory items and proteins, including extracellular protease.2,3 Characterize essential virulence factors and and have the capability to effect on IgA and IgG immunoglobulins.4

Two main potent proteases have been isolated, portrayed and widely studied Pseudomonas elates (PE) and alkaline protease (PAP). Pseudomonas, isolated from an assortment of ecological sources and tissues infected patients secret these protease,5 which are probably instrumental in imitating and controlling the tissue invasion and necrosis characteristic of pseudomonas infections.6

Separate of cancer cells industriously affects strong tumors or submerge the blood with abnormal cells. Cell division is a typical method used via the body for repair and growth.7 Parent cells divided to make two daughter cells. And these daughter cells are used to form novel tissue, or to altercation cells that have died consequently of aging or harm. Stop splitting of healthy cells when there is no lengthy required for more daughter cells, but cells of cancer remain to yield copies. They are also able to spread starting with one a player in the body then onto the next in a procedure known as metastasis.8

MATERIAL AND METHODS

P.aerogenosa isolates: they were isolated from wound infections.

Bacterial isolation

Every one of the synthetic compounds has gotten from sigma concoction Co. (USA) and (BDH England). The cotton swap of wound examples was spread on blood agar and McConkey agar plates, brood at 37°C medium-term. At that point, single states which are non-lactose aged and gives G +ve response on oxidase test and catalase test, were exchanged to Macconkey agar and blood agar to get an unadulterated culture, at that point various biochemical tests and even VITEK2 minimized framework were done.9

Protease production assay

Qualitative method

CLED or Nutrient agar plates having skim milk (5–10%) inoculated with bacterial overnight developing a culture at 37°C. The lysis cleared area around the growing colonies

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represent the positive result of protease production.\textsuperscript{10} Then the Azocasimase assay, were used to define the enzyme activity and,\textsuperscript{11} method for determination of specific activity and protein concentration.

**Preparation of partially purified protease**

Incompletely partially protease were set up as indicated by\textsuperscript{12} by inoculation of sterile dialysis tubing on the surface of blood agar by a swab with medium-term societies (37°C) of Proteus vulgaris 16 from nutrient broth. The plates were brooded medium-term at 37°C. The bacteria were scratched from the films with a glass slide and suspended in 50Mm (tris-HCL (pH 8.0) containing 0.04% NaN\textsubscript{3}. The layers were then completely washed in this cradle, and the washings were added to the bacterial suspension, centrifugation, the supernatant containing extracellular protease was added to 60% (wt/vol) Ammonium sulfate mixed at 4°C medium-term, the hastened proteins were gathered by centrifugation and redissolved in tris–HCL and 100 M NaCl (pH8.0) and connected to the section DEAE with 100Mm NaCl.

**CELL LINE GROWTH AND CYTOTOXICITY ASSAY**

Cell culture: Hep2 (Human Larynx Epidermoid carcinoma), RD (Rhabdo–myasarcoma) and Ref (Rat fetus fibroblast) cell lines were acquired from Iraqi community research and restorative hereditary, Mustansiriyah University. The cells were developed in RPMI–1640 medium containing 10% fetal calf serum and brooded at 37°C to blended monolayer.

The connected cells washed firstly with PBS and gathered from the tissue culture flask by treatment with trypsin versine solution. The cells were counted by trypan-blue (about 95% viability), 200µl of cell suspension were harvested in each well of microtiter plate and incubated in CO\textsubscript{2} incubator for 24h at 37°C then 200µL of altered concentrations of purified protease represented in (0.16, 0.8, 0.4, and 0.2, 0.1, 0.05) µg/mL were set up in serum free media (SFM) and added to cells, then re-incubate the plates for additional 48 hours. Moreover, each concentration were duplicated in three wells and negative control wells were treated with SFM just. Toward the finish of introduction period, the cells were recolored by 100µl of crystal violet solution and incubated at 37°C for 30 minutes.\textsuperscript{13}

The optical density of each well was read by micro–ELISA reader at 492 nm. The percentage of inhibition proportion was calculated agreeing to.\textsuperscript{14}

**Statistical analysis:** The experiments data were analyzed using statistical software SPSS version 16, significant differences between treatments were assessed using students T- test and p values < 0.05 were considered significant.

