SHORT COMMUNICATIONS

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in vitro ANTILEUKEMIC ACTIVITY OF *Euphorbia echinus* EXTRACT

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Aim. Cancer continues to pose a serious threat to human health. Euphorbia plants are rich in phenolics, aromatic esters, steroids and several bioactive compounds. Studies have shown the presence of a large number of bioactive compounds in *E. echinus* including flavonoids, phenolics, and proanthocyanins.

Method. There it was investigated cytotoxic effects of *E. echinus* methanolic extract on K562, HL60, Ishikawa, Raji and SH-SY5Y cells.

Results. The *E. echinus* extract was found to be highly cytotoxic against HL60 and K562 (79.78 and 76.44% cytotoxicity, respectively). DNA fragmentation was exclusively observed in K562 cells indicating that the reduction of viable cells following treatment with *E. echinus* extract is due to apoptosis.

Conclusions. Our results suggest that *E. echinus* extract might have a drug potential against leukemic cells.

Key words: Euphorbia echinus; cancer; K562 cells; HL60 cells.

Cancer continues to pose a serious threat to human health. Cancers are characterized by the presence of one or more malignant tumor formed from transformation of an initially normal cell [1].

It was reported that different plant extracts can induce cytotoxicity and apoptosis against various cancer cells [2, 3]. *Euphorbia echinus* is a native plant from south of Morocco popularly called "Daghmous" [4]. *Euphorbia* genus is characterized by various terpenoids and steroids [5]. It was shown that genus *Euphorbia* has cytotoxic, antitumor, antibacterial, anti-inflammatory, and antiviral effects [6]. We aimed to investigate cytotoxic effects of *E. echinus* methanol extract on cancer cells *in vitro*.

Materials and Methods

Preparation of plant extract

The whole plant of *E. echinus* was collected from Sbouya Sidi Ifni, Southern Anti-Atlas, Morocco and was authenticated by Prof. El Oualidi Jalal (Scientific Institute, Mohammed V University in Rabat, Morocco) and Prof. Raouane Mohammed (Department of Biology Mohammed V University in Rabat, Morocco). It was extracted three times with 70% ethanol under agitation at room temperature. After

filtration, the extract was evaporated under vacuum on a rotary evaporator at 45 °C. Then it was diluted to different concentrations to be used in the cytotoxic assays.

Cell culture

Cytotoxic effects of Euphorbia echinus extract were assessed on HL60 (human promyelocytic leukemia cell line), K562 (human chronic myeloid leukemia cell line), Ishikawa (human endometrial adenocarcinoma cell line), Raji (human lymphoblastoma cell line) and SH-SY5Y (human neuroblastoma cell line). ECV304 (human umbilical vein endothelia cell line) was used as a non-cancerous cellular control. The cell lines were purchased from ATCC (USA). RPMI medium was used for suspension cells and DMEM medium for adhesive cells. Both media were supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% L-glutamine.

Cytotoxicity assay

Cytotoxic effects of *E. echinus* extract were determined by MTT test as described earlier [7]. Stock solution of *E. echinus* extracts was used in concentration of 10 mg/ml. The final concentrations of the extract were 1000, 500, 100, 50, and 10 μg/ml. Medium without extract was used as a positive control, and medium without cells and extract was used as a negative control. The experiment was repeated 6 times.

DNA fragmentation assay

After cell viability tests, the extract caused cytotoxicity above 50% for K562 and HL60 cells. Therefore, K562 and HL60 cells were incubated with the extract in the highest concentration for 24 h. DNA fragmentation assay was conducted as described earlier [8].

Statistical analysis

Statistical analysis was performed by using Student's t-test. Differences at $P < 0.05$ were considered statistically significant.

Results

Anticancer activity of *E. echinus* was investigated on HL60, K562, SH-SY5Y, Ishikawa and Raji cell lines. As shown in Fig. 1, our extract was more cytotoxic against HL60 and K562 cells among other examined cells. It caused 79.78% and 76.44% cytotoxicity at concentration of 1000 μg/ml against HL60 and K562, respectively. *E. echinus* extract had moderate effect on Ishikawa (42.16% mortality), while showing low cytotoxicity on Raji (36.4%) and SH-SY5Y (17.84%). Moreover, the results have shown that our extract has proliferative effect on ECV 304 used as control cell lines.

