BioBacta

Journal of Bioscience and Applied Research
https://jbaar.journals.ekb.eg/

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Anticancer Activity of Cytosine Deaminase Enzyme Purified from Local *Saccharomyces cerevisia* Bread Yeast

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DOI: 10.21608/jbaar.2024.268103.1036

Abstract

The study aimed to extract and purify the enzyme cytosine deaminase from locally manufactured bread yeast *Saccharomyces cerevisia*. To achieve the goal of the study, bread yeast was made locally and transported to the laboratory for an enzyme extraction process using toluene as an organic solvent for yeast wall rapturing. A crude enzyme was extracted with cold distilled water. Steps for enzyme purification started with a precipitation step with ammonium sulfate at 60% saturation, and then the ion exchange purification method ended with a gel filtration purification process applied. In each step, the supernatant volume, enzyme activity, specific enzyme activity, protein concentration, and percentage of enzyme yield were recorded. The cytotoxic effect of different enzyme concentrations in all purification steps toward the lung cancer cell line A549 and human breast cancer line MDA cell line beside normal cell line REF cells was investigated. The specific activity of the crude extract of locally produced bread yeast reached 13.205 units/mg protein, and for ammonium sulfate enzyme precipitation the specific activity amounted to 17.68 units/mg protein, for ion exchange was 216.66 units/mg protein, while for purification with gel filtration, the specific activity reached 571.428 unit/mg protein. The cytotoxic effect of the extracted enzyme in all steps; crud, ion-exchange, and gel filtration purified enzyme on the lungA549 and breast AMD cancer lines beside the REF normal cells were should a range of toxic effects at different enzyme concentrations. The toxicity was reduced till diminished as the enzyme was more purified.

Keywords: Cytosine deaminase, A549 cell line, MDA cell line, *Saccharomyces cerevisia*.

Introduction

Bread yeast, *Saccharomyces cerevisia*, is a single-celled microorganism that belongs to the Ascomycetes class, while others belong to the Basidiomycetes class, one of the most widespread species in nature. Therefore, it is used as a good model for eukaryotic cells, because it does not possess virulence factors and does not produce any type of toxin that causes harm to humans and animals (1). It is a valuable tool for most aspects of basic research for these organisms, which often simplifies matters because all the biological functions found in eukaryotes are present and preserved in yeast, and what has enhanced its importance is its entry into the field of industrial fermentations and the production of proteins, vitamins, and fats, as well as the production of enzymes (2,3). It was found that dry yeast gives

Received: February 5, 2024. Accepted: March 6, 2024. Published: June 18, 2024

less fermentation activity than fresh yeast due to some inhibitors and high sugar concentration. Also, appropriate storage conditions preserve its enzymatic activity, and its shelf life is 15 days when stored at a temperature of 4°C. However, if there is a need for longer storage, the temperature at 1°C is sufficient (4, 5). Bread yeast produces a group of enzymes, including the enzyme cytosine deaminase, which belongs to the hydrolytic deamination group. The presence of this enzyme was observed in prokaryotic cells and fungi, and not observed in higher eukaryotic organisms (6,7). The enzyme cytosine extracted from baker's yeast works to convert 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU) in the pyrimidine catabolism pathway by the enzyme cytosine deaminase. because (5-FC) compound is a fluorinated pyrimidine which is an analog of the natural nitrogenous base uracil, it has been used for more than 30 years as an anti-cancer substance to treat cancerous tumors such as skin, breast, lung, and colon cancer. Therefore, many researchers have suggested giving (5-FC) orally and implanting the extracted enzyme capsule near or at the site of treated or affected tissues of the cancerous tumor, the enzyme implanted in the site of the injury in the form of a capsule will act to convert(5-FC) to(5-FU), which is one of the most famous chemotherapy agents that has a role in treating cancer, by remove the amine group from the base 5-fluorocytosine to turn into 5-fluorouracil (8, 9, 10). For this reason, there are several attempts to extract the enzyme from microscopic organisms and implant it in the form of a capsule in the skin. Therefore, extracting large quantities of it is necessary and required. Accordingly, the optimal conditions for producing the enzyme were studied to obtain its best effectiveness to obtain enough of the thermally stable enzyme to be used clinically in the treatment of cancer (11,12) which gave the importance of this enzyme. It has been found that there are a number of factors that cause cancer, including factors dealing primarily with the environment and specific factors for the affected person, the most important of which are genetic predisposition to infection and a weak immune system, as well as lifestyle and other factors specific to the tumor itself. (13,14,15). As for other factors, they are external, interfering, and affect the progression of the disease, such as the person's exposure to Carcinogenic environmental factors (16,17). All of these factors explain the difference in the variation of cancer from one person to another, even if they are exposed to the same risks (18,19, 20). The study aimed to extract the enzyme from bread yeast, purify it, and study its effectiveness through biological applications as an anticancer *in vitro*.

