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The effectS of *Brassica oleracea* plant extracts on tow type of leukemia cells (Invitro study)

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Abstract: *Cold water hot water and ethanol extracts from Brassica oleracea plant they were prepared and tested for their antioxidant activity and efficacy against leukemia cells. All of the extracts showed significant antioxidant activity. All the extracts could kill the majority (50-75%) of abnormal cell among primary cells harvested from 3 patients with acute lympho-blastic leukemia (ALL) and 3 with acute myeloid leukemia (AML). DNA fragmentation patterns were detected within treated cells and inferred targeted cell death by apoptosis.The metabolites within the ext-racts may act as tumor inhibitors that promote apoptosis. In addition the plant extracts may be used to supplement or replace established drugs treatments .*

Key words: *Plant Extract, leukemia cells, Invitro study*

I. Introduction

In recent years, chemoprevention has attached two considerable attentions as a mean of blocking malignant transformation in its early stages and disease progression in laterstages [1, 2]. Herbal medicin is still the most common source for primary health care of about 65-80% of the world's population ,mainly in developing countries, because of better cultural acceptability, better compatibility with the human body and fewer side effects. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines [3]. The medicinal values of these plants lie in their components which produce definite physiological actions on the human body [4, 5]. The most important of these components are alkaloids, tannins, flavonoids and phenolic compounds [6].

Brassica genus is native in the wild in western Europe, the Mediterranean and temperate regions of Asia. In addition to the cultivated species, which are grown worldwide, many of the wild species grow as weeds, especially in North America South America, and Australia [7,8]. It is important genus in the Brassicaceae ZWSZ2family, several species and types of Brassicas are significant oilseed crops, vegetables ,forage crops, and are used in the production of condiments, such as mustard, Brassica species are widely used in the cuisine of many cultures and recognized as a valuable source of dietary.

Fiber and contain little fat, and source of vitamins and minerals [9]. They contain a large number of novel photochemical, some of which protect against carcinogenesis. Hence, Brassicas are believed to be useful in the prevention of cancer [10,11, 12].

Aqueous extracts from willow (Salixsp.) (Saliceae) leaves prevented proliferation of three cancer cell types acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and Ehrlich a scites carcinoma cells [12]. Alcohol extra-cts of Ganoderma lucidum induced apoptosis in MCF-7 human breast cancer cells. In many cases the complex mixtures in crude extracts were more effective than single purified compounds [13].

Leukemia is one of the most common cancers . The greater prevalence of leukemia in the modern world may be due to the reduction of incidence of most infectious diseases and the increased life span of humans. Treatments for cancer diseases are expensive with no assurance that even simple leukemia can be cured .For developing countries the use of endogenous medicinal plants as cures against leukemia and other cancers is attractive[14].

The objectives of the study were to study the cytotoxic effects of Brassica extract against two leukemia cell types.

II. Materials And Methods

The plant collection

The plant Brassica oleracea identifid by Dr. Ali Hussein Al-Musawi (Department of Biology, College of Science, University of Baghdad).The plant was collected from a botanical garden located in Palestine Street (Baghdad) in September 2012.

Extract preparation

The extraction used 1 g of dried, powdered leaves suspended in 10 ml of hot water, cold water, or 80% (v/v)ethanol. Extracts were stirred mechanically for 12 h at room temperature (25ºC) except the hot water extract (80ºC) that was made in 30 min . Solids were removed by centrifugation (4,000 g,10min)and the supernatant collected. The resulting extracts were completely dried in a rotary evaporator at 40 ºC and the lyophilized extracts stored at 4ºC for further process.

Antioxidant activity

The antioxidant activity of the plant extracts was evaluated by using the 2.2'diphenylpicrylhydrazyl (DPPH) assay [15]. The extracts (5-20 g in 50 l) were added to 5 ml of a 0.004% (w/v) of DPHH in methanol (100% v/v). After, a 30 min incubation period at room temperature the absorbance at 517 nm was compared to DPPH in methanol without an extract sample (blank) and quercetin was used as positive control. The percent inhibition of free radical formation (I %) was calculated as

I% = (A blank _ A sample / A blank) × 100 Where; A blank is the absorbance of the control reaction (containing all reagents except the extract), and A sample is the absorbance of the mixture containing the extract. The experiment was carried out out in triplicate.

