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In vitro antiproliferative activity of saffron extracts against human acute lymphoblastic T-cell human leukemia

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Cancer is still considered as one of the most life threatening cause responsible for a huge number of annual deaths around the world. Particularly, leukemia is difficult to be cured. In this context, free radicals are one of the factors that cause or predispose to cancer. Hence, they should be controlled in the body by prophylactic or curative treatments. The aim of this study was to evaluate the antiproliferative effect against human acute lymphoblastic T-cell leukemia (*Jurkat* cell line) of the Lebanese saffron (*Crocus sativus* L.; Family Iridacea), and to detect which components of saffron are responsible for the growth inhibitor. Lebanese saffron decreased cell growth of *Jurkat* cells in a dose dependent manner. A mixture of crocin and safranal also decreased the number of *Jurkat* cells and the IC_{50} value of this mixture was lower than that of the whole saffron extract.

Keywords: Lebanese saffron, Antiproliferative activity, Acute lymphoblastic leukemia, Lebanon

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Leukemia is characterized by an abnormal increase of immature white malignant blood cells called "blasts" and affecting people at any age. In the year 2015, approximately 54,270 males and females in USA developed some form of leukemia, and 24,450 died of their disease[1]. About 90 % of all leukemia cases are diagnosed in adults[2]. For the identification of agents of interest for treatment of T-cell leukemias, the Jurkat cell line has been employed. This cell line is derived from human T-cell leukemia and used in the recent past to determine the mechanism of differential susceptibility to anti-cancer drugs and radiation (Medical subject heading). *Jurkat* cells may be used to study T-lymphocyte behavior, cell signaling and expression of chemokine receptors. They are also important in producing interleukin 2. The *Jurkat* cell line was established in the late 1970s from the peripheral blood of a 14- year- old boy with T cell leukemia. Different derivatives of the *Jurkat* cell line can now be obtained from cell culture banks that have been mutated to lack certain genes[3].

Acute lymphoblastic leukemia (ALL) is a malignant proliferation of lymphoid cells blocked at an early stage of differentiation. ALL is a biologically heterogeneous disorder, so that morphologic, immunologic, cytogenetic, biochemical, and molecular genetics characterizations of lymphoid leukemia are needed to correctly diagnose and classify the various ALL subtypes[4]. Approximately 75 % of all cases of childhood leukemia are ALL. About 3,000 children in the United States and 5,000 children in Europe are diagnosed with ALL each year. The peak incidence of ALL occurs between 2 and 5 yrs of age. The incidence of ALL is higher among boys than girls. T-cell ALL represents approximately 10-15% of ALL cases in developed countries and it is characterized by male predominance compared with B-cell precursor ALL[5].

Saffron is a spice derived from the dried stigmas of *Crocus sativu*s L. (Family Iridaceae), which was known by ancient civilizations. The flowering period of *Croccus sativus* extends over 2-3 weeks in October or November (depending on geographical differences), in which the flowers are picked by hands. Their red stigmas are manually separated and then dried in order to obtain the saffron spice. The stigmas from about 100,000 flowers are required to produce 1 kg of pure dried saffron[6].

Research on saffron experienced a renaissance in the last decade, and a growing body of evidence indicates that saffron and its characteristic components possess anticarcinogenic and antitumor

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activities both *in vivo* and *in vitro*. Saffron extract inhibited or retarded tumorigenesis in a variety of experimental models *in vivo*. Topical application of saffron extract (100 mg/kg body weight) inhibited two-stage initiation/promotion dimethylbenz [a] anthracene (DMBA)-induced skin carcinogenesis and oral administration of saffron extract in the same dose restricted 20-methylchloanthrene (MCA)-induced soft tissue sarcomas in mice[7]. In addition to this, Saffron extract has significantly prolonged (almost 3-fold) the life span of cisplatin-treated (2 mg/kg body weight) mice and partially prevented the decrease in body weight, hemoglobin levels, and leukocyte counts[8]. Taken together, these studies indicated that saffron may be a promising agent for reducing cisplatin-toxic side effects, including nephrotoxicity.

