Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

Evaluating Therapeutic Characteristics of Anticancer L - Glutaminase Enzyme on Ethylenimine Induced Mutagens in Rats.

Gulbahar F. Karim[1], Karkaz M. Thalij[2]
[1]College of Nursing, University of Kirkuk, Kirkuk, Iraq.
[2]Department of Food Sciences, College of Agriculture,University of Tikrit, Tikrit, Iraq.
[1]gfkarim22@gmail.com, [2]krkzmoh@yahoo.com

Abstract

This study include, the partial purification of L-glutaminase enzyme from *E.coli* by ammonium sulphate precipitation (40%-80%) fractions, and the enzyme activity elevated to 75 IU/ml following dialysis. Pharmacological evaluation of L-glutaminase enzyme was used for the in vivo studies on sixty rats as an animal models. The hematological parameters for rats in the treated group (T2) showed significant changes at the level ($P<0.05$) when compared with normal control group (T1). The WBCs was found to be increased with the reduction in the RBCs and Hemoglobin (Hb.) concentration. However there were no significant changes of these parameters in the other treated groups (T3, T4, T5, and T6) in contrast to (T1). The serum total protein, globulin and, albumin were found to be decreased significantly for the (T2) in comparison with the (T1). However, their level had been relatively normalized in comparison to (T2) as a result of administrating doses of 0.5, and 1.0 ml L-glutaminase in combination with 0.5 mg ethylenimine /Kg. body weight for T5 and T6 respectively. Concerning the level of serum globulins, there were a significant elevation in the serum globulins value for T5 and T6 which were treated with doses of 0.5 and 1.0 ml L-glutaminase in combination with 0.5 mg ethylenimine/Kg. body weight respectively. While, a significant decrease in the values of serum albumin detected in all rats' treated groups and the L-glutaminase efficacy was not achieved completely compared with the T1.The Liver enzymes ALT, AST and, ALP for T2 showed significant elevation at the level ($p<0.05$) when compared with T1. The treatment with L-glutaminase at doses of 0.5 and, 1 ml /kg body weight in combination with 0.5 mg of ethylenimine/kg body weight in T5 and, T6 groups respectively, seem to reverse the changes to word near normal level. There were a

significant elevation ($p<0.05$) in the relative weight of Liver, kidney and spleen of rats in T2 in comparison with T1. However, there weight decreased significantly ($p<0.05$) in T5 group in comparison to (T2). Whereas there were no significant differences in the organs' relative weight for the T6 group from that of T1. The finding of the present study improved the preventive role of the L-glutaminase against the mutagenic, and carcinogenic effect of ethylinimine, and it was dose dependent.

Keywords: L-glutaminase, Anticancer, Ethylenimine, *E.coli.*

DOI: http://doi.org/10.32894/kujss.2018.13.4.1

تقييم الخاصية الدوائية للأنزيم L-glutaminase المضادة للسرطان على طفرات المستحثة بمادة Ethylenimine في الجرذان

كولبهار فتح الله كريم[1]، كركز محمد ثلج[2]
[1]كلية التمريض، جامعة كركوك، كركوك، العراق.
[2]قسم الصناعات الغذائية، كلية الزراعة، جامعة تكريت، تكريت، العراق.
[1]gfkarim22@gmail.com, [2]krkzmoh@yahoo.com

الملخص

تم تنقية انزيم L-glutaminase باستخدام طريقة ترسيب كبريتات الامونيوم للأجزاء (80−40) وارتفع النشاط الانزيمي الى 75 IU/ml بعد إجراء عملية الديلزة. تم بيان تأثير الحقن الوريدي لإنزيم L-glutaminase على بعض المتغيرات الفسلجية والكيموحيوية في دم الجرذان واوزان بعض اعضائها لأجل التقييم الدوائي لهذا الأنزيم ودوره في تثبيط أو الحد من التأثير التطفيري والمسرطن لمادة Ethylenimine على مجاميع الجرذان التي تضمنت ستون جرذاً تحت الدراسة. تراوح اوزانهم بين mg (200-250) لمدة 15 اسبوعاً. اظهرت النتائج ارتفاع معنوي ($P<0.05$) في العدد الكلي لكريات الدم البيض TWBCs مع انخفاض في العدد الكلي لكريات الدم الحمر TRBCs وتركيز الهيموكلوبين Hb concentration للمجموعة المعاملة T2 نتيجة التجريع الفموي لمادة Ethylenimine مقارنة بمجموعة السيطرة T1 في حين لم يطرأ تغيرا" معنويا" على تلك المتغيرات في المجاميع الاخرى، كما حصل انخفاض معنوي ($P<0.05$) للبروتين الكلي والكلوبيولين والالبومين في مصل