Viability of tumor cells

The study was performed on cells harvested from adult leukemia patients or healthy relatives admitted to the National Center for Treatment and Research of Blood Diseases.

International protocols governing the ethical treatment of patient were followed. The experimental samples were taken from healthy volunteer relatives (3 samples) and leukemia patients that included 3 ALL (acute lymphoblastic leukemia) and 3AML (acute myeloid leukemia, immature monocytes) patients. ALL and AML had been diagnosed by peripheral blood andbone marrow examination and cytochemistry (with immunological markers used in two cases). Mononuclear cells were separated from other blood cells by Ficoll hypaque density gradient(Pharmacia, Uppsala Sweden).

The cells were then washed with three changes of PBS. The cell counts were adjusted so there were 105 cells in 0.1 ml (counting both mature and immature cells). The culture medium was prepared using modified Earle's salt with 1.2g/l sodium carbonate and L-glutamine (Gibco, Grand, USA), 10%(v/v) inactivated fetal bovine serum (Gib- co) , 100 g /ml penicillin and 100 g/ml streptomycin was added. The medium was filtered through 0.22 m Millipore filter, one ml of which was transferred into a 1.8 ml screw-capped sterile plastic tube. Next, 0.1 ml of the cell suspension containing 105 cells was added to each of 5 tubes per extract. To three of the tubes, 0.1 ml of the extract was added, while the other two tubes served as negative and positive controls. Culture medium was used instead of the extract for the negative control and the extract was added to the cells from healthy volunteers as a positive control .The tubes were incubated at 37°Cin the presence of 5% (v/v) CO2 for 24 h (dark condition, humid- ified air). The cells were tested for their viability using the trypan blue exclusion test [16]. Two hundred cells were counted, and the percentage of viable cells was estimated.

DNA Extraction

DNA was extracted from mature (normal cells) and immature white blood cells (leukemic cells) before and after treatment with each extract. Cells were washed with PBS and then lysed in cold lysis solution (5 mM of Tris, pH 7.4, 20 mM of EDTA, 0.5% (v/v) Triton X- 100) for 20 min (Gao et al., 2002). Cell lysates were centrifuged at 27,000 g for 15 min, and DNA was extracted from the aqueous phase with phenol: chloroform: isoamyl alcohol (25:24:1, v:v:v) containing 0.1% (w/v) hydroxyquinoline. DNA was precipitated with 0.3 mM of sodium acetate and 2 cm3 of cold 100% (v/v) ethanol. Agarose gel electrophoresis (1% w/v) at 30 mA for 2 h followed by UV fluorescence was used to determine the degree of DNA fragmentation [17,18].

III. Results And Discation

DPPH radical scavenging activity

The plant extracts each showed a concentration dependent scavenging activity by quenching DPPH radicals (Table 1).

As judged by this assay, the ethanol extract showed high antioxidant activity at 68% inhibitionof radical formation compared to 37% for the cold water extract at 1g/ml (Table 1). In the other hand, the positive control (quercetin) was tested and had the antioxidant activity at 58% inhibition of radical formation. This high antioxidant capacity may be due to the high concentration of phenolics and flavonoids in ethanol extracts.

Table 1. Measurement of antioxidant activity of plant extracts using DPPH.

Extracts (1 µg/ml)	Antioxidant activity (%)
Cold water	37 ± 0.38
Hot water	59 ± 0.89
Ethanol Extract	68 ± 0.47
Quercetin	58± 0.35
LSD	1.054

Each value represents the mean ± S.D (Standard Division) and mean of three replicates (P 0.05).

Anti leukemic effect

After 24 h incubation of the mononuclear ALL cells with plant extracts, a remarkable destruction of lymphoblast's (86%, only 14% were viable) occurred (Table 2 .a ,b,c).

Table 2.a. The effect of the plant samplesCold water on the percentage of viable ALL cells after 24 h of incubation.

