

Effect of *Colocynthis (Citrillus colocynthis)* plant extract on leukemic cells

دراسة تأثير نبات الحنظل على الخلايا السرطانية

Khulood W.ALSamarrae Bashar A.jawad* Farah T.Abdullah
Wessam A. Hussen**

Biotechnology Research Center/AI-Nahrain University

*College of science/Babil University

**Ministry of health/Ghazi Al-Harreri hospital for special surgery

خلود وهيب السامرائي بشار عوده جواد* فرح ثامر عبدالله وسام عبداللطيف حسين**

مركز بحوث التقنيات الأحيائية / جامعة النهريين

*كلية العلوم / جامعة بابل

**مستشفى غازي الحريري للجراحات التخصصية / وزارة الصحة

Abstract

The present research concentrated on evaluating the biological activity of *Colocynthis (Citrillus colocynthis)* plant oil extract on peripheral blood cells from leukemia patients using some cytogenetic parameters. when it is added to this cell in culture media in different concentration and incubated for 72 hours at 37C, the results pointed out that cell exposed to the *Citrillus* extract in culture media have mitotic index near to normal ranges, and number of chromosomal aberrations in cells exposed to the *Citrillus* extract is less than in the leukemic cells. This study considered as an attempt in survey for anti-tumor agents in medicinal plants.

المستخلص

يهدف البحث الحالي الى تقييم بعض التأثيرات الحياتية للمستخلص الزيتي لنبات الحنظل على خلايا الدم المحيطي للمرضى المصابين بمرض ابيضاض الدم باستخدام بعض المعايير الوراثية وذلك عند اضافته لمزارع خلايا الدم السرطانية بتركيز مختلفه و بحرارة 37 درجة مئوية لمدة 72 ساعة . اشارت النتائج بأن الخلايا الطبيعية المعرضة للمستخلص الزيتي لنبات الحنظل في المزارع الخلوية ذات معدلات انقسام طبيعي وكان عدد الانحرافات الكروموسومية لهذه الخلايا اقل من الخلايا السرطانية . تعتبر هذه الدراسة كمحاولة في مجال البحث عن نباتات طبية ذات فعالية مضادة للسرطان .

Introduction

Leukemia is a hematological malignancy that involves bone marrow. This disease is socially important, as it is among the major causes of death in children. Acute lymphoblastic leukemia (ALL) is the most common form, accounting for about 80% of all acute leukemia cases. Acute myeloblastic leukemia (AML) accounts for 15% of leukemia cases in children [1,2]. The causes of the acute leukemia have not yet been identified. The current concept of their etiology and pathogenesis suggests an important role for chromosome aberrations, which may be inherited or induced by external factors [3].

Colocynthis (Citrillus colocynthis) is well known in medicinal plants, grows naturally in the western Iraqi desert and in many other tropical and subtropical countries [1]. It is indigenous to Turkey and found also in Sri lanka, Egypt, Syria, and the Arabian Gulf [2]. Its fruits have been recommended for indigestion and diabetic people in traditional medicine. *Colocynthis* is an annual plant, with a whitish root, and prostate,

angular, hispid stems. The leaves are alternate, chordate, ovate, many-lobed, and white, with hair beneath the lobes obtuse [3].

Vomiting, bloody diarrhea, colitis, kidney irritation and damaging of the liver follow the intake of toxic dosage (0.6-1g), and then increased diuresis, leading on to anuria [1]. The main active compounds of *Citrillus* is L-glucoside (Chlorogenicacid derivatives), saponins, tannins and linoleic acid (which is important for human nutrition) [2,4].

The bitter principle of the extract is obtained in an impure form called Colocynthin; Colocynthin is a glucosid, the action of diluted acids resolving Colocynthin, into resinous body, and sugar.

A crystalline substance, Colocynthin, is insoluble in water, and cold absolute alcohol, but soluble in ether and boiling alcohol. Colocynthin is insoluble in chloroform, and petroleum ether [5].

In recent years there has been much interest in developing new oil seed crops which could be used in food, and for medicinal and industrial purposes [6].

Material and Methods

Tow hundreds grams of plant seeds (obtained from college of pharmacy/Baghdad University) were dried overnight at 50C⁰ and grinded into powder then mixed with 150 ml hexane and the extraction carried on by soxhlet for 16hrs.

The extract was evaporated by rotary evaporator; the residue was mixed with olive oil with the ratio 1:1 and used for other steps [6].