Oral administration of saffron extract (200 mg/kg body wt) induced a dose-dependent growth inhibition of sarcoma-180 (S-180) ascites tumors Ehrlich ascites carcinoma (EAC) and Dalton's lymphoma ascites (DLA) in mice, and significantly increased (2-3fold) life spans of tumor-bearing mice[9,10]. Interestingly, liposome-encapsulated saffron extract injected i. p. into mice considerably increased the antitumor effects of this extract towards several solid animal transplantation tumors[11]. Thus, saffron and its constituents are suggested as promising anticancer drug candidates. Alone or in combination with established anticancer drugs, they may have the potential for treatment of cancer. Different hypotheses on the modes of anticarcinogenic and antitumor actions of saffron and its components have been proposed. One mechanism is the inhibition of DNA and RNA synthesis. A fourth suggested mechanism is the interaction of carotenoids with DNA topoisomerase II[12]. A second suggested mechanism is its inhibitory effect on free radical chain reactions. A third proposed mechanism is the metabolic conversion of naturally occurring carotenoids to retinoid[13,14]. It is common sense that oxidative stress contributes to various diseases, including leukemia. Hence, antioxidants could play a role to fight leukemia[15,16].

Based on the above acquaintance, we have tested the effect of saffron of *Crocus sativus* native from Lebanon against acute lymphoblastic leukemia cells.

Material and methods

Chemicals

Chemicals and reagents used to study antiproliferative activities were purchased from Sigma-Aldrich Co. (Beirut, Lebanon) while the other chemicals, solvents, and reagents were purchased from Alpha Co. (Beirut, Lebanon). Only the fetal bovine serum was obtained from CELBIO (Milano, Italy).

Plant materials

Crocus sativus L. stigmata were collected at full flowering stage in November 2012 from El- Qaa of the North Bakaa valley (Lebanon) at the altitude 1000m above the sea. Voucher specimen No 750 was Botanically authenticated by Dr K Saade, Biology Department, Faculty of Sciences II, Lebanese University and deposited in the Biology Department Herbarium, Faculty of Sciences II, Lebanese University.

Sample extraction

The dried and ground sample (2 gm) of stigmas from *Crocus sativus* L. was extracted with 40 mL of methanol by maceration during 24 hrs. The material was filtered using Whatman paper No 1, whereas the extract plant was transferred into 50 ml round bottom flask, and then taken with rotary vacuum evaporator. Moreover, these crude extracts were freeze-dried by lyophilization. The mass yield of saffron extracts from *Crocus sativus* was 7.5% W/W[15].

Phytochemistry

The stigma of the saffron flower contains different chemical substances such as Carbohydrates, minerals, mucilage, vitamins (riboflavin and thiamine), and pigments such as crocin, anthocyanin, carotene, lycopene, zigzantin, flavonoids, amino acids, proteins, starch, gums, and other chemical compounds[16,17,18]. The saffron stigma has a distinct unique color, flavor, and aroma and some of the groups of chemical compounds that are responsible for each of these identified properties[19]. The main pharmacologically active metabolites are: crocetin, crocin, picrocrocin, safranal (Fig. 1).

Cell lines and culture condition

Jurkat cells (T lymphocyte cells): The cells are suspension lymphoblasts. The growth medium was Advanced RPMI 1640 (Gibco/Invitrogen), 10% fetal bovine serum (Hyclone), 10 mM Hepes 100 U/mL penicillin, 100 µg/mL streptomycin, 5 % CO_2 (37˚C). Liquid Nitrogen Storage: Complete growth medium supplemented with 5% (v/v) DMSO in 1 mL aliquots of approximately 5 x 10^6 cells. RPMI 1640 medium for suspension cells with fetal bovine serum (FBS)[20,21].

Cell viability assay

Jurkat cells (20,000 cells/dish) were seeded in 24-well plates. Two wells were prepared for each concentration. Cells treated with saffron extract at different concentrations were compared with untreated controls. Furthermore, *Jurkat* cells were treated with saffron extract at different concentrations as well as a combination of both crocin and safranal.The plates were incubated for 3 days. The cytotoxicity was detrmined by trypan blue staining. Cell suspension (20 mL) was taken from the well and mixed with 20 mL traypan blue. Dead cells appear in blue color, while living cells appeared under the microscope. Then, 10 mL of this mixture were used for cell counting[22].

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± SD. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by Dunnett's test. Differences were considered significant at $^{**}p < 0.01$. The 50% inhibitory concentration (IC_{50}) was calculated by nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows [GraphPad Software, San Diego, CA, USA (www.graphpad.com)].