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

الدم للمجموعة المعاملة T2 مقارنة بالمجموعة T1 بينما الحقن الوريدي للانزيم بمقدار 0.5 و 1 ml مع تجريع 0.5 mg من Ethylenimine للمجموعتي T5 و T6 على التوالي ادى الى تغير في مستوى البروتين الكلي باتجاه الطبيعي تقريبا" مقارنة بالمجموعة T2، أما بالنسبة لمستوى الكلوبين في المصل فقد لوحظ إرتفاعا" معنويا" ($P<0.05$) في مستواها لمجموعتي T3 و T4 بعد الحقن الوريدي لأنزيم L-glutaminase بتركيز 0.5 و 1 ml على التوالي، وسجل اعلى ارتفاع لمستوى الكلوبين للمجاميع T5 وT6 بعد معالجتها بالحقن الوريدي لأنزيم L-glutaminase بتركيز 0.5 و 1 ml مع تجريع 0.5 mg من Ethylenimine في حين لوحظ إنخفاظا" معنويا" في مستوى الالبومين لكل مجاميع الدراسة ولم يظهر دور الأنزيم بصورة تامة مقارنة بالمجموعة T1. كما بينت النتائج إرتفاعا" معنويا" في مستوى ALT و AST و ALP للمجموعة T2 مقارنة بالمجموعة T1. بينما ادى الحقن الوريدي لأنزيم L-glutaminase بتركيز 0.5 و 1 ml مع 0.5 mg من Ethylenimine الى تعديل مستوى الأنزيمات اعلاه بإتجاه الطبيعي تقريبا" للمجاميع T5 و T6 على التوالي، كما إن هناك إرتفاعا" معنويا في وزن التقريبي لأعضاء الكبد والطحال والكلية للجرذان في T2 مقارنة بالمجموعة T1 بينما أدى الحقن الوريدي لأنزيم L-glutaminase بتركيز 1 ml و 0.5 mg و تجريع 0.5 من Ethylenimine الى إنخفاض اوزان الاعضاء لمجموعة T5 مقارنة بالمجموعة T2 لكن لايزال عاليا" معنويا مقارنة بالمجموعة T1 في حين ليس هناك فروقات معنوية في أوزان تلك الأعضاء لمجموعة T6 مقارنة بمجموعة السيطرة T1.

الكلمات الدالة: الانزيم L-glutaminase، مضاد للسرطان، Ethylenimine، E.coli.

DOI: http://doi.org/10.32894/kujss.2018.13.4.1

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

1. Introduction:

Enzymes are selective catalytic proteins which produced by living cells and may or may not contain prosthetic group [1]. L-glutaminase (L-glutamine amidohydrolase EC 3.5.1.2) catalysis the hydrolysis of L-glutamine to glutamic acid and ammonia [2]. The L-glutaminase enzyme plays a major role in the cellular nitrogen metabolism of both prokaryotes and eukaryotes, and can be derived from plant, animal tissues and microorganisms including bacteria, yeast and fungi [3]. The L-glutaminase produced by various microorganisms but the enzyme with the best properties have been purified from the family of enterobacteriaceae especially from E.coli [4]. The E.coli attracts the attention of many investigators, and they studied its ability for the production of such enzyme[4 -9]. The advantage of using microorganisms for the production of L-glutaminase is the economical bulk production capacity, and also the microbes are easy to manipulate to obtain enzymes of desired characteristics [3].

Over a few decades, L-glutaminase attracted more attention in both food industrial and pharmaceutical applications. It used as enzyme therapy for cancer especially for acute lymphocytic leukemia [10], it also used in biosensors for monitoring the glutamine levels in mammalian and hypridoma cells. However in 1993, Pseudomonas species recombinant glutaminase was patented for its activity against cancer, and HIV virus therapy [11]. Hence the aim of the present study is to evaluate the therapeutic capacity of L-glutaminase enzyme for preventing the mutagenic, and carcinogenic effect of ethylinmine in vivo using rat groups as an animal model.