The IC_{50} values of *E. echinus* on the cancer cell lines were shown in the Table. According to IC_{50} values calculations, it was found 162.99 and 306.33 μg/ml for HL 60 and K562 respectively. Since its cytotoxicity against Ishikawa and SH-SY5Y under 50%, their IC_{50} values are above 1000 μg/ml.

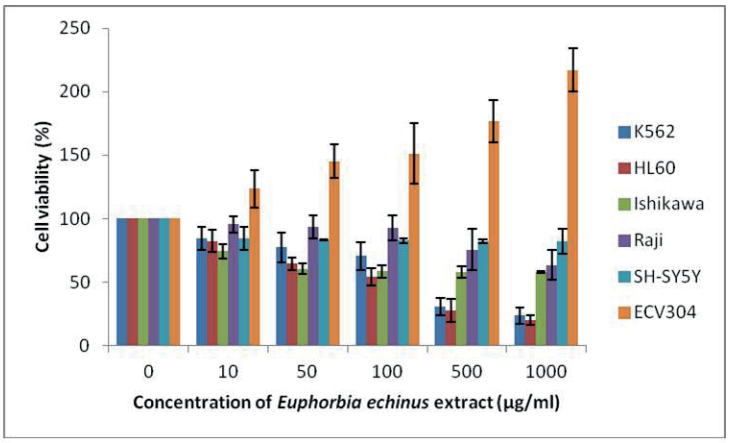
After cytotoxicity experiments, to evaluate apoptotic effect of *E. echinus* extract, the most sensitive cells (HL60 and K562) were selected for DNA fragmentation assay. According to the results, apoptotic effects of the extract was observed in exclusively K562 cells (Fig. 2).

Discussion

In this work, we studied the cytotoxic effects of *E. echinus* extract on HL60, K562, SH-SY5Y, Ishikawa, and Raji cell lines. HL60 was the most sensitive to the extract with an IC50 value of 162.99 μg/mL Ishikawa was inhibited moderately by the extract. The extract has shown a weak cytotoxic effect against Raji and SH-SY5Y since the percentage of survival cells was > 50%. Interestingly, non cancerous cell lines ECV304 were not susceptible to the extract.

In other studies, anticancer effects of some *Euphorbia* species on gastric AGS cell lines at the concentration of 200 μg/ml [9]. Also, it was found that *E. helioscopia* extract is cytotoxic for Hep-2, T-47D, and PC-3 cancer cell lines [10]. In another study, it was shown that *E. herba* extract has cytotoxic effect on MCF-7 as well [11].

In order to study the apoptotic effect of *E. echinus* extract against HL60 and K562 cell lines, DNA fragmentation assay was made and obtained results are presented in Fig. 2. According to the results, there were DNA fragments in only K562 cells treated with *E. echinus* extract. No ladder formation was observed in untreated and HL60 cells. This indicates that *E. echinus* extract selectively induced apoptosis in, K562 cells. For various cell lines, some species of genus *Euphorbia* induced DNA damage. It was found that *E. hirta* extract has genotoxicity effects against MCF-7 [12]. *Euphorbia* plants are rich in phenolics, aromatic esters, steroids and several other bioactive compounds [13]. Studies have shown the presence of a large number of



Cell lines	1000 µg/ml	500 µg/ml	100 µg/ml	50 µg/ml	10 µg/ml
K562	23.56 ± 6.23	30.73 ± 6.80	70.53 ± 11.26	77.32 ± 11.89	84.44 ± 9.01
HL60	20.22 ± 3.79	27.53 ± 8.94	54.20 ± 6.89	64.50 ± 5.13	82.43 ±8.79
Ishikawa	57.84 ± 0.69	57.77 ± 4.46	58.45 ± 4.90	60.61 ± 4.31	74.24 ± 5.86
Raji	63.60 ± 11.85	75.69 ± 16.07	92.92 ± 9.75	93.67 ± 8.99	95.53 ± 6.60
SH-SY5Y	82.16 ± 9.53	82.22 ± 1.22	82.62 ± 2.07	83.32 ± 0.6	84.59 ± 9.24
ECV304	217 ± 17.18	176.47 ±16.68	151.35 ± 23.72	145.28 ± 13.37	123.69 ± 14.73