Material and Methods

Extraction of cytosine deaminase enzyme from baker's yeast local *S. cerevisiae:*

Crud enzyme extraction steps

Extraction and purifying of the enzyme cytosine deaminase from locally produced *Saccharomyces cerevisia* were described by *Cerignani and Ipata* (1978) method with some modifications, (21). Briefly, about 100 grams of ready-made bread yeast was suspended with 50 ml of toluene for cell wall rapturing to extract the enzyme cytosine deaminase from the yeast with the aid of gentle heating at 45 ^{0} C for an hour to be left at room temperature for 2-3 hours then transferred to separator funnel and 100 ml cold water was added. The mixture was incubated at 4 ^{0} C for 18 hours to separate the aqueous layer from the organic one. The aqueous layer had been centrifuged in a cool centrifuge with10000 rpm for 20 minutes at 4°C. The supernatant represented the crud extract for the enzyme which had been subjected to different steps for enzyme purification employing measuring of specific enzyme activity and protein content in each step.

Purification steps

The enzyme was purified through purification steps that included both precipitation with

ammonium sulfate salts and dialysis. The dialysis process was carried out for the enzyme resulting from the precipitation stage. Other Purification steps were applied using two chromatographic techniques: the DEAE-Cellulose ion exchange technique and gel filtration using a Sephadex G-200 column as a final purification step. The presence of enzyme was detected by the disc method to react with a specific substrate. (22,23). The enzyme was stored at -20 degrees Celsius until the other purification stages were performed

Purification of the enzyme with Ion-exchange chromatography

The diethyl amino ethyl cellulose exchanger was prepared according to the method described by (24,25), in which about 20 g of the exchanger (DEAE - Cellulose) purchased from Whatman Company was suspended in a graduated cylinder in a liter of distilled water and left to stagnate for the material to precipitate. The exchanger was activated with 500 ml of NaOH 0.25 M for 24 hours, washed with pH stabilized at 7, and by additional 500 ml HCL solution 0.25 M for another 24 hours. The flow speed was adjusted to 30 ml/hour, with a pH of 7.2 using a buffer solution of 0.05 M. After performing the dialysis process, 10 ml of the concentrated protein solution resulted from the ammonium sulfate precipitation step. Each 5 ml elute was collected from the column in separated tubes followed by an absorption reading at a wavelength of 280 nm. The proteins bound to the column were recovered with a 0.1-0.5 salt gradient using a 0.05 M base buffer solution with a pH of 7.2 containing 0.5 M potassium chloride with a flow rate of 30 ml/hour and collecting the eluted portions in parts of 5 ml/part, followed by measuring the absorbance in each recovered part at a wavelength of 280 nm (22). Enzymatic activity, enzyme-specific activity, and protein concentration were estimated for the collected portions that possessed the best absorption peak (5).