2. Materials and Methods:

2.1 Purification of L-glutaminase; Ammonium Sulphate Precipitation and Dialysis:

Finely powdered ammonium sulphate was slowly added to crude enzyme purified from E.coli isolate from clinical sample, so as to reach 40% saturation. The whole content was stirred at 4°C on magnetic stirrer. The precipitated protein was removed by centrifugation at 10,000 rpm at 4°C for 20 minutes. Fresh ammonium sulphate was added to the supernatant to increase the concentration to 50%. The obtained precipitate was resuspended in a minimal volume of 0.01M phosphate buffer at (pH 8). The precipitated protein was removed by centrifugation, as described earlier. The fresh ammonium sulphate was added again to the supernatant to increase the concentration to 80%.The obtained enzyme precipitate was resuspended in a minimal volume of 0.01M phosphate buffer,pH 8 [12]. and dialyzed against 0.01M phosphate buffer (pH 8) for 48-

72 hours at 4°C and the buffer were changed occasionally [13]. Finally, L-glutaminase activity of dialysate was assayed according Imada, et.al [14].

2.2 In vivo testing therapeutic characteristic of L-glutaminase:

Laboratory Animals Initialization:

Sixty male rats had 200-250g weight, 4 weeks-old (Albino-Sprague Dawley Rats) were individually weigheted, wing banded and housed in heated battery brooders under 12 hrs. Fluorescent lighting daily, with feed and water provided ad libitum. Rats were fed the optimal formula according to [15]. The experimental design consisted of six dietary treatments by the intravenous injection from partially purified L-glutaminase enzyme with 75 IU/ml activity. The Rats were administrated Ethylenimine 0.5 mg (dissolved in d.w)/kg. body weight each 72 hrs., for four weeks in the treated group (T2). The rats were randomly assigned to the following treated groups Table 1. There were two replicates of five rats per dietary treatment and the rats were maintained on these treatments for 15 weeks period.

Table 1: Experimental protocol for the study anti-carcinogenic effect of glutaminase on ethylenimine induced liver cancer in rats.

Group	Treatment	Conc.	Period duration(weeks)		
			0-4	5-8	9-15
T1	CD	0.0	CD	CD	CD
T2	E	0.5mg/kg of.Body weight	CD	CD+E	CD
T3	Gt	0.5 ml/	CD	CD+Gt	CD+Gt
T4	Gt	1 ml	CD	CD+Gt	CD+Gt
T5	Gt + E	0.5ml+0.5 mg/ kg. of Body weight	CD	CD+Gt+E	CD+Gt
T6	Gt + E	1ml+0.5 mg/kg. of Body weight	CD	CD+Gt+E	CD+Gt

E =Ethylenimine; CD = control diet; Gt = L-glutaminase.

3. Parameters Studies:

3.1 Haematological parameters:

Following 15 weeks of treating rats with L-glutaminase, and Ethylenemine, and following deep ether anesthesia, the ventral side of treated rats were cutted, then five milliliters of blood were collected from posterior vena cava, dispensed in two tubes, one of them without EDTA for biochemical tests, and the other with EDTA for hematological parameters determination according to (16). All blood samples were labeled and immediately analysis. For measuring

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

hemoglobin (Hb.) concentration, the manual Sahli's (Acid hematin) method has been used. Total Red Blood Cells (TRBC) count has been measured by standard manual technique. For measuring the Total White blood cells(TWBC) count the direct method has been used [16].

4. Biochemical parameters:

The blood samples without EDTA were placed at room temperature for about one hour at room temperature, then centrifuged at 3000 rpm for 15 minutes. Follow that the supernatant (serum) was collected by using micropipette and transferred to ependorf tubes, then kept in a freezer at -20 °C for one week for biochemical tests. serum separator tubes were used for serum collection. Serum concentration of total protein, albumin, and globulin were determined according to Kits from Biolabo (France). The Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) were determined according to the manufacturers recommended procedure from Biomerieux-France. The ALT test applied immediately following sample collection. ALT estimated immediately.

5. Statistical Analysis:

Data were analyzed by the ANOVA analysis, using the general linear model of the Statically Analysis System. Significant treatment differences were evaluated using Duncan’s multiple-range test. All statements of significance are based on the 0.05 level of probability.

