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The Effects of Extracted Peptide from Skin of Iraqi Frog (*Rana ridibunda*) on Human Leukemic Lymphocytes

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Abstract:

The purified frog skin peptides were tested on leukemic patients lymphocytes, which revealed effects of cytotoxicity. Four frogs (*Rana ridibunda*) were stimulated by single intra-peritoneal injection of norepinephrine-HCl . Five different peptides;1(18) A, 2(19) L, 3(20) I,4(21) E and 5(22) Y were isolated and quantified. The peptide 3(20)I had 5.87% of hemolysis, while healthy human lymphocytes cytotoxic activity was for 2(19)L with inhibition(-10.4%).All peptides were subjected to polyacrylamide gel electrophoresis. The results revealed peptides 1(18)A, 2(19)L, 3(20)I which appeared as low as 10 KDa marker. Theoretically, the whole polypeptide had a molecular weight 7488.61 Dalton and contained on 62.405 amino acid (a.a). The peptide 4(21)E had a highest inhibitory effect(46%) on tumor cell line L20B. Furthermore , peptides effects on acute and chronic myeloid lymphocytic leukemia patients cell cultures revealed peptides selectivity in their action according to their net charge and functional group as reactant proton donor by the evidence of peptide 5(22)Y, 16.22 Dalton so it was either N—terminus (--NH_2) or C—terminus (--OH) that led to cross cell membrane then acted as antigen mediated and activated cells in a high significant value (-142.37± 47.69)for acute myeloid lymphocytic leukemia .Both of peptides 3(20) I and 2(19) L were revealed a highly significant differences within Chr.40 and Chr. 22 of inhibition effects by testing volumes 15 µl and 10 µl . Those inhibition effects were due to peptides reaction with mitochondrial membrane which led to apoptosis . Conclusion; Frog skin peptides have a therapeutically worth for malignant diseases. Also some of peptides were activated lymphocytes may to cure immunodeficiency.

Key words: Frog, Leukemia, Lymphocytes, Peptides, Skin

Introduction:

The amphibians are creatures lacking to protect themselves against predators, in which they are likeable meal to a variety of predators (1). In order to maintain themselves from potential foes, the amphibians have developed their physiological and morphological capabilities (2). The skin of frogs and toads has two types of glands onto dorsal surface, head, and neck. Mucous glands secrete snotty materials to keep their skin moist and cutaneous respiration. The granular (poison, serous)glands act as a defense mechanism when frogs stimulated by an injury, stress and microbial invasion(3) .Frogs skin secretions vary according to the species and their toxicity grade (4). The researchers reported that skin slimy secretion contained antimicrobial peptides (AMPs) (5). Esculentin is frog skin peptide and has insulinotropic action (6).

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The frog skin (*Rana ridibunda*) peptides can eradicate some of both multi drug resistance (MDR) gram positive ,gram negative pathogenic bacteria and parasites as their therapeutic agents(7, 8). Frogs skin (AMPs) have immunomodulation activity (9,10), so they can activate lymphocytes proliferation by increasing IL-2 and IL-12 levels *in vitro*(11).The purified octadeca peptide pLR *Rana pipiens* can activate histamine releasing *in vitro* (8).Chinese doctors advise leukemic patients to drink Liu Shen Wan as a patent medicine which contains toad secretions. They observed that juice has effects of curing leukemia (12, 13).The aim of this study is to focus on cytotoxic effects of Iraqi frog skin peptides on leukemic lymphocytes. These peptides need further *in vivo* studies to assess their effects as to therapeutic trail for many diseases so they have a future.

Materials and Methods:

The experiment was carried out with some modifications by ignoring frogs gender, while their housing was in animal house which started with half October and ended in half December 2017. The experiments were done in Biotechnology Research Center laboratories of Al-Nahrain University, Baghdad, Iraq.

Four Iraqi frogs (*Rana ridibunda*) of different weights (20, 30, 35 and 45gm) were stimulated to release secretions from skin glands (14).

Isolation of Secretion

Each frog intra-peritoneal was injected by norepinephrine-HCl 40 ng/gm of body weight, then it was left for 15 minutes in 150 ml of 0.1 M NaCl solution containing 0.01 EDTA as a washing solution (15).