Extract con.	1Mg	5Mg	10Mg	20Mg
time	**Dead %**	**Dead %**	**Dead %**	**Dead %**
6h	2 ± 0.12	36 ± 2.6	48 ± 4.3	67 ± 5.1
18h	5 ± 0.36	30 ± 3.1	56 ± 2.8	73 ± 2.3
24h	**4 ± 0.29**	**20 ± 2.3**	**59 ± 3.1**	**77 ± 5.1**
L.S.D. (0.05)	1.02	2.1	3.56	1.83

Table 2. b. The effect of the plant samples Hot water on the percentage of viable ALL cells after 24 h of incubation..

Extract con.	1Mg	5Mg	10Mg	20Mg
time	**Dead %**	**Dead %**	**Dead %**	**Dead %**
6h	8 ± 0.3	41 ± 4.1	56 ± 2.3	81 ± 1.9
18 h	13 ± 2.1	39 ± 3.1	62 ± 1.3	76 ± 4.2
24 h	**16 ± 1.8**	**47 ± 2.3**	**76 ± 1.6**	**84 ± 3.1**
L.S.D. (0.05)	0.87	1.68	3.21	4.24

Table 2.c. The effect of the plant samples Ehtanol Extracton the percentage of viable ALL cells after 24 h of incubation.

Extract con.	1Mg	5Mg	10Mg	20Mg
time	**Dead %**	**Dead %**	**Dead %**	**Dead %**
6h	19 ± 1.2	45 ± 1.2	57 ± 3.1	76 ± 5.3
18h	± 3.6	36 ± 3.2	59 ± 2.8	74 ± 3.8
24 h	**36 ± 2.5**	**52 ± 4.6**	**71± 4.9**	**86 ± 3.5**
L.S.D. (0.05)	1.25	2.1	4.23	3.2

Destruction was dose, extract and time dependent ranging from 9 to 86% across the treatments. Responses were not linear, higher doses and longer times could not kill all the leukemia cells. However, cell death was significantly higher than that in the cells treated with media alone (8%, not shown). Similar results were observed, with AML cells, the mean viability of extract treated cells was 30% (70% destroyed) when compared to the cells treated with media alone 93% (Table 3 .a,b,c)

Table 2.a. The effect of the plant samplesCold water on the percentage of viable AmL cells after 24 h of incubation.

Extract con.	1Mg	5Mg	10Mg	20Mg
time	**Dead %**	**Dead %**	**Dead %**	**Dead %**
6h	9 ± 1.2	32 ± 2.023	39 ± 3.25	58 ± 5.3
18 h	26 ± 2.5	35 ± 1.92	47 ± 4.23	66 ± 2.1
24 h	37 ± 1.9	49 ± 3.1	53 ± 1.64	72 ± 1.3
L.S.D. (0.05)	1.09	3.55	2.13	1. 8

Table 2. b. The effect of the plant samples Hot water on the percentage of viable AmL cells after 24 h of incubation.

Extract con.	1Mg	5Mg	10Mg	20Mg
time	**Dead %**	**Dead %**	**Dead %**	**Dead %**
6h	18 ± 2.2	29 ± 1.45	53 ± 4.1	62 ± 2.1
18 h	15 ± 1.4	36 ± 1.23	63 ± 2.6	71 ± 1.57
24 h	34 ± 3.1	55 ± 3.1	61 ± 3.1	75 ± 1.34
L.S.D. (0.05)	3.11	2.13	1.89	2.8

Table 2.c. The effect of the plant samples Ethanol on the percentage of viable AmL cells after 24 h of incubation.

Extract con.	1Mg	5Mg	10Mg	20Mg
time	**Dead %**	**Dead %**	**Dead %**	**Dead %**
6h	22 ± 2.2	36 ± 1.25	59 ± 2.12	64 ± 1.23
18 h	31± 2.1	39 ± 0.87	68 ± 3.2	77 ± 4.21
24 h	28± 3.1	51 ± 3.2	73 ± 3.12	82 ± 5.2
L.S.D. (0.05)	3.23	2.21	4.2	3.26