Results and discussion

Maceration of *Crocus sativus* (saffron extract) yielded 7.5 % w/w. In order to evaluate the Lebanese saffron extract, a mixture of crocin and saffranal, and human acute lymphoblastic leukemia (ALL) cells were seeded at initial cell concentration 20,000 cells/mL and then cultured for 3 days in the presence or absence of saffron extract and mixture at concentrations ranging from 0.1-500µg/mL. The Lebanese saffron extracts and the mixture of crocin and saffranal were able to exert antiproliferative activities against human acute lymphoblastic leukemia (ALL) cells with IC50 values 71±2.50µg/mL and 39±1.70µM, respectively (Figs. 2 & 3). These results are of interest when considered together with other studies focusing on the effects of saffron extracts on a variety of biological functions.

Natural substances from vegetables, herbs and spices could be beneficial in the prevention or treatment of a variety of cancers. Crocin is a carotenoid compound derived from saffron extract exhibits antitumor activity against many human

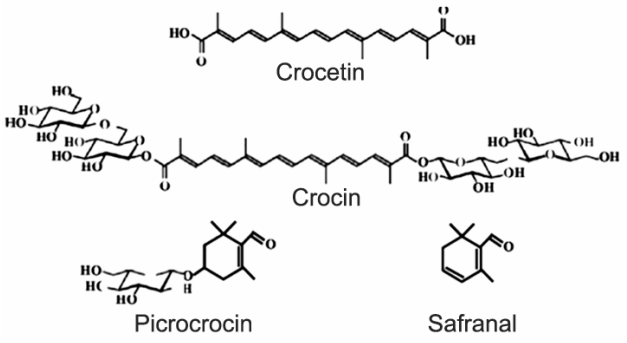

Fig. 1-Chemical structure of the main saffron components

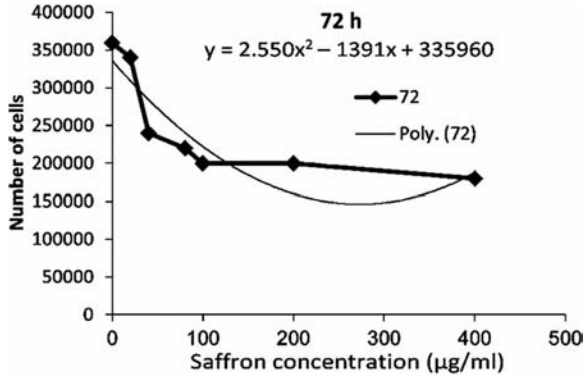

Fig. 2-Treatment with saffron extract after 72 hrs

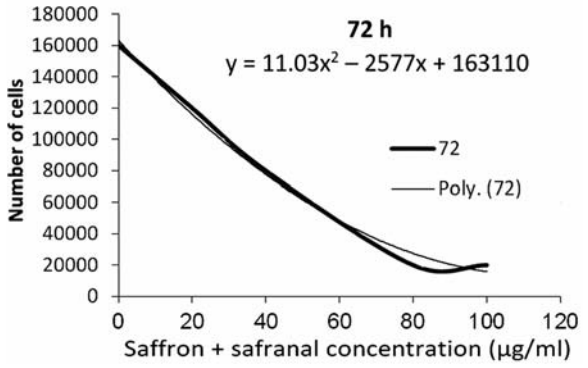

Fig. 3-Treatment with mixture (saffron + safranal) after 72 hrs

tumors, noting here that it has been used as a traditional ancient medicine against different human diseases long time ago. However, the effects of Crocin *in vivo* and *in vitro* exhibits the proliferation and tumorigenicity on human HL-60 cells, which may be mediated by the induction of apoptosis and cell cycle arrest and the regulation of Bcl-2 and Bax expression[23]. Moreover, crocin exhibited cytotoxic effects on MOLT-4 leukemia cell line which might be mediated through the increase of DNA fragmentation[24]. On other side, crocin, crocetin,

picrocrocin and safranal isolated from saffron extracts (*Crocus sativus)* inhibit the growth of cancer cells *in vitro*. Considering its water solubility and high inhibitory growth effect, crocin proved to be the more promising saffron compound to be assayed as a cancer therapeutic agent[25].