Peptide Purification

Gel filtration chromatography on Sepharose 6B was used for protein detection. This gel was prepared according to Pharmacia catalogue instructions (Merck KGaA, Darmstadt, Germany). The isolated secretions were loaded slowly over the Sepharose 6B gel. The protein was eluted from color by 20 mM sodium citrate buffer (pH5) and flow rate was adjusted to give 40 ml per hour. 5 ml for each fraction was collected. Activity and absorbance were determined spectrophotometricaly at 280 nm for each fraction by using UV detector.

Protein Electrophoresis

The gel filtrated peptides(Protein X) were assayed to measure their molecular weight by using Sodium Dodecyl Sulfate Polyacrylamide gel (SDS-PAGE) according to (16). Each fraction (80µg) was loaded with 18% SDS-PAGE for each well. Following electrophoresis, the gel was fixed over night at room temperature in 40% methanol, 7% acetic in deionized water and staining etc. Protein ladder (Promega) was for standard proteins with molecular weights (150,100,75,60,35,25,16 and 10KDa) that used for calibration.

Determination of Protein Concentration

Bradford method (17) was applied to determine peptides concentration for each of fractions (595 nm) by using the standard curve or the equation (y=0.0046x+0.0596) as shown in Fig.(1).

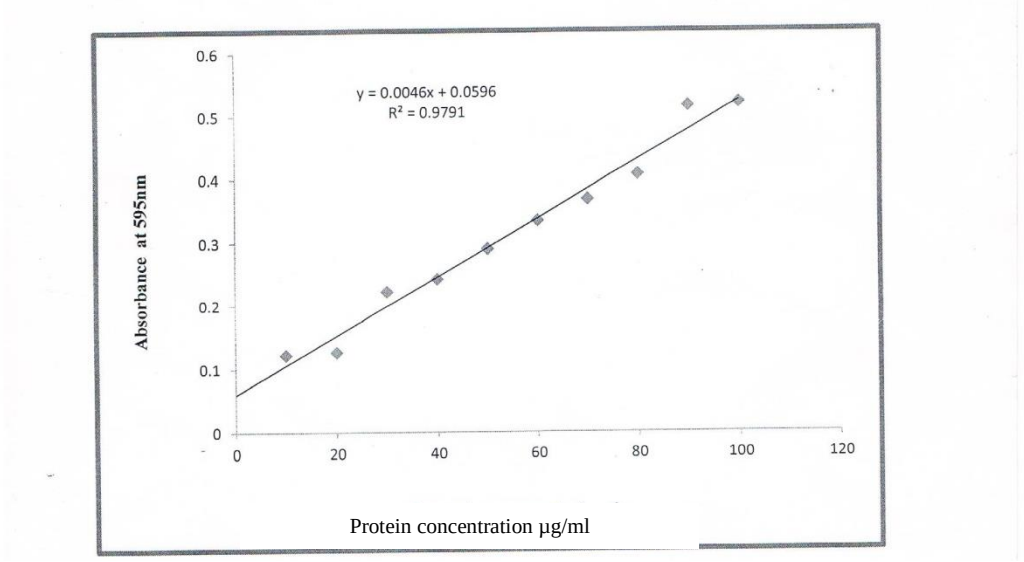

Figure.1. Standard curve for protein concentration determination. Note, y=Protein fraction absorbance (595 nm), x=Protein concentration (µg/ ml) .

Determination of peptide concentration by using Biospec-nano (Shimazu -BIOTEC, Japan).

The reading designed to read the absorbance at 280 nm and peptides concentrations reading were in micromole (µM).

The molecular weight of peptide fraction was theoretically calculated using the formula:{Peptide concentration (µg/ml)}/{Peptide Mole fraction(µM)} X 1000= Peptide molecular weight (Dalton).

The number of amino acids in peptide fraction was mathematically calculated using the formula**:** Peptide molecular weight (Dalton) /Average of amino acid molecular weight (120Dalton) according to (18).

Patients under Study

The patients under study were newly admitted to Al-Imamayn Al-Kadhomiyayn Medical City – Department of blood diseases. The study involved four adult male patients suffered of symptoms (fever, fatigue weight loss, night sweating, recurrent nose bleeds and swollen lymph nodes) with ages 22, 40, 43,and 74 years. The suspected patients

were examined by a specialist physician in blood diseases. The examinations involved blood film and bone marrow.