6. The Results and Discussion:

- **Purification of L-glutaminase**

The Partial purification of the enzyme using ammonium sulphate precipitation showed that the best fraction was (80%) in contrast to the crude enzyme, 40% and 50% fractions. It produced the maximum value of enzyme activity that was 75 IU/ml following dialysis which estimated by Nesslerization process [14]. The finding of the present study was in agreement with that of other investigators who found that 80% ammonium sulphate was produced the highest yield of L-glutaminase activity from Streptomyces avermitilis [17].

7. Pharmacological Evaluation of L-glutaminase Enzyme:

The partial purified L-glutaminase produced by E.coli isolate obtained from wound sample was used for in vivo study in rat models to investigate the effect of L-glutaminase enzyme on some hematological, chemical parameters and the relative organs weight of treated animal.

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

7.1 Effect of Ethylenimine and L-glutaminase on The Hematological Parameters:

Table 2, illustrates the effect of intravenous injection of L-glutaminase enzyme and oral administration of ethylenimine on some hematological parameters in treated rat groups. The hematological parameters of rats in the treated group (T2) which were administered 0.5mg Ethylenimine orally /Kg. body weight showed significant changes at the level ($p<0.05$) when compared with normal control group(T1). The (TWBC) count was found to be increased with the reduction in the (TRBC) count and Hemoglobin (Hb.) concentration which was 9.7, 4.9($\times 10^3$ cell/ mm^3) and 8.1gm/dl respectively when compared with the control group (T1) that were 7.2, 7.58 ($\times 10^3$ cell/ mm^3), and 12.8gm/dl respectively. However there were no significant changes of these parameters in the treated groups; T3 and T4 which are received intravenous doses of 0.5 and 1.0 ml L- glutaminase respectively. The above doses of L-glutaminase achieved negative effects on orally administration of ethylenimine, and there were no significant changes of WBC count. Whereas there were significant increase of RBC count, and Hb. concentration in the treated groups; T5 and T6 which became 7.3, 7.67 ($\times 10^3$ cell/ mm^3) and 12.5gm/dl respectively for the T6 group. These results revealed the role of L-glutaminase in inhibiting the effect of Ethylenimine which might stimulate the host immune system, and suppress the erythropoisis or increase the hemolysis above the normal rate. However its effect was dose dependent.

Table 2: Interaction effects between L-glutaminase and ethylenimine on the hematological parameters of rats dietary for 15 weeks.

Treatment type	WBC ($\times 10^3$ cell / mm^3)	RBC ($\times 10^3$ cell / mm^3)	Hb (dl/ml)
T1	7.2± 0.81 [a]	7.58±0.93 [a]	12.8±1.05 [a]
T2	9.7± 0.68 [b]	4.91 ±0.38 [b]	8.1 ± 0.95 [b]
T3	6.9± 0.59 [a]	7.60 ±0.46 [a]	12.8±1.16 [a]
T4	7.2± 0.88 [a]	7.62 ±0.61 [a]	12.9±1.21 [a]
T5	7.7± 0.63 [a]	6.95 ±0.57 [c]	11.4±0.86 [c]
T6	7.3± 0.79 [a]	7.67 ±0.74 [c]	12.5±1.03 [d]

T1: control group; T2: 0.5mg Ethylenimine/kg; T3: Glutaminase 0.5ml/kg; T4: Glutaminase 1ml/kg; T5: 0.5ml/kg Glutaminase+0.5mg Ethylenimine/kg; T6: 1ml/kg Glutaminase+0.5mg ethylenimine/kg.(rats body weight). a, d: Values within columns followed by different letters differ significantly at ($p<0.05$).

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

7.2 Effect of Ethylenimine and L-glutaminase on Serum Proteins:

The interaction effects of the intravenous injection of L-glutaminase and the oral administration of Ethylenimine on biochemical parameters of rat groups were investigated. According to the results illustrated in Table 3, the serum total protein, globulin and, albumin were found to be decreased significantly ($p<0.05$) for the treaded group (T2) which became 5.34, 3.04, and 2.30mg/dl respectively compared with the control group(T1) which were 6.82, 4.21 and 3.61 mg/dl respectively. The administration of 0.5 and 1.0 ml L-glutaminase/Kg. body weight caused significant increase in the level of serum total proteins.