Purification of the enzyme with Gel filtration chromatography

The gel filtration chromatography technique was employed for further enzyme purification. Using a Sephadex G-200 filled column prepared according to the manufacturer's instructions (Pharmacia Fine Chemicals), suspending 10 g of Sephadex G-200 powder in 200 ml distilled water and heated in a water bath for 5 hours at 90°C and left to cool, had been applied in this step. The suspended matrix was washed twice with a Tris-base buffer solution of 0.25 M and pH 7.2. A process of removing air and bubbles was carried out using a laboratory vacuum pump. The gel was filled in a glass column with dimensions (1.5 x 80) cm and the material was left to settle. The column was calibrated using a 0.25 M Tris-base buffer solution with a pH of 7.2 and a flow rate of 30 ml/hour to collect an elution of 5 ml each in separate tubes. An Aliquot of 5 ml from concentrated semi-purified enzyme in the ion exchange step was placed gradually on the internal walls of the gel filtration column. The collected 5 ml elution was followed by an absorption reading at 280 nm. Enzymatic activity, enzyme-specific activity, and protein concentration were estimated after each step and disc method for enzyme detection (9).

A cytotoxic Activity of Cytosine deaminase enzyme on cancerous and normal cells

Cancer lines and normal cells were supplied by the laboratories of the Biotechnology Research Center / Al-Nahrain University / Department of Molecular Medical Biotechnology. The cells for the lines were grown in RPMI culture medium supplemented with 10% fetal bovine serum. When a confluent monolayer formed, the cells were treated with an amount of trypsin-version solution. 2-5 milliliters for 5-10 minutes to prepare the secondary culture, after which the cells are prepared to be treated with the materials under study. The study included the following lines:

- *Lung Cancer cell line* (A 549): these cells are a basal epithelial adenocarcinoma of human pulmonary alveoli and constitute a cell line that was first developed in (17, 26). By removing and transplanting cancerous lung tissue into the transplanted tumor of a 58-year-old Caucasian man. These cells are used to study lung cancer and develop drug treatments against it. It was the 16th pass processed by the Biotechnology Research Center.

- *Human Breast cancer cell line (MDA*): The 25-pass cancer line, a universal epithelial breast cancer cell line, was generated from the epithelial cells of a 51-year-old Caucasian woman who developed metastatic mammary adenocarcinoma and is one of the most commonly used breast cancer cell lines in medical research laboratories (27).

-*Natural line Rat Embryo Fibroblast (REF) cells*: The natural cell culture of the rat embryo represented one of the important and basic sources to provide a culture of normal, undifferentiated fibroblast cell culture. In the current study REF cells were used in passage No. 12, the line was supplied from the Biotechnology Research Center/Al-Nahrain University.

- Maintaining and preparing cancer cell lines

The cancer cell lines used in the study, MDA, A549, and the normal rat fibroblast (REF) were maintained according to (Freshney, 2012) (28). This is done by observing these cells, and when a complete monolayer was formed, the secondary culture was carried out by removing the old growth medium, then the cells were washed with sterile phosphate buffer (PBS), after which 2-3 ml of trypsin-versin' TV solution was added to the tissue culture vessels of Falcon tissue culture container of 25 cm size, containing the cell lines, so that the surface of the cells is covered when the bottle is placed horizontally, when kept in the incubator for 5-10 seconds, with gently moving to break the adhesion of the cells from each other to the wall of the culture container, then the culture medium is added to get rid of the TV and to redistributed in the culture container. Complete information is then written on each container (cell type, new passage number, and date of secondary transplantation for each cell line).