Blood films were stained by Leishman and examined by a physician. The diagnosed films were for newly acute and chronic myeloid lymphocytic leukemia patients (before administration of any medication) as shown in Fig. (2) which was for acute myeloid lymphocytic leukemia patient. There were abnormal increasing in white blood cells count and immature leukocytes, also alterations in red blood cells shape and count. Lymphocytes of patient was cultured without treatment and incubated in CO_2 incubator for 24 hrs. and additive four days at 37Cº to observe any blood cells deformities as shown in Fig. 3 and 4**.**

Preparation of Healthy Human and Leukemic Patient Lymphocytes

The preparation of lymphocytes was practiced according to (19).

Hemolysis Activity

Preparation of healthy human red blood cells (hRBCs) was carried out as described previously by (20, 21).

Peptides Biological Activity

The biological activity of different purified peptides(18A,19L,20I,21E and22Y) against L-20B cell line ,healthy human lymphocytes and healthy human red blood cells were evaluated separately. The colorimetric cell viability MTT was used as described by (19, 22) at absorbance 620nm.

A- L-20B cell line was assayed by transferring sterile micropipette 200 µl of this cells line suspension and it was put in sterile ELISA plastic plate wells by 4 replicates both of tested and control wells (200µl/ well) then 10µl were pipetted of each of the tested peptides (18A, 19L ,20I,21E and 22Y) and they were put in columns of wells(10µl/well) respectively but not for control wells. The plate was covered and incubated for 24 hrs.at 37Cº in CO_2 incubator

B- Hemolysis was assayed by transferring sterile micropipette 200 µl of hRBCs suspension and it was put in sterile plastic plate wells by 4 replicates for both of tested and control wells (200µl / well) then 10µl were pipetted of each of the tested peptides (18 A, 19 L, 20 I, 21 E and 22 Y), and they were put in columns of wells (10µl/well) respectively but not for the control. The plate was covered and incubated for 24 hrs.at 37Cº in CO_2 incubator.

C- The prepared healthy human lymphocytes suspension was tested by transferring sterile micropipette 200µl of healthy lymphocytes suspension and it was put in sterile ELISA plastic wells by 4 replicates for both of tested and control wells then 10µl was pipetted of each the tested peptides 18A,19L,20I,21E and 22Y and put their in columns of wells (10µl/well) respectively but not for the control. The plate was covered and incubated for 24 hrs.at 37Cº in CO_2 incubator.20µl of MTT solution (5 mg/ml) was added to each of wells in the ELISA plates (A,B and C) and incubated at 37Cº for 24 hrs. Finally,50µl of DMSO (dimethylsulfoxide) was added to each of wells in three plates (A,B and C) and incubated at 37Cº for 10 minutes .The absorbance was measured for each well at 620nm by using an ELISA reader .The live cells percentage of viability and inhibition were calculated according to the formula :

GI% = (OD of control wells –OD of test wells) / (OD of control wells) X 100 .

The cytotoxic efficacy of different concentrations as volumes (30µl,15µl,10µl and 5µl) of frog skin purified peptides (18A,19L,20I,21E and 22Y) were done by using the colorimetric cell viability MTT assay as described by (19,22). The peptide fractions were named by letters A, L, I (Ali); E (Evan) and Y (Yousif).

Statistical Analysis

Data were statistically analyzed by using the program Statistical Analysis System SAS (23) to study the coefficient on the studied traits. A comparison of the significant differences between the values was done by using the least-squared means (lsm).