However, their level had been relatively normalized in comparison to control group as a result of administration doses of 0.5 and 1.0 ml L-glutaminase in combination with 0.5 mg ethylenimine/Kg. body weight by T5 and T6 respectively, and levels became 5.64, and 5.86 gm/dl respectively. Concerning the level of serum globulins, there were no significant increase observed in these value for T3 and T4 which were received 0.5 and 1.0 ml L-glutaminase intravenously. However, there were a significant elevation in the serum globulins value in comparison to treated group(T2) for T5 and T6 which were treated with doses of 0.5 and 1.0 ml L-glutaminase in combination with 0.5 mg ethylenimine/Kg. body weight and became 3.43 and 3.74gm/dl respectively.There were a significant decrease in the values of serum albumin in all rat groups and the L-glutaminase efficacy was not achieved completely when compared with the control group. The oral administration of ethylenimine caused significant increases in incidences of pulmonary adenomas and hepatomas in male and female B6C3F1 mice and B6AKF1 males [18]. So it affect on the level of proteins made by liver including albumin, which is the main component of total proteins [19]. The remaining fraction is called globulin (including the immunoglobulin's). Albumin levels are decreased in liver disease[20]. The hypoalbuminemia may also occur in protein malnutrition, nephritic syndrome, chronic protein losing enteropathies [21], as well as acute, and chronic inflammatory disorders, in addition to most cases in hepatocellular diseases result from different causes as increased immunoglobulin concentrations, and direct inhibition of synthesis by toxins and alcohol [22]. High serum globulin levels may be indicative of some chronic inflammatory, infectious disease, an autoimmune disease such as lupus, rheumatoid arthritis, liver disease, kidney disease, or carcinoid tumors, leukemia, or other bone marrow disease [23].

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

Table 3: Interaction effects between L-glutaminase and ethylenimine on the serum proteins levels of rat's dietary for 15 weeks.

Treatment type	Total proteins	Globulin	Albumin
	(mg/dl)		
T1	7.82± 0.34[b]	4.21 ±0.23[a]	3.61±0.26[a]
T2	5.34±0.13[d]	3.04±0.37[c]	2.30±0.33[c]
T3	6.95±0.55[a]	4.22±0.25[a]	2.73±0.30[b]
T4	6.96±0.61[a]	4.24±0.34[a]	2.72±0.27[b]
T5	5.64±0.36[c]	3.43±0.24[b]	2.21±0.12[c]
T6	5.86 ±0.48[c]	3.74 ±0.33[b]	2.12 ±0.15[c]

T1: control group; T2: 0.5mg Ethylenimine/kg; T3: Glutaminase 0.5ml/kg; T4: Glutaminase 1ml/kg; T5: 0.5ml/kg Glutaminase+0.5mg Ethylenimine/kg; T6: 1ml/kg Glutaminase+0.5mg ethylenimine/kg. a, e: Values within columns followed by different letters differ significantly at ($p<0.05$).

7.3 Effect of Ethylenimine and L-glutaminase on Serum Enzymes:

As shown in Table 4, the liver enzymes of rats in the treated group (T2) which were administered 1 mg Ethylenimine /Kg. body weight, showed significant changes at the level($p<0.05$) when compared with the normal control group(T1). There are an elevation of liver enzymes; Alanine aminotransferases(ALT), aspartate amino tranferases (AST), and Alkaline phosphatase (ALP) which became 214, 82 and 53.1 IU/L respectively compared to control group(T1) which were 124, 38 and 32 IU/L respectively. Whereas, the intravenous injection of 0.5 and 1.0 ml L-glutaminase in combination with oral administration of 0.5 mg ethylenimine/Kg. body weight for treated groups T5 and T6 respectively, seem to reverse the changes to word near normal level, and they became 133, 40 and 33.4 IU/L respectively for T6 group. These results were demonstrated that L-glutaminase improve the levels of the serum ALT, AST and ALP when compared to the treated group (T2) which administered ethylenimine ($p<0.05$), this finding may be attributed to the effect of L-glutaminase which may stabilize hepatocyte cell membrane and prevent delivery of ALT, AST, and ALP to the extracellular fluid. Since the elevated values of ALT and, AST in serum is indicative of liver damage [24]. ALT is elevated in acute liver damage and, is a more reliable marker of liver integrity than AST [25]. Since AST enzyme is not specific for liver, it is also present in RBCs, and cardiac with skeletal

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

muscles [26]. Alkaline phosphatase (ALP) is other liver enzyme. the elevation of ALP is indicate the damage of hepatic cells. Since this enzyme exists in cytoplasm, and it releases to circulation after liver damage [27], also the development of tumor causes tissue damage and leads to increase ALP level [28].