Blood Samples: (2 – 5) ml were collected from International Center for Blood Diseases by vein puncture using plastic disposable syringes 5 ml coated with heparin (0.5 ml) clotting is prevented by gentle mixing, then placed in cool – box to be transported to laboratory. About 0.3ml or 6 – 7 drops of heparin zed whole blood was inoculated in RPMI 1640 (Rossel Pank Memorial Institute 1640) culture medium supplied with 10% ml fetal calf serum and 0.3 – 0.5 ml of crude PHA (Phyto Hem Agglutinin), and then the cultures were incubated at 37C⁰ with frequent shaking every 24 hr.

After 71 hr of incubation, 0.1 ml of colcemide was added to each tube with mild shaking then transferred back to incubator. After that harvesting procedure was done including the following steps [7]:

1. The culture was centrifuged at 1500 r.p.m. for 10 minutes.
2. The supernatant was discarded by pipetting off media, leaving as little medium as possible over the cell pellet.
3. The pellet was re-suspended in 5 ml of hypotonic solution (KCl) with continuous gently shaking, the hypotonic solution mostly pre-warmed to 37C⁰ before use.
4. The culture tubes were incubated in the incubator at 37C⁰ for 20 min.
5. The culture tubes were again centrifuged at 1500 r.p.m. for 10 min; then again the supernatant was discarded by pipetting off media, leaving the media as little as possible over the cell pellet.
6. Then fixative solution (Glacial acetic acid and Absolute methanol 1:3) was added to the remaining media drop by drop with continuous shaking.
7. The culture tubes were again centrifuged at 1500 r.p.m. for 10 min.

8. Step 6 and 7 should be repeated several times until the cell suspension became clear.
9. After the final centrifugation, the cells were suspended in a small volume of fixative (approximately 0.5 – 1 ml) depending on the size of the cell pellets to give a slightly opaque suspension.
10. Slides for the cell samples were prepared, then stained with Gimsa stain and examined by microscope to observe the chromosomes at metaphase.

Cytogenetic analyses were performed on the samples of five groups as shown in Table (1). These analyses include mitotic index (MI) and chromosomal variation test.

Result and Discussion

One of the current test systems to evaluate the cytotoxic effect is based on chromosome analysis.

Table (1): shows the effect of Citrillus extract on mitotic index (MI) in normal and leukemic blood cells.

Parameter	MITOTIC INDEX PERCENTAGE %	
	NORMAL CELLS mean% ± SE	LEUKEMIC CELLS mean% ± SE
Group 1	36 ± 4.35	3.9
Group 2	57 ± 3.21	30 ± 3.09
Group 3	61 ± 3.46	31 ± 3.21
Group 4	65 ± 3.46	28 ± 3.46
Group 5	74 ± 3.21	26 ± 3.21

According to table (1):

Groups (1,2) representing the mitotic index percentage for normal and leukemic cells without Citrillus extract (control), while group (3, 4, 5) representing cells with (0.1, 0.2, 0.3) ml concentration of extract respectively.

According to result of group (1) there is marked effect of citrillus extract on MI in both leukemic and normal cells. There is an increase in mitotic index in normal cell but leukemic cells, appeared with decrease in MI percentage.

Cytogenetic analysis also includes the effect of Citrillus extract on percentage of chromosomal aberration.

Tables (2): illustrate the cytogenetic analysis of leukemic blood cells treated with Citrillus extract.

Parameter	Chromatid Break mean% ± SE	Acentric Fragment mean% ± SE	Dicentric Chromosome mean% ± SE
Group 1	53 ± 4.35	22 ± 7.93	18 ± 3.21
Group 2	42 ± 2.30	16 ± 1.73	16 ± 2.08
Group 3	37 ± 2.30	14 ± 2.08	14 ± 1.73
group 4	24 ± 2.88	13 ± 3	11 ± 1.73

Group 1 representing untreated leukemic cell

Group 2 representing leukemic cell treated with 0.1 ml of extract

Group 3 representing leukemic cell treated with 0.2 ml of extract

Group 4 representing leukemic cell treated with 0.3 ml of extract

There is a clear effect in MI in both normal and leukemic cell after treatment with the extract, marked increase in MI for normal cells and decrease in MI in leukemic cell is

noted, this indicate that Citrillus extract showed immunomodulatory activities which could stimulate the immune system by increasing chemotaxic activity [7].