Results:

The blood films examination of acute myeloid lymphocytic leukemia patients revealed anomalies in both of patients erythrocytes and leukocytes so as an increasing in leukocytes count, immature leukocytes, lymphocytes shape alteration and patients nucleated red blood cells as shown in Fig. 2

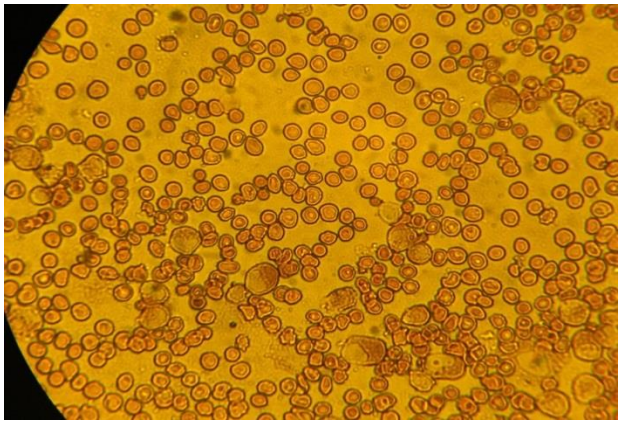

Figure 2. Blood film for acute myeloid lymphocytic leukemia patient (acute 74 year), which contains nucleated RBCs with their attachment and large size of lymphocytes Leishman stain (Oil lens).

While patients lymphocytes cell culture revealed large number after 24 hrs. of incubation period as shown in Fig.3.Hence, day 5 of incubation period revealed different shapes and sizes of lymphocytes as shown in Fig. (4).

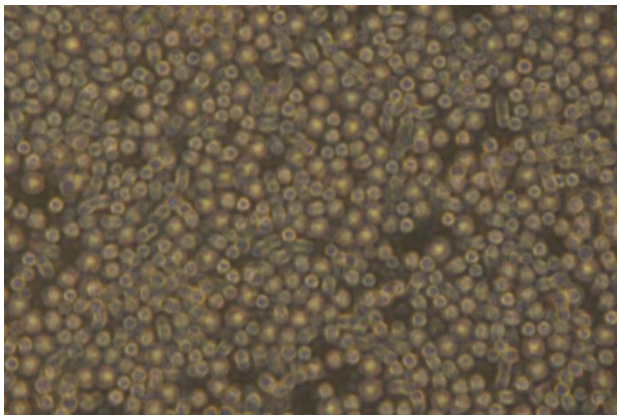

Figure 3. Acute myeloid lymphocytic cell culture after 24 hrs. of incubation without treatment, which shows large number of lymphocytes. (40X)

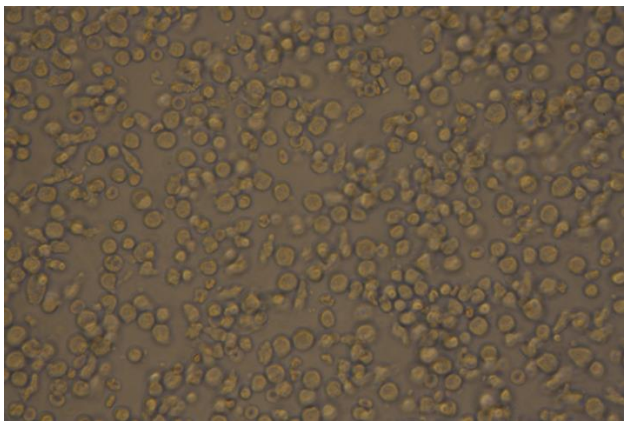

Figure 4. Acute myeloid lymphocytic leukemia cell culture at day 5 of incubation without treatment, Which contains different shapes of lymphocytes, large, small rounded cells and bipolar fusiform attached cells. (60 X)

The purification of isolated secretions revealed protein substance which consisted of five protein fractions 18 A,19 L,20 I,21 E and 22 Y as shown in Table, 1 and Fig. 5. Hence, the highest absorbance 0.369 nm was for protein fraction 3(20)I and the lowest absorbance 0.296 nm was for protein fraction 1(18)A.

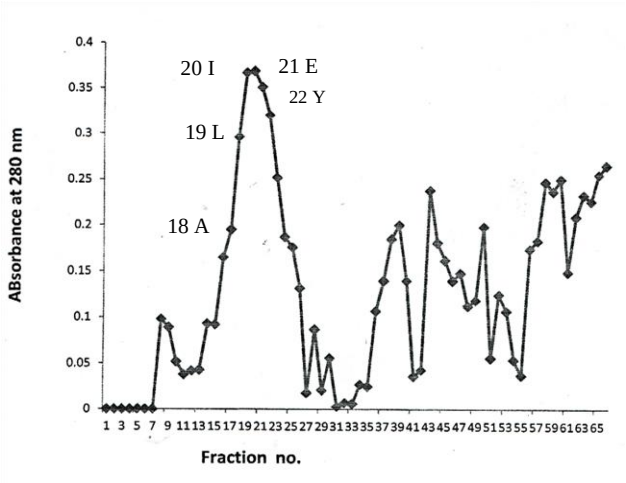

Figure 5. The isolated peptide fraction, 18(A), 19(L), 20(I), 21(E) and 22(Y), that obtained from frog skin secretion by using column chromatography.