- Cytosine deaminase toxicity test

The following tissue culture steps were performed under sterile conditions according to (28) to investigate the toxicity of the enzyme to the cell lines under study:

A - Seven concentrations of the enzyme cytosine deaminase extracted from bread yeast were prepared for all steps of enzyme purification, including the crude enzyme, the enzyme purified by ion exchange, and the enzyme purified by gel filtration, using sterilized serum-free media SFM in preparing the concentrations of (100 - 50 - 25 - 12.5 - 6.250 - 3.125 - 1.5625) mg/ml. All dilutions were sterilized with a 0.22 mm Millipore filter, all steps were held under aseptic conditions, and the prepared concentrations were used immediately after completing the preparation process.

B - The prepared normal and cancerous cells suspension in RPMI medium containing 5% serum after being de-attached from tissue containers with TV solution and after a new culture medium was added to it, a seeding process was employed by transfer 100 microliters of the cell suspension to each of the 96-wells tissue culture plate with aid of a micropipette. The seeded tissue culture plates are placed in the incubator at a temperature of 37°C for twenty-four hours until the cells adhere to the wells and a confluent monolayer is formed.

C - The treatment step is done by disposing of the old culture medium in the wells, and 100 microliters of new serum-free culture medium is added, then 100 microliters of the enzyme cytosine deaminase solutions, previously prepared of different concentrations were added, in three

replicates for each concentration, in addition to that 12 replicates were prepared for the control, which contains only the cancerous lines and the medium as a negative control. All treated plates were incubated at 37°C in a 5% CO_2 incubator for 24 hours.

D - After the 20th hour before the exposure period ended, the plates were taken out of the incubator, their contents were poured, and the cells were then washed with a phosphate buffer solution. Then 0.1 milliliters of MTT (2mg/ml) dye solution were added to each well in the plate, and they were re-incubated at 37°C for an additional four hours. At last, 50 microliters of DMSO solution were added to each well, and the resultant color was read with a microplate-ELISA reader device at a wavelength of 620 nm.

E - The inhibition rate percentage (IR %) of cell lines treated with each extracted cytosine deaminase enzyme solution in the plate was calculated according to the following equation:

* Percentage of cell viability = Average absorbance reading of treated cells for each concentration / Average absorbance reading of control cells x 100.

* Percentage of cell inhibition (IR%) =Average absorbance reading of control cells - Average absorbance reading of treated cells for each concentration / Average absorbance reading of control cells x 100. (29).

Statistical analysis:

Data were analyzed using the statistical analysis system- SAS (2018) program to detect the effect of different factors on study parameters. The values with $P < 0.05$ were considered significant. The least significant difference LSD test (Analysis of Variation-ANOVA) was used to significantly compare between means in this study (30).

Results

Extraction of cytosine deaminase enzyme from local baker's yeast *S. cerevisia*

Crud enzyme extraction steps: The specific activity of crude enzyme extracted from the local type of *Saccharomyces cerevisia* yeast was (13.2) units/mg. The extraction result was compatible with the study of (5) in which the crude extracting the enzyme cytosine enzyme from manufactured yeast type, had a specific activity of (9.6) units/mg of protein.

Precipitation step for the enzyme extraction result: Results showed that the best saturation percentage was (60%), as it gave the highest specific activity reaching 17.68 units/mg of protein, number of purifications times1.341, and enzymatic yield of 11,335%. These results were agreed with (5) study where different saturation percentages of ammonium sulfate were used to precipitate the enzyme cytosine extracted from Turkish-type bread yeast including (20-40-60-80) % to determine the best saturation percentage. The results showed that the best saturation rate was 60%, giving the highest specific activity of 12.5 units/mg of protein, with a number of purification times of 1.302 times, and an enzymatic yield of 4.82%.

- Ion exchange chromatography step for the enzyme purification result: The specific activity of the cytosine enzyme in this step reached 216.6 units/mg protein, with 16.407 purification times, and an enzyme yield of 1.475%. Also, this result was conducted with (5) study on commercial bread yeast type in which the specific activity was 358 units/mg protein, with a number of purification times of 37.29 times, and enzymatic yield of 2.55%.