Table 4: Interaction effects between L-glutaminase and ethylenimine on enzyme activity in rats dietary for 15 weeks.

Treatment Type	Enzyme Activity (IU/L)		
	ALP	AST	ALT
T1	104±4.28 [c]	38±1.3[c]	32±1.73[a]
T2	214±9.18[a]	82±1.1[a]	53.1±1.29[b]
T3	129±3.94[c]	38±0.8[c]	31.25±0.75[c]
T4	130±6.38[c]	39±1.6[c]	32.55±1.04[c]
T5	202±4.85[b]	52±1.2[b]	41.1±1.11[b]
T6	133±5.92[c]	40±1.7[c]	33.4±1.23[c]

T1: control group; T2: 0.5mg Ethylenimine/kg; T3: Glutaminase 0.5ml/kg; T4: Glutaminase 1ml/kg; T5: 0.5ml/kg Glutaminase+0.5mg Ethylenimine/kg; T6: 1ml/kg Glutaminase+0.5mg ethylenimine/kg. a, e: Values within columns followed by different letters differ significantly at ($p<0.05$).

7.4 Effect of Ethylenimine and L-glutaminase on Relative Organ Weights:

The relative weight of Liver, kidney and spleen organs of the experimental animal had been weighted following treatment period as presented in Table 5. There were a significant elevation in the relative weight of above organs of rats in treated group (T2) that administrated 1mg ethylenimine /Kg body weight and became 7.69, 5.74 and 2.83 respectively in comparison with control group (T1) which were 4.35, 3.86 and 1.65 respectively. There were no significant changes in the weight of above organs of rats in the T3 and T4 groups which received 0.5 and 1 ml intravenous doses of L-glutaminase. However, their weight decreased significantly in T5 group as a result of administering 0.5ml L-glutaminase in relation with 0.5 mg ethylenimine/kg body weight and became 5.72, 4.90 and 2.14 respectively in comparison to treated group (T2), but still high significantly when compared with control group (T1). Whereas the organs' relative weight were 4.47, 3.98 and 1.78 respectively for the T6 group, and there were no significant differences from that of control group. These observations explained the importance of L-glutaminase in preserving the organs from damage by toxic effect of Ethylenimine. Furthermore,

Kirkuk University Journal /Scientific Studies (KUJSS)
Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

the protective effect of this enzyme was dose dependent, since 1ml intravenous doses of L-glutaminase produce more valuable, and beneficial results than 0.5ml.

Table 5: Interaction effects between L-glutaminase and ethylenimine on the organs relative weights of rats dietary for 15 weeks.

Treatment type	Liver	Kidney	Spleen
	mg/kg. body weights		
T1	4.35± 0.66[c]	3.86 ±0.47[c]	1.65±0.31[c]
T2	7.69± 0.95[a]	5.74 ±0.73[a]	2.83±0.48[a]
T3	4.29± 0.82[c]	3.72 ±0.51[c]	1.58±0.35[c]
T4	4.38± 0.75[c]	3.81 ±0.37[c]	1.49±0.11[c]
T5	5.72± 0.58[b]	4.90 ±0.48[b]	2.14±0.20[b]
T6	4.47± 0.81[c]	3.98 ±0.50[c]	1.78±0.18[c]

T1: control group; T2: 0.5mg Ethylenimine/kg; T3: Glutaminase 0.5ml/kg; T4: Glutaminase 1ml/kg; T5: 0.5ml/kg Glutaminase+0.5mg Ethylenimine/kg; T6: 1ml/kg Glutaminase+0.5mg ethylenimine/kg. a, c: Values within columns followed by different letters differ significantly at ($p<0.05$).

The potential carcinogenicity of ethylenimine was evaluated by International Agency for Research on Cancer IARC [29] and concluded that ethylenimine is possibly carcinogenic to humans. Because ethylenimine is a direct-acting alkylating agent that is mutagenic in a large number of test systems, including bacteria, insects, mammalian cells in culture, and mice (assessed in vivo by the dominant lethality test). The finding of the present study improved the preventive role of the L-glutaminase against the mutagenic effect of ethylinimine, and it was dose dependent. Pandian, *et.al.*[30] reported that purified L-glutaminase from Alkaligene faecalis KLU102 exhibit a dose-dependent cytotoxic activity against *HeLa* cells, with an IC50 value of 12.5µg/ml. The role of L-glutaminase in reducing cell viability contributes greatly to its anticancer activity by arresting the progression of the disease.