The present experiment of SDS -PAGE showed bands and revealed five peptides as shown in Fig.(6) and collectively forms a polypeptide in which they appeared as faint band on the gel so the used series of these fractions are shown in Fig. (5), that leads to hypothesized that each fraction is a part of whole polypeptide.

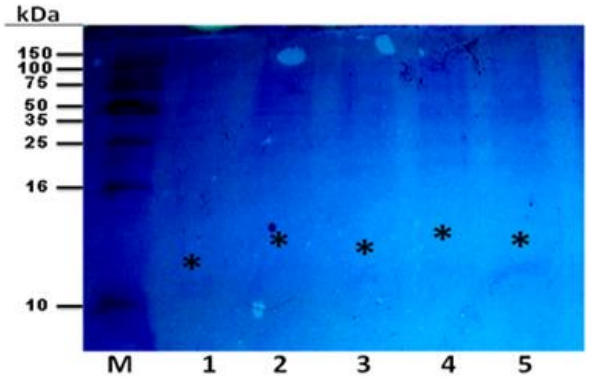

Figure 6. 18% SDS-PAGE. The protein X was purified by gel filtration, and the fractions were loaded on 18% SDS-PAGE. Lane M: is the PageRuler™ Plus Unstained Rec. Protein Ladder (Promega); lane 1: fraction A; lane 2: fraction L; lane 3: fraction I; lane 4: fraction E; lane 5: fraction Y. The protein for each fraction was indicated by black asterisk.

Of calculations, the peptide fraction 3(20)I revealed large molecular weight 3651.2 Dalton and contained 30.43 a.a. While a small molecular weight was for peptide fraction 5(22)Y as shown in Table 1.

Table 1. Some of chemical and physical properties of extracted peptide fractions from frog skin.

Peptide fraction number	Absor. (280nm)	Absor. (595nm)	Peptide conc. (µg/ml)	Mole fraction. (µMole)	Molec. Weight (Dalton)	Number of amino acids
1(18)A	0.296	0.067	1.61	1.69	952.7	7.94
2(19)L	0.362	0.085	5.52	8.811	626.5	5.22
3(20)I	0.369	0.114	11.83	3.24	3651.2	30.43
4(21)E	0.351	0.077	3.78	1.686	2242	18.68
5(22)Y	0.320	0.06	0.08	4.933	16.22	0.135

Theoretically, the whole polypeptide had a calculated molecular weight 7488.61 Dalton and contained 62.405 amino-acid. The polypeptide fractions 1(18)A, 2(19) L , 3(20)I , 4 (21) E and 5 (22) Y contained 7.94, 5.22 , 30.43 , 18.68 and 0.135 amino acid respectively. The peptide fraction 5(22) Y showed low molecular weight (16.22Da), that leads to predict that this is an initiator as N-terminus (--NH_2) of the peptide or hydroxide ion (--0H) as shown in Table 1.Therefore,the SDS –page showed final band near to 10KDa compared to ladder as shown Fig.(6). This result indicated that the fractions 1(18)A, 2(19)L and 3(20)I appeared as low as 10KDa marker. The biological activity of 10ul for protein fractions revealed lowest hemolysis 5.87% on healthy hRBCs was to peptide fraction 3 (20 I). While the highest inhibition effect 26.1% on healthy human lymphocytes was to protein fraction 1(18)A. Furthermore, the results revealed the highest inhibition 46% on tumor cell line L-20B was to protein fraction 4 (21)E as shown in Table 2 .