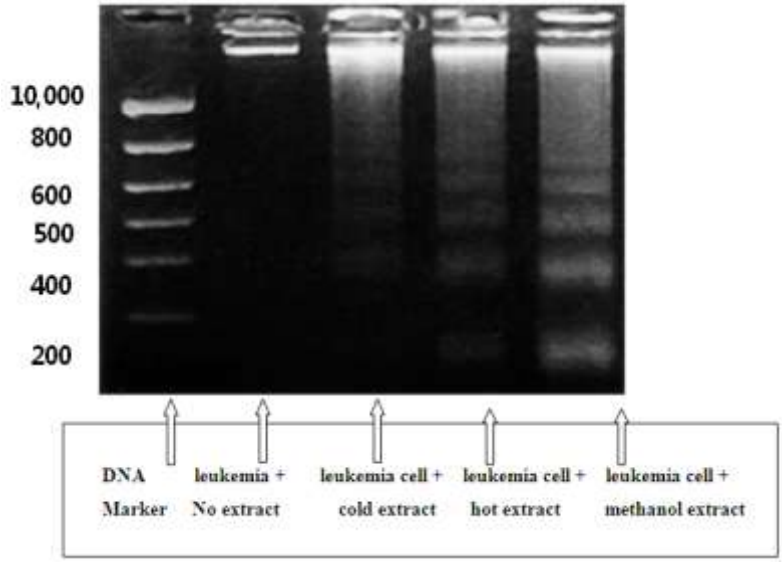

Figure 3. Agarose gel electrophoresis of DNA extracted from AML cells treated with plant extracts. Lane 1 shows a DNA ladder Lane 2 shows AML cells treated with culture fluid but no extract. Lane 3 shows the cold water extract treatment effect. Lane 4 shows the hot water extract treatment effect. Lane 5 shows the ethanol extract treatment effect.

In addition , the extracts were incubated with normal mononuclear cells from healthy volunteer(was no significant difference in killing healthy cells (mean15.3%) when compared to the7% caused by the media addition control (negative control; data not shown).

Therefore, leukemia cells were more vulnerable to the extract than healthy cells 3 samples). There was no significant difference in killing healthy cells (mean15.3%) when compared to the 7% caused by the media addition control (negative control; data not shown).Therefore, leukemia cells were more vulnerable to the extract than healthy cells .Phenolic and flavonoid compounds are common in medicinal plants, spices, vegetables, fruits ,grains , pulses and other seeds. These compounds are an important group of natural antioxidants with beneficial effects on human health [19] . They can participate in protection against the harmful action of Reactive oxygen species, mainly oxygen free radicals.

Phytochemicals, especially the phenolics found in medicinal plants, fruits and vegetables, have been proposed as the major bioactive compounds providing the health benefits associated with diets rich in plant foods [20].

In this context, redox and antioxidant systems are among the most promising targets for functional food science . For this reason, many functional foods aim to increase human intake of antioxidants to reduce the risk of chronic diseases linked to oxidative stress. Among the most common dietary sources of natural antioxidants are grapes and berries that are rich in phenolic compounds and particularly flavonoids [20,21].

The results of this study suggest extracts of the herbs like Brassica oleracea can substitute for grapes and berries .In the earlier reports anti leukemic plant extracts, the all amandine derivatives that are extracted with water and/or ethanol from All Amanda catharica, L (Apocynaceae) showed significant activity in vivo against the p-388 leukemia in the mouse [22]. In addition, willow leaves showed highly active against ALL and AML cells [23,24]. probably related to salicylic acid derivatives . The resveratrol induced DNA fragmentation in 32Dp210 leukemic cells. Resveratrol (a phenolic) induced apoptosis in 32Dp210 cell as shown by the induction of internuleosomal DNA fragmentation and the cleavage of procaspase3 in resveratrol treated leukemic cells. Here, a major destructive effect on AML and ALL cells was obtained by the ethanol extract (Tables 2 and 3). That extract could be used as a natural antitumor medicine.The active ingredient(s) may be phenolic compounds because most glycosides

and many types of tannin will dissolve in ethanol solutions [25,26,27]. Whatever the active factor of the extract appears to promote cell apoptosis since DNA damaged in leukemic cells incubated with ethanol extract (Figure 3).

In comparison to established treatments for leukemia,th extract were equally effective in killing 80% of diseased cells within a few hours .Therefore, this plant extract used alone or in combination with other extracts or other drugs has the potential to kill all leukemia cells whilst leaving healthy cells viable. The plant extracts may provide low cost treatments for cancers and new treatments for drug resistant cancers.

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