It has been found that glutaminase purified from a gram positive coccus and other sample from gram negative rods with considerable lower km value resulted in marked inhibition of Ehrilich ascites carcinoma when given 24 hours after tumor implantation and increase the survival time of tumor bearing animals [10]. Roberts, et.al.[31] reported a seven day established asparginase- resistant Ehrilich ascites carcinomas regressed completely when tumor-bearing

animal injected with purified bacterial glutaminase or Glutaminase–asparginase preparation. Sinph, and Banik, [32] were reported the gradual inhibition in growth of hepatocellular carcinoma (Hep-G2) cell lines by L-glutaminase produced from Bacillus cereus MTCC1305. However, the purified intracellular L-glutaminase from Penicillium brevicompactum NRC829 inhibited the growth of human cell line hepatocellular carcinoma (Hep-G2) with IC50 value of 63.3μg/ml [33].

The advantage of microbial glutaminase as their stability, large scale production over other sources, their role in biotechnological industries and their medical application as anticancer agent made microorganisms represent a preferable source for producing the enzyme and lead to continuous searching of high potential microorganisms strains [34]. To our knowledge, the present study on protecting effect of L-glutaminase enzyme against mutagenic and carcinogenic activity of Ethylenimine represents the novel one at least in Iraq, since all attempt failed to find related articles.

References

[1] L. A. Underkofler, R. R. Barton and S. S. Rennert, "***Production of microbial enzymes and their applications***". Applied Microbiology., 6(3), 212 (1958).

[2] R. Nandkumar, K. Yoshimunek, M. Wakayama and M. Moriguchi, *"Review:* ***Microbial glutaminase: Biochemstry, Molecular approaches and applications in the food industry***". Journal of Molecular catalysis B:Enzymatic. 23, 87 (2003).

[3] K. V. Sarada, "***Production and application of L-glutaminase using fermentation technology***". Asian pacific Jornal of research. 1(8), 1 (2013).

[4] S. Pruisner, J. N. Oavis and E. R. Stadtman, "***Regulation of glutaminase B in E.coli***", Journal of Biological Chemistry, 251, 3447 (1976).

[5] A. Meister, L. Levintow, R. E. Greenfield and P. A. Abenscheim, "***Hydrolysis and transfer reactions catalyzed by w-amidase preparations***". Journal of Biological Chemistry, 215, 441 (1955).

Volume 13, Issue 4, December 2018 , pp. (1-16)
ISSN: 1992-0849 (Print), 2616-6801 (Online)

[6] H. E. Wade, H. K. Robinson and B. W. Phillips, **"*Asparaginase and Glutaminase activities of bacteria*"** Journal of General Microbiology 69, 299 (1971).

[7] S. Prusiner, **" *Regulation of glutaminase level in Escherichia coli*"** Journal of Bacteriology, 123(3), 992 (1975).

[8] T. Yokotsuka, T. Iwasa, S. Fujii and T. Kakinuma, **"*Studies on Temperature Digestion of Shoyu Koji*"**. Part I., Journal of Japanese Soy sauce Research Institute, 13, 18 (1987).

[9] A. Sabu, **"*Sources, properties and applications of microbial therapeutic enzymes*"**. Indian Journal of Biotechnology, 2(3), 334 (2003).

[10] J. Roberts, J. S. Holcenberg and W. C. Dolowy, ***"Antineoplastic activity of highly purified bacterial glutaminase*"**. Nature 227, 1136 (1970).

[11] A. Sabu, T. R. Keerthi, Rajeev, S. Kumar and M. Chandrasekharan, **"*L- glutaminase production by marine Beauveria species Under solid state fermentation*".** Process Biochemistry, 35, 705 (2000).

[12] A. Sabu, K. M. Nampoothiri and A. Pondey, **" *L-Glutaminase as a therapeutic enzyme of microbial origin. In: Baredo, Methods in Biotechnology: Microbial Enzymes and Biotransformations*"**. Humana Press Inc, Totowa, 75 (2005).

[13] L. Davidson, D.R. Brear, P. Wingard, J. Hawkins and G.B. Kitto, **"*Purification and Properties of an L-Glutaminase- Asparaginase from Pseudomonas acidovorans.*** Journal of Bacteriology, 129, 1379 (1977).