Table 2. Some of biological activities by testing the concentration of peptide fractions, **that isolated from Iraqi frog skin secretion *Rana ridibunda*.**

Peptide fraction no.	Peptide conc. (µg/ml)	Hemolysis% of healthy hRBCs	Inhibition % of healthy lymphocytes	Inhibition % of tumor cell line L20B
1(18)A	1.61	7.04	26.1	**25**
2(19)L	5.52	6.26	- 10.4	**43.5**
3(20)I	11.83	5.87	- 16.5	**40.3**
4(21)E	3.78	8.8	- 146.9	**46**
5(22)Y	0.08	8.4	- 550.4	**0.8**

(-)=Activation effect by decreasing inhibition%.

In this study , results revealed an obvious significant difference in inhibition percentage of effects for protein fraction 3(20) I on leukemic lymphocytes in cell culture of acute myeloid lymphocytic leukemia patients with the highest mean 7.65 ± 6.63 so it was the best one in inhibition mean from the other 3 treatments, in contrast with the lowest mean -142.37±47.69of protein fraction 5(22) Y, P< 0.01. While there were percentages of inhibition for protein fractions 2(19)L 41.87±2.57, 3(20)I 38.62 ± 2.57 and 38.10±2.59,P< 0.01 in cell culture of chronic myeloid leukemia patients but not significant as shown in Table3**.**

Table 3. Statistical differences between types of peptides effect as inhibition rate% in acute and chronic leukemic patients cell cultures .

	Acut.74 year	Chr. 40year	Chr. 22year	Chr. 43year
Prot. Frac.	Mean ±S.E	Mean ±S.E	Mean ±S.E	Mean ±S.E
Frac. 1(18)A	-14.67± 6.17 [b]	39.57± 10.59 [a]	28.15± 7.21 [a]	26.82± 4.38 [a]
Frac. 2(19)L	1.42± 9.48 [b]	28.95± 14.57 [a]	41.87± 2.57 [a]	31.62± 5.78 [a]
Frac. 3(20)I	7.65± 6.63 [b]	38.10± 2.59 [a]	32.50± 6.69 [a]	38.62± 2.24 [a]
Frac. 4(21)E	-4.02± 7.01 [b]	34.10± 4.27 [a]	35.85± 7.24 [a]	10.40± 26.79 [a]
Frac. 5(22)Y	-142.37± 47.69 [a]	16.15± 6.95 [a]	30.00± 2.70 [a]	-5.35± 24.97 [a]
Significant	**	N .s	N.s	N .s

N.S. =Non-significant ** = P< 0.01

Acute myeloid lymphocytic leukemia for 74 years old male patient (Acute 74).**Chronic myeloid lymphocytic leukemia for male patients(Chr.40,22,43) . (-) = Activation effect by decreasing in inhibition rate.**

Also the assessment of the effective protein fractions concentrations (volume) on leukemic lymphocytes in cell culture of chronic myeloid lymphocytic leukemia revealed to a highly significant of inhibition 43.6± 3.94 was to volume 5µl with patient cell culture **(**Chr.40 **)**in comparison with volume 30 µl by the mean value 10.22± 8.68, P<0.01**.**While the highly significant inhibition

41.18 ± 4.73) was to tested volume 30ul in comparison to lowest inhibition 22.84 ±4.04 P<0.05 was for the tested volume 5ul in (chr.22) patient as shown in Table 4 .

There were no statistical significant differences between the tested protein fractions (peptides) volumes (30µl, 15µl, 10µl and 5µl **)** on leukemic patients lymphocytes of(acut.74) and (chr.43) in despite of mathematical differences between volumes effects but not significant as shown in Table 4.

Table 4. Statistical differences between types of peptides concentration (volume) effect as inhibition rate in acute and chronic

Tested	Acut . 74	Chr.40	Chr.22	Chr.43
Conc.	Mean ±S. E	Mean ±S. E	Mean ±S. E	Mean ±S. E
Vol. 1(30µl)	-59.98± 51.96 [a]	10.22± 8.68[a]	41.18± 4.73[b]	39.16± 2.86 [a]
Vol. 2(15µl)	-37.40± 32.47 [a]	33.58± 4.94[b]	38.50± 1.87 [b]	33.04± 3.44 [a]
Vol. 3(10µl)	-16.62± 20.12 [a]	38.10± 5.75[b]	32.18± 5.41[ab]	6.74± 21.07 [a]
Vol. 4(5µl)	-7.60± 10.84 [a]	43.60± 3.94[b]	22.84± 4.04[a]	2.76± 19.37 [a]
Significant	N. s.	**	*	N. s.