- Gel filtration chromatography step for the enzyme purification result: The specific activity of the cytosine enzyme using gel filtration reached 571.428 units/mg protein, and the number of purifications was 43.273 times, with an enzymatic yield of 0.647%. A separation column, Sephadex

G-200 was used in several studies after the ion exchange step in purifying the cytosine deaminase enzyme, as (5) study in which the specific activity of the enzyme reached 400 units/mg protein with several purification times of 41.6 times, with an enzymatic yield of 1.19. %.

Cytotoxic Activity Assay for Cytosine Deaminase Enzyme:

- Cytosine deaminase anticancer effect on lung cancer A549 lines

The effect of crude extract, purified enzyme by ion exchange method, and gel filtration method on lung cancer A549 passage 21 when treated for 24 hours were illustrated in the following: -

Cytosine deaminase crud enzyme anticancer effect on Lung cancer A 549 line: A comparison between seven concentrations of crud enzyme and the inhibition rates of A549 lung cancer cells was shown in Table (1).

All crud enzyme concentrations caused a significant inhibition rate towed the lung cell line A549. The potent inhibition rate for the crud enzyme as shown in Table (2) had been showing at a concentration of 6.25mg/ml reaching IR%=70.56% significantly.

Cytosine deaminase anticancer effect of Ion Exchange different concentrations after 24 hours exposure on Lung cancer A 549 line: A comparison between seven concentrations for semi-purified enzyme by ion exchange chromatographic method and the percent inhibition rates of A549 lung cancer cells was shown in Table (2).

The effect of semi-purified cytosine deaminase enzyme extracted from local handmade *S. cerevisia* bread yeast showed a concentration-dependent inhibition effect. The potent anticancer activity had been shown at the highest enzyme concentration (25mglml) which gave a significant inhibition rate of 71.67%.

Cytosine deaminase anticancer effect of Gel filtration different concentrations for 24 hours on Lung cancer A 549 line: A comparison between seven concentrations for semi-purified enzyme by gel filtration chromatographic method and the percent inhibition rates of A549 lung cancer cells was shown in Table (3).

As shown in Table (3) only the highest concentration 25mg/ml of the purified enzyme by gel filtration chromatography possessed an inhibition effect on Lung cancer A 549 line reached 68.7%.

Table 1: Effect of Crud Enzyme different concentrations on Lung cancer line as % IR after 24 hours

Concentration (mg/ml)	Mean ± SE of IR%
50	60.87 ±0.36 bc
25	67.33 ±0.88 a
12.5	67.00 ±2.51 ab
6.25	70.56 ±0.72 a
3.125	59.00 ±2.08 c
1.5625	44.56 ±1.78 d
0.78125	24.00 ±4.62 e
0 (control)	0.00 ±0.00 f
LSD value	6.414 *
This means having the different letters in the same column differed significantly, * (P≤0.05).	

Table 2: Effect of Ion Exchange concentration in IR after 24 hours

Concentration (mg/ml)	Mean ± SE of IR%
25	71.67 ±0.88 a
12.5	43.63 ±8.34 b
6.25	11.17 ±4.76 c
3.125	31.30 ±5.34 b
1.5625	0.00 ±0.00 c
0.78125	7.00 ±5.56 c
0.39	0.00 ±0.00 c
0 (control)	0.00 ±0.00 c
LSD value	13.096 *
This means having the different letters in the same column differed significantly, * (P≤0.05).	

Table 3: Effect of Gel filtration concentration in IR after 24 hours

Concentration (mg/ml)	Mean ± SE of IR%
25	68.70 ±1.30 a
12.5	0.00 ±0.00 b
6.25	0.00 ±0.00 b
3.125	0.00 ±0.00 b
1.5625	0.00 ±0.00 b
0.78125	0.00 ±0.00 b
0.39	0.00 ±0.00 b
0%(control)	0.00 ±0.00 b
LSD value	1.377 *
This means having the different letters in the same column differed significantly, * (P≤0.05).	