Fig. 1. **Cytotoxic effects of *E. echinus* extracts against HL60, K562, Raji, Ishikawa, SH-SY5Y and ECV304**
The graph represents cell viability (%) according to different concentration (µg/ml) of extract.
Values represent means ± SD

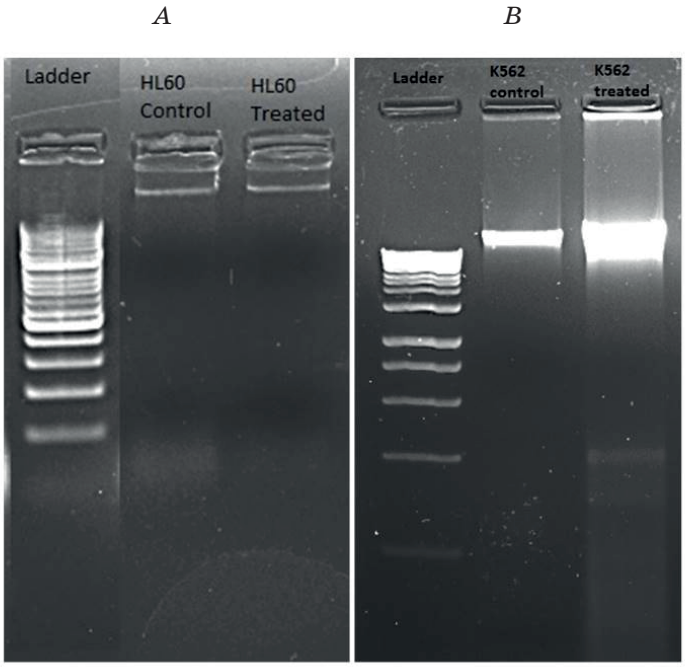


Fig. 2. **Analysis of apoptotic effects of *E. echinus* against HL60 (*A*) and K562 (*B*) with DNA fragmentation method**
DNA fragmentation was only observed for K562 cells.

Table 1. **IC_{50} values (μg/ml) of methanolic extract of *E. echinus* on the cell lines**

Cell lines	IC_{50} values (μg/ml)
K562	306.33
HL60	162.99
Ishikawa	> 1000
Raji	> 1000
SH-SY5Y	> 1000

bioactive compounds in *E. echinus* including flavonoids phenolics, and proanthocyanins [14] which could be responsible for its anticancer activity. Different extracts have been reported to induce apoptosis and/or cytotoxicity in cancer cells [15, 16]. These findings have revealed that the methanol extract of *E. echinus* exerts cytotoxic effects against cancer cell lines. Comparing with constituents of different extracts, its chemical constituents can shed a light to find new agents for cancer cells.

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in vitro ПРОТИВОЛЕЙКЕМИЧЕСКАЯ АКТИВНОСТЬ ЭКСТРАКТА *Euphorbia echinus*

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Мета. Рак становить серйозну загрозу здоров'ю людини. Рослини молочаю багаті фенольними речовинами, ароматичними ефірами, стероїдами та кількома біоактивними компонентами. Дослідження показали наявність великої кількості біологічно активних сполук у *E. echinus*, включаючи флавоноїди, феноли та проантоціани.

Метод. Досліджували цитотоксичну дію метанольного екстракту *E. echinus* на клітинах K562, HL60, Ishikawa, Raji та SH-SY5Y.

Результати. Екстракт *E. echinus* виявився високоцитотоксичним проти HL60 і K562 (79,78 і 76,44% цитотоксичності відповідно). Фрагментація ДНК спостерігалася лише в клітинах K562, що вказує на те, що зменшення кількості життєздатних клітин після оброблення екстрактом *E. echinus* відбувається через апоптоз.

Висновки. Наші результати показують, що екстракт *E. echinus* може мати лікарський потенціал проти лейкозних клітин.

Ключові слова: *Euphorbia echinus*; рак; K562 клітини; HL60 клітини.