N .s = Non-significant, * = P< 0.05, ** = P< 0.01
Chronic myeloid lymphocytic leukemia (Chr.), (-) = Activation effect by decreasing in inhibition rate.

Discussion:

Peptides were secreted from the skin glands of stimulated Iraqi frogs. In this study , five peptide fractions were isolated and purified by using Sepharose 6B gel as an alternative method for protein purification instead of Sephadix G50 or Sepharose FF which were used by the other researchers (1, 2, 24). Furthermore, the purified peptide under study had molecular weight 7488.61 Dalton and less than 10 KDa in agreement with (24).The obtained peptide contained 62.405 amino acid residues in agreement with (25) in their mimicry, who published that the peptide containing 63 amino acid residues.

The biological activity of purified peptides on hRBCs may lead to predict peptide 2(19)L which contain 5.22 amino acids that may possess the specific amino acid that capable to react with cell membrane and trans-membrane channels of hRBCs because of hydrophobic interactions thereby caused an increase in cells membrane permeability with low hemolytic activity in agreement with (1, 12, 21, 26).

The study suggested , that peptides are differed among themselves in their bioactivity and of their mode of action according to the initiator functional group , as on N-- terminus of amine group (--NH_2) or hydroxide ion (--OH) of C--terminus in first amino acid , which appeared in 5 (22) Y peptide with a molecular weight 16.22 Dalton in agreement with (9). Those functional groups have a reactant proton , which may have reacted with outer, inner cell membrane and mitochondrial membrane of under studied cell cultures in agreement with (25) .

All tested peptides revealed inhibitory effects for tumor cell line L20B that can be attributed to cells sensitivity and they have a certain mechanism to defend against the tested peptides that led to predict, the peptide 2(19) L which contained 5.22 amino acid residue crossed cell membrane, thereby reacted with mitochondrial membrane then caused death in agreement with (27, 28).The outer lymphocytic leukemia cells membrane had phosphatidyl serine (3-9%), which led to their negatively charged and predict an electrostatic interactions with our tested peptide 2(19) L 5.22 a.a and 3 (20) I 30 a.a. Finally, these peptides penetrated cell membrane and caused apoptosis for both of acute and chronic myeloid cells in agreement with (13, 29, 30).While healthy human lymphocytes revealed activation and proliferation at the same peptide because of its capability to stimulate lymphocytes to secret interleukins (IL-2*) in vitro* in agreement with (9, 11, 31, 32, 33). Furthermore, this peptide may have a capability for differential recognition between healthy lymphocytes cell membrane and L-20B tumor cell membrane in agreement with (8).

In this study, the peptides possess inhibition effects on chronic myeloid lymphocytic leukemia cell culture, but not significant. While both peptides 2(19)L and 3(20)I inhibit acute myeloid lymphocytic leukemia cell culture in agreement with (34) who published their using of isolated peptide(3200 Dalton), which inhibited the inducted tumor in mice . Hence, the effect of tested peptide may directed for disruption of leukemic cell membrane , also the effect may involve induction of apoptosis and cell death in agreement with (10).

The peptides 3(20)I (30.43 a.a.) and 2(19)L(5.22 a.a.) reveal the most selective effects on chronic myeloid lymphocytic leukemia cell culture by concentrations 11.83 µg/ml and 5.52 µg/ml respectively so as their tested volumes 15µl, and 10

µl, they have been analogues for maganin-2 peptide by its effect on hematopoietic and solid tumors at concentration as low as 12µg /ml in agreement with (35).

In this study, the tested peptides 1(18)A 7.94 a.a ,4 (21) E 18.63 a.a and 59(22) Y 0.135 a.a may act as antigenic effect for activation to acute myeloid leukemia cells that lead to proliferation by reaction of peptides with cell membrane receptors in agreement with (10, 34,36).