- ***Cytosine deaminase anticancer effect on Human breast cancer MDA lines***

The effect of crude extract, purified enzyme by ion exchange method, and gel filtration method on human breast cancer MDA passage 11 when treated for 24 hours were illustrated in the following: -

Cytosine deaminase crud enzyme anticancer effect on human breast cancer MDA line: A comparison between seven concentrations of crud enzyme and the inhibition rates of MDA breast cancer cells was shown in Table (4).

The crud enzyme inhibited the growth of the breast cell line in all concentrations except the last concentration (0.78125mg/ml) with no significance. The highest inhibition rate concentration was 64.49% at a concentration of 25mg/ml.

Cytosine deaminase anticancer effect of Ion Exchange different concentrations after 24 hours exposure on Human breast MDA cancer line: A comparison between seven concentrations for semi-purified enzyme by ion exchange chromatographic method and the percent inhibition rates of MDA breast cancer cells was shown in Table (5).

Even though there was no significant difference in the inhibition rat present for both concentrations (25 and 12.5) mg/ml; the latest showed the highest IR% of 67.46%.

Cytosine deaminase anticancer effect of Gel filtration different concentrations after 24 hours exposure on Human breast cancer MDA line: A comparison between seven concentrations for semi-purified enzyme by gel filtration method and the percent inhibition rates of MDA breast cancer cells was shown in Table (6).

The cytosine deaminase enzyme purified by gel filtration showed the highest inhibition rate at a concentration of 25 mg/ml reaching 66.2% as a toxic effect on the MDA cancer cell line.

- ***Cytosine deaminase cytotoxic effect on Rat Embryo Fibroblast (REF) normal cells***

The effect of crude extract, purified enzyme by ion exchange method, and gel filtration method on Rat Embryo Fibroblast (REF) normal cells passage 12 when treated for 24 hours as illustrated in the following:

*Cytosine deaminase crud enzyme cytotoxic effect on Rat Embryo Fibroblast (REF) normal cell*s: A comparison between seven concentrations of crud enzyme and the inhibition rates of REF cells was shown in Table (7).

Crud enzyme solution affected the rat embryo fibroblast normal cells REF in almost all concentrations might illustrate the toxic effect of cytosine deaminase crud enzyme on other normal cells.

Cytosine deaminase cytotoxic effect of Ion Exchange different concentrations after 24 hours exposure on normal REF cells: A comparison between seven concentrations for semi-purified enzyme by ion exchange chromatographic method and the percent inhibition rates of REF cells was shown in Table (8).

As in the crud solution, the semi-purified enzyme by ion-exchange chromatography technique showed a toxic effect on these normal cells, but with lower potency.

Cytosine deaminase cytotoxic effect of Gel filtration different concentrations after 24 hours exposure on normal REF cells: A comparison between seven concentrations for semi-purified enzyme by gel filtration method and the percent inhibition rates of REF cells was shown in Table (9).

Table 4: Effect of Crud concentration in IR / MDA: 11

Concentration (mg/ml)	Mean ± SE of IR%
50	62.72 ±0.54 a
25	64.49 ±2.61 a
12.5	60.23 ±1.89 a
6.25	38.90 ±8.30 b
3.125	36.50 ±6.17 b
1.5625	0.00 ±0.0.00 c
0.78125	0.00 ±0.00 c
0 (control)	0.00 ±0.00 c
LSD value	11.502 *
This means having the different letters in the same column differed significantly, * (P≤0.05).	

Table 5: Effect of Ion Exchange concentration in IR / MDA: 11

Concentration(mg/ml)	Mean ± SE of IR%
25	63.27 ±2.21 a
12.5	67.46 ±0.73 a
6.25	42.67 ±1.20 b
3.125	22.96 ±1.23 c
1.5625	0.00 ±0.00 d
0.78125	0.00 ±0.00 d
0.39	0.00 ±0.00 d
0 (control)	0.00 ±0.00 d
LSD value	12.224 *
This means having the different letters in the same column differed significantly, * (P≤0.05).	