The difference in MI between treated and non-treated cells may be related to mitogenic substances found in Citrillus extract which have ability to stimulate the treated cells to divide without addition of the mitogen [5]. Colocynthin is a glycoside, however many types of mitogen are glycosides in nature such as lectin.

The low increase in MI for leukemic cells may be explained in a way that leukemic cells have lost part of mitogenic receptors which located on cell surface as a part of leukemogenic process [3], this process considered as an attempt to escape from the immunosystem so this process will prevent the adherence of protein to the cell receptors resulting in reduction in the cell division rate [4]. The results described above led us to postulate that inhibition of cell growth induced by Citrillus extract might be a result of apoptotic cell death. *In vivo* and *in vitro* study on the cruciferous vegetable, possess cancer prevention potential attributed their activity to their content in thioglycoside conjugates, namely, glycosinolates [5]. Previous study on breast, prostate and cervical colon cancer were also found to suppress proliferation and to induce apoptosis, partially mediated by cell-cycle arrest in G1 phase [6]. Cytogenetic analysis also revealed that the Citrillus extract has ability to reduce the Chromosomal aberration rate, marked decrease in different chromosomal aberrations in treated groups are clarified in Table (2) in comparison with untreated groups.

As we know most of the chromosomal aberration occur in G1 phase of cell cycle, thus leukemic factor obstruct against DNA repair system.

The marked reduction in the simple chromosomal aberration rate in cells treated with the extract may be related to its ability to prevent accumulation of DNA lesion through enhancing the activity of DNA polymerase. The extract acts as a substance for the DNA repair machinery in S phase [8].

So activity may be retained to the active compounds, in the extract, one of these compounds are the fatty acids (Oleic & Linoleic acid) and phytosterols glycoside which were found in seeds [6]. These compounds are acting as DNA repair system machinery [9]. It is important to say that this extract effect is dependent.

Other suggestions pointed that these compounds may exert antigenotoxic activities by scavenging DNA damage agents such as hydroxyl, oxygen or other compounds which may act as desmutagens and bioantimutagens [10]. In general, most antileukemic drugs act by Perturbing enzymes or substances which are related to DNA or RNA synthesis and this largely affects cell division [11]. Clearly, further studies are still required before considering Citrillus extract as a potential antileukemic agent, for instance, elucidation of the mechanism involved in Citrillus extract -induced death will shed some light on the molecular basis in the relative selectivity toward leukemic cells displayed by these compounds.

References

1. Diwan, F.H.; Abdel-Hassan, I.A.; and Mohammed, S.T. (2003). Effect of saponin on mortality and histopathological changes in mice. *Easten Mediterranean Health Journal*.6:345-351.
2. PDR for herbal medicines. First edition 1998.P:753.

3. Felter, H.W.; and Liou, J.U. (1898). *Colocynthis* (U.S.P) Colocynth. Kings American Dispensatory.1:118.
4. Duke, J. A. (1983).*Citrillus colocynthis* (L.)SCHRAD. Hand Book of energy Crops.
5. Bankole, S. A. and Jola, A. O. (2004). Effect of lemon grass (*Cymbopogon citrates* stap f) powder and essential oil on mould deterioration and aflatoxin contamination of melon seeds (*Citrillus colocynthis*). African Journal of Biotechnology.3 (1): 52-59.
6. Yaniv, Z.; Shabelsky, E.; and Schafferman, D. (1999).Colocynth: Potential arid land oilseed from an ancient cucurbit. Journal Janick(ED).257-261.
7. Shubber, E.K.; and Juma, A. S.(1999).Cytogenetic effects of plant extract of *Urtica dioica* on mice somatic cells. Agriculture and Biological Research Center. 42 (3):182-187.
8. . Sugimura, T.; Sato, S. (1983).Mutagens and carcinogens in food. Cancer research.43:2415-2421.
9. Carrano, A.V.; Thompson, L.V.; and Stetka, D.(1979).DNA cross linking, sister chromatid exchanges and specific locus mutation. Mutant Res.63:175-188.
10. Ames, B.N. (1983). Dietary carcinogens and ant carcinogen. Science 22: 1256-1266.
11. Blijinski, M.(2002).The anticancer agent PB-100 Selectivity active malignant cell line inhibits multiplication of sixteen malignant cell lines, even multidrug resistant. Genet. Mol. Biol., 23:224-235.