[14] A. Imada, S. Igarasi, K. Nakahama and M. Isono, **" *Asparaginase and glutaminase activities of microrganisms*",** Journal of General Microbiology, 76, 85 (1973).

[15] NAS-NRC (National Research Council Recommended), ***"Dietary allowance"***. 15th Edition, National academy Press. Washington (2002).

[16] H. Theml, , H. Diem, and T. Haferlach, ***"Color Atlas of Hematology, Practical Microscopic and Clinical Diagnosis",*** 2nd Ed.., Stuttgart. New York (2004).

[17] N. A. Abdallah, S. K. Amer, and M.K. Habeeb, ***Production, purification and characterization of L-glutaminase enzyme from Streptomyces avermitilis.*** African Journal of Microbiology Research, 7(14), 1184 (2013).

[18] BRL (Bionetics Research Labs). ***"Evaluation of Carcinogenic, Teratogenic, and Mutagenic Activities of Selected Pesticides and Industrial Chemicals"***. National Cancer Institute, Bethesda, MD, (1968).

[19] S. B. Rosalki, and N. Mcintyre, ***"Biochemical investigations in the management of liver disease. Oxford textbook of clinical hepatology",*** 2nd Ed., Press, New York (1999).

[20] A. Mizuno, T. Uematsu, and S. Gotoh, ***"The measurement of caffeine concentration in scalp hair as an indicator of liver Disease"***, Journal of pharmacy and pharmacology, 48, 660 (1996).

[21] S.P. Daniel, and M. K.Marshall, (1999). ***"Evaluation of the liver: laboratory tests. Schiff's diseases of the liver",*** 8th Edition. Lippincott, USA(1999).

[22] N.W. Tietz, ***"Fundamentals of Clinical Chemistry"***. 6th Edition, Elsevier (2008).

[23] A. Henry, M.D. Teloh, ***"Serum Proteins in Hepatic Disease ",*** Annals of clinical and laboratory science, 8(2),127 (1978).

[24] P.T. Giboney, ***"Mildly elevated liver transaminase levels in the asymptomatic patient"***. American Family Physician, 71(6), 1105 (2005).

[25] O. A. Ojiako, and H. U. Nwanjo ***Is Vernonia amygdalina hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats.*** African Journal of Biotechnology, 5(18), 1648 (2006).

[26] D. W. Moss, and A. R. Handerson, ***"Tietz Textbook of clinical chemistry"***. 3rd eddition. Saunders, USA (1999).

[27] R. R. Sallie, S. Tredger and R. Williams, ***"Drugs and the liver part 1: Testing liver function. Biopharm"***. Drug Dispos., 12(4), 251 (1991).

[28] J. Iqbal, M. Minhajuddin, and Z. H. Beg, **"*Suppression of diethylnitrosamine,and2–acetylaminofluorene–induced hepatocarcinogenesis in rats by tocotrienol-rich fraction isolated from rice bran oil*".** European journal of cancer prevention13(6), 515 (2004).

[29] IARC (International Agency for Research on Cancer). **"*Aziridine. in Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide*".** International Agency for Research on Cancer, France 71, 337 (1999).

[30] S. R. Pandian, V. Deepak, S. D. Sivasubramaniam, H. Nellaiah, and K. Sundar, ***"Optimization and purification of anticancer enzyme L-glutaminase from Alcaligenes faecalis KLU102".*** Journal of Biologia 69(12), 1644 (2014).

[31] J. Roberts, J. S. Holcenberg and W. C. Dolowy, ***"Glutaminase induced prolonged regression of established Ehrlich carcinoma".*** Life Sciences. 10(25), 1 (1971).

[32] P. Snigh, and R. M. Banik, **"*Biochemical characterization and antitumor study of L-glutaminase from Bacillus cereus MTCC1305*".** Applied Biochemistry and Biotechnology. 171(2), 522 (2013).

[33] A. M. Elshafei, M. M. Hassan, N. H. Ali, M. A. Abouzeid, D. A. Mahmoud, and D. H. Elghonemy, **"*Purification, Kinetic properties and antitumor activity of L-glutaminase*

from Penicillium brevicompactum NRC 829". Journal of British Microbiology Research, 4(1), 97 (2014).

[34] M. K. Habeeb, "***Microbial production of glutaminase enzyme***", Journal of Research in Biology 3(1), 775 (2013).