Table 6: Effect of Gel filtration concentration in IR / MDA: 11

Concentration(mg/ml)	Mean ± SE of IR%
25	66.19 ±0.78 a
12.5	61.96 ±1.18 a
6.25	46.76 ±7.48 b
3.125	13.67 ±3.69 c
1.5625	0.00 ±0.00 d
0.78125	0.00 ±0.00 d
0.39	0.00 ±0.00 d
0 (control)	0.00 ±0.00 d
LSD value	8.986 *
This means having the different letters in the same column differed significantly, * (P≤0.05).	

Table 7: Effect of Crud concentration on REF normal cell as IR% after 24 hours

Concentration (mg/ml)	**Mean reading**	**Mean IR%**
50	0.056	80
25	0.057	79.7
12.5	0.055	80.5
6.25	0.060	78.6
3.125	0.058	79
1.5625	0.251	11
0.78125	0.281	0.00
0 (control)	0.281	0.00
Chi-Square (χ^2)	---	21.307 **
** (P≤0.01).		

Table 8: Effect of Ion Exchange concentration on REF normal cell as IR% after 24 hours

Concentration (mg/ml)	Mean reading	Mean IR%
25	0.288	0.00
12.5	0.232	17
6.25	0.130	54
3.125	0.124	56
1.5625	0.115	59
0.78125	0.311	0.00
0.39	0.441	0.00
0 (control)	0.281	0.00
Chi-Square (χ^2)	---	16.522 **
** (P≤0.01).		

Table 9: Effect of Gel filtration concentration on REF normal cell as IR% after 24 hours

Concentration (mg/ml)	Mean reading	Mean IR%
25	0.578	0.00
12.5	0.599	0.00
6.25	0.400	0.00
3.125	0.307	0.00
1.5625	0.288	0.00
0.78125	0.337	0.00
0.39	0.399	0.00
0%(control)	0.281	0.00
Chi-Square (χ^2)	---	0.00 NS
NS: Non-Significant.		

Discussion:

All extraction results were compatible with many studies and the best saturation rate was 60%, giving the highest specific activity. Also in the ion exchange chromatography step, results were conducted with other studies that employed the commercial bread yeast. Besides, in several studies for gel filtration chromatography enzyme purification step, the separation column, Sephadex G-200 had been used, after the ion exchange step in purifying the cytosine deaminase enzyme (5, 20. 31). The cytotoxic effect of the extracted enzyme possessed potent anticancer activity at the highest enzyme concentration used in this study, for all purification steps that gave a significant percent inhibition rate toward Lung cancer cell line A549. Cytosine deaminase anticancer effect on Human breast cancer MDA lines showed the same results**.** Enzyme toxicity had appeared also at almost all concentrations, especially in crud extract on the normal cells REF cells. Thus, enzyme toxic effects on normal cells could be decreased as more purification steps were employed, so the toxicity of gel filtration purified enzyme had vanished.

Conclusion:

The handmade local yeast has the potential of combating soil fungi and certain fungal diseases, this important yeast of particular emphasis on its application in treating conditions such as cancer is a focus point for researchers and industrial application strategies.

Funding: The research hadn't received any financial support from an Institution

Author Contributions:

All authors confirm their contribution to the paper as follows: study conception and design: Zainab Y. and Muna.A.; data collection analysis and interpretation of results by; Muna, A. and Milad A. Mezher. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgments:

The authors would like to acknowledge the Biotechnology Research Center/Al-Nahrain University/ Ministry of Higher Education and Ibn Sina Research Center/Corporation of Research and Industrial Development/ Ministry of Industry and Minerals for support in completing all work requirements.

Conflicts of Interest: The authors affirm that there are no conflicts of interest.

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