**RESULTS & DISCUSSION**

From the acquired outcomes, extraction of protease from bacterial cells, it could be presumed that the isolate no 5 demonstrated the higher capacity for protease generation of (26mm) in width of lysis zone on skim drain agar medium with higher protein concentration of 0.16 mg/ml by Bradford technique.\textsuperscript{22} as in Table 1.

**Purification of pseudomonas aerogenosa protease**

**Ammonium sulfate preparation:**

It was seen that pseudomonas aerogenosa protease action was the most concentration of ammonium sulfate (20–90%) but it was more in 50, 60, 70 and 80% saturation.

**Chromatography Exchange Ion**

The results depicted Figure 1 that there was one protein peak showed up in the washing step with 50 kDa molecular weight, while three primary protein peaks appeared in the elution by gradient concentrations of sodium chloride, the washing fraction contained most of the enzyme activity while there was no activity in the eluted fraction with Nacl solution.

**Cell line Growth and Cytotoxic Assay**

To examine the effects of pseudomonas aeroginosa protease enzyme on the growing of two cell lines (Hep and RD) and standard cell line (Ref), the cells cultures exposed to six different concentrations of the purified protease enzyme (0.05, 0.1, 0.2, 0.4, 0.8, and 0.16) µg/mL. The results showed a significant differences (p < 0.05) in percentages of inhibition rate (IR) on Hep2 cell line as compared to control, depending on the concentration of the enzyme the IR was 18.75% at a concentration 0.1µg/mL than increases to 52.64% at a concentration 0.4 µg/mL, than it decreased at higher concentrations of the extract Figure 2. The same pattern of inhibition rate was revealed in the growth of RD cell line, the results illustrated that IR was 45.3% at concentration of 0.1 µg/mL and increased significantly (p < 0.05) to 80.28% at a concentration of 0.4 µg/mL, and the inhibition rate decreases by increasing concentration of the extract (Figure 3) from these

**Table 1:** Showed the protease production and protein concentration of Pseudomonas aeruginosa isolates.

<table>
<thead>
<tr>
<th>Isolate no</th>
<th>Diameter in mm</th>
<th>Protein concentration mg/mL</th>
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<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0.10</td>
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<tr>
<td>2</td>
<td>18</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.10</td>
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<tr>
<td>5</td>
<td>26</td>
<td>0.16</td>
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<tr>
<td>6</td>
<td>22</td>
<td>0.11</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>0.9</td>
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<tr>
<td>8</td>
<td>18</td>
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<td>9</td>
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<td>10</td>
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results it is clear that the effects of the active compound obvious in low concentrations. Cultured normal cell line (Ref) showed a significant differences (p < 0.05) in percentage of inhibition rate, the highest IR was from 66.6% at low concentration 0.05 µg/mL, and decreased significantly as the concentration of the extract increased, and the lowest IR was 1.64% at concentration 0.16 µg/mL the normal call line (Figure 4). the results were closely related to the results obtained, that is the addition of 50µg/ml of plant increase thymocyte viability by 79.4%-84.3%. It is also proved by that some bacterial enzymes are cytotoxic at a higher concentration toward human normal cells.

From the results obtained it can be concluded that the strongest cytotoxicity of the extract was at a concentration 0.8 µg/ml, since it is effective against Hep and RD cell line and less effective against normal cell line Ref. These results come in agreement with the previously improved results which used the Rhus spp. extract as antitumor and antioxidant. The addition of the protease enzyme 5 Ref to the cultured cell line promote cell cycle arrest at either the G0/G1 or G2/M phase rather than causing cell damage damage directly, and eventually induced apoptosis in cell line It was believed that the induction of apoptosis is related to the inhibition of the activity of signal transduction molecules involving in the cell cycle, since certain antioxidants, such as flavonoids, have been reported to exert inhibitory effects on P13-Kinase. As well as may bacterial enzymes have an effect on human cells especially on cancer cells in addition to their effect on human IgG.

REFERENCES

biological functions, and diagnostic and therapeutic potentials.

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