Previous data demonstrate that *Crocus sativus* extract and its major constituent, crocin, significantly inhibited the growth of colorectal cancer cells but not affecting normal cells[26]. On the contrary, Safranal is the major compounds isolated from saffron extract and has been used in traditional medicine as food additive for its color and taste. Meanwhile, flow cytometry results revealed the induction of apoptosis by safranal. It might be concluded that safranal could be involved in saffron induced cell death in HeLa and MCF7 cells. Safranal and particularly its liposomal form could be investigated as promising chemotherapeutic agents in cancer treatment[27]. However, DNA fragmentation and apoptosis, induced by safranal in human prostate cell line (PC-3), appear to have potential therapeutic agents[28]. Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde, C10H14O) is an active ingredient in the saffron. Thus, histogram of treated cells compared to control cells indicates that apoptotic cell death is involved in safranal toxicity. Recent pre-clinical study demonstrated anti-tumor activity of safranal against neuroblastoma cells[29]. On the other hand, crocetin, a carotenoid compound derived from saffron, has long been used as a traditional ancient medicine against cancer. Previous study indicates that crocetin has a significant antitumorigenic effect, both *in vitro* and in *vivo.* Crocetin inhibits pancreatic cancer cell proliferation and tumor progression in a xenograft mouse model[30]. Cancer dramatically impacts quality of life and human life expectancy; *Crocus sativus,* is used as a folk medicine for treating human diseases and showed cancer chemoprevention potential. Saffron possesses free radical-scavenging properties and exhibits the proliferation and showed antitumor activities.

Significant cancer chemo-preventive effects have been shown in both *in vitro* and *in vivo* models. Based on data, saffron and its ingredients could be considered as a promising candidate for clinical anticancer trials[31]. Saffron has been reported to have inhibitory effects on tumoral cells. A previous study evaluated that saffron aqueous extract has inhibitory certain effects on the growth on both human transitional cell carcinoma (TCC) and mouse non-neoplastic[32]. Human lung cancer is the most common form of cancer. *Crocus sativus* L. (Saffron) stigma aqueous extract induces apoptosis in alveolar human lung cancer cells through caspase-dependent pathways activation[29]. Previous studies on the pharmaceutical effects on chemical components and medicinal value of saffron (*Crocus sativa*), possess anticancer activity against a wide spectrum of tumors, such as leukemia, ovarian carcinoma, colon adenocarcinoma, rhabdomyosarcoma, papilloma, squamous cell carcinoma, and soft tissue sarcoma[33]. Recent studies indicate the anticancer and antitumor properties of saffron against human cancer. The synergic effects of *Crocus sativus* L. and Low Frequency Electromagnetic Field on VEGFR2 Gene Expression could be considered as a promising chemotherapeutic agent in breast cancer treatment.

Traditional impact of study

Cancer patients seek remedies such as traditional medicinal plants that are believed to be a safe and effective treatment in order to overcome and face the risk of the side effects of conventional therapy and reduced efficacy of modern chemotherapeutic drugs such as tamoxifen and other anticancer synthetic substances[34]. Moreover, surgery and radiotherapy are not always useful, and the clinical results are not acceptable. They are highly effective methods of cancer treatment which exert severe side effects in use[35,36]. Moreover, the research on medicinal plants has become more significant with essential oils, and natural extracts, or their bioactive components being shown to prove anticancer activities *in vitro* and *in vivo*. Saffron was used as a folk medicine, ancient time ago, to treat different kinds of diseases including cancer. Several monoterpenoids and carotenoids isolated from the petals of saffron, among them, some identified compounds: Safranal, Crocin, Crocetin possessed anticancer activities[16]. An optimal concentration of Cisplatin drugs combined with Crocin can provoke synergistic apoptotic effect and increase the risk of Cisplatin side effect[9]. It comes to our attention that the traditional significance of this study is to reduce the side effects of anti cancer synthesized compounds when they are mixed with active natural products, and to discover new natural anticancer substances agents for fighting against malignancies and also to eliminate the side effects like hair loss, vertigo, vomiting, and anemia after chemotherapy treatment by anticancer synthetic substances.

Conclusion

This study exhibits that the Lebanese saffron has an anti-proliferative activity against acute lymphoblast Leukemia (*Jurkat* cells). All of those activities are due to the composition of saffron that contains many different substances. This study must be followed by more experiments and research in order to test the activity of saffron against the remaining type of leukemia. Nowadays, and despite all the efforts deployed by the scientific community, it has been noticed that the cancer cases are increasing year after year, which is an interesting issue to explore new methods of treatment. Among the available and advanced methods, the using of plant compounds, known as phytotherapy, has shown a high activity against cancer cells. In this framework, the exploration of Lebanese saffron activities against cancer should have all our attention. By going back to the natural ways of life, and to avoid diseases, especially against cancer, man finds hope in nature through its richness in plants like saffron, which paved the way to a new horizon in cancer treatment.

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