Conclusions:

Iraqi frogs skin (*Rana ridibunda*) peptides are five bioactive molecules so they have low molecular weight with a number of amino acids which contain either ---NH_2 or ---OH functional group which reacts with lymphocytes and mitochondrial membranes. The peptides have significantly selective effects on leukemic patients cell cultures and have low toxic effect on human red blood cells. Some of peptides have cytotoxic effect as on lymphocytes inhibition rate versa peptides which are activated lymphocytes. Peptides are reacted as antigens sequel to cell mediated antigen immune response. Also peptides concentrations differ in their effects. Seemingly, frog skin peptides possess therapeutic benefits for malignant diseases and immunodeficiency.

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Conflicts of Interest: None.

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تأثيرات الببتيدات المستخلصة من جلد الضفدع العراقي *Rana ridibunda* على الخلايا اللمفاوية لمرضى ابيضاض الدم

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الخلاصة:

لقد اختبرت ببتيدات منقات من جلد الضفادع العراقية على الخلايا اللمفاويه لمرضى ابيضاض الدم بان لها تأثيرات قاتله للخلايا اللمفاوية . فقد تم تحفيز اربعة ضفادع(*Rana ridibunda*) بحقنها في تجويف البطن بجرعه مفرده عقار نورابينفرين - HCl.تم تشخيص خمسة ببتيدات وهي Y (22) 5 , E (21) 4 , I (20) 3, L (19) 2 , A (18) 1 ثم قدرتركيزها. كان للببتيدات قابلية على تحلل خلايا دم حمراء للانسان سليم ووجد ان اقل نسبة تحلل عند ببتيد I(20)3 وكانت نسبته 5.87% ، بينما كانت اقل نسبة تثبيط للخلايا اللمفاوية السليمه كتأثير سام للخلايا عند ببتيد L(19)2 ونسبة (10.4%-).اختبرت الببتيدات بتقنية الترحيل الكهربائي للبروتينات باستخدام بولي اكريل امايد جل (18%SDS). اظهرت النتائج بان الببتيدات I(20)3, L (19)2, A(18) 1 بان وزنها الجزيئي اقل من البروتين المعلم (10KDa). لقد وجد نظريا بان عديد الببتيدات لها وزن جزيئي 7488.61دالتون وتحتوي على 62.405حامض اميني . ان الببتيدE(21)4 له اعلى تاثير مثبط (46%) على الخط الخلوي السرطاني L20B. والاكثر من ذلك الببتيدات المختبرة على الزروع الخلوية لكل من مرضى ابيضاض الدم النخاعيني اللمفاوي الحاد والمزمن، حيث اظهرت النتائج بان الببتيدات لها فعالية انتخابية وطبقا الى صافي شحنة الببتيد والمجموعة الفعالة الواهبة للبروتون التفاعلي وبدليل الببتيد Y(22)5وزن الجزيئي 16.22 دالتون الذي يمثل اما نهاية امينية (NH_2--) او نهاية هيدروكسيلية (OH--)والتي تؤدي الى عبورها الغشاء الخلوي وبعد ذلك الخلايا تنشط للتضاعف عندما يعمل الببتيد كمستضد وسيط، حيث كانت اعلى قيمة معنوية (47.69 ± 142.37-) للتنشيط في زروع ابيضاض الدم النخاعيني اللمفاوي الحاد .ان كل من الببتايدات I(20)3 ذات الوزن الجزيئي 3651.2 دالتون، وايضا الببتايد, L (19)2 ذات الوزن الجزيئي 626.5 دالتون اظهرتا اعلى فروق معنوية للتاثير المثبط على خلايا المرضى Chr.40و .Chr 22 وباستخدام الحجوم المختبرة µl 15و10µl,ان التاثيرات المثبطة للخلايا اللمفاوية بواسطة الببتيدات المختبرة قد يكون بسبب تفاعل الببتيدات مع غشاء المايتوكندريا والذي يؤدي الى موت الخلية المبرمج .الاستنتاج، ان ببتيدات جلد الضفدع ربما لها قيمة علاجية للامراض الخبيثه واخر له القابلية لتنشيط الخلايا اللمفاوية ربما تستعمل لمعالجة مرض العوز المناعي .

الكلمات المفتاحية: الضفدع، ابيضاض الدم، الخلايا اللمفاوية، الببتيدات، الجلد.