Dr. Yasir Al-Juraisy
Lecturer, Biol. Dept., Coll. of Science,
Al-Mustansyriah Univ.
Dr. Nahi Y. Yaseen
Director General, ICCMGR.
Dr. Badry Al-Ani
Professor Emeritus, Biol. Dept.
Coll. of Science, Univ. of Baghdad.
badryani@hotmail.com

Effect of crude extracts of fruits and pits of date palm

(Phoenix dactylifera L. cv. Zahdi) on some cancer cell lines in vitro and treatment of transplanted mammary adenocarcinoma in mice

Abstract

The present investigation represents a preliminary study of the effect of crude extracts of fruits and pits of date palm (Phoenix dactylifera L. cv. Zahdi) on two malignant cell lines (human laryngeal carcinoma-Hep2 and murine mammary adenocarcinoma-AMN3) and one normal cell line (rat embryo fibroblast-REF). The study also includes evaluation of the effect of these extracts on several cytogenetic parameters such as mitotic index (MI%), blast index (BI%) and chromosomal aberrations (CA) after in vitro culture of peripheral blood lymphocytes. This work also includes a study of the therapeutic potential of two extracts, one from fruits and the other from pits in the treatment of transplanted murine mammary adenocarcinoma in mice.

The in vitro cell growth assay showed that there was time- and concentration-dependent cytotoxic effects of crude extracts of date palm fruits and pits on Hep2 and AMN3 cell lines. The highest significant effect of these extracts was achieved after 72 hrs of exposure with the highest concentration (10000 µg/ml). Both aqueous extract of fruits (AF) and ethanolic extract of pits (EP) caused growth inhibition percentage (76.3% , 89.4%) for Hep2 and (84.1% , 93.4%) for AMN3 respectively. However, 72 hrs exposure to crude extracts of fruits and pits at concentration of 10000 µg/ml caused slight inhibitory effect on REF cell line, reaching 21.1% and 17.7% for AF and EP respectively.

On the other hand, all crude extracts of fruits and pits caused significant reduction in the mitotic index and blast index of peripheral human lymphocytes, but without any structural or numerical chromosomal aberrations. Also these extracts neither replaced phytohemagglutinin (PHA) as mitogenic agent, nor colcemide as mitotic arresting agent at metaphase.

The therapeutic doses of both AF and EP were determined according to LD50 in mice. The results indicated high effectiveness of both extracts in a dose- and time-dependent manner. The highest therapeutic doses of AF and EP (1.2 and 1 gm/kg B.wt. respectively) showed the best therapeutic effect by reducing the tumor volume in mice to about 73.9% and 83.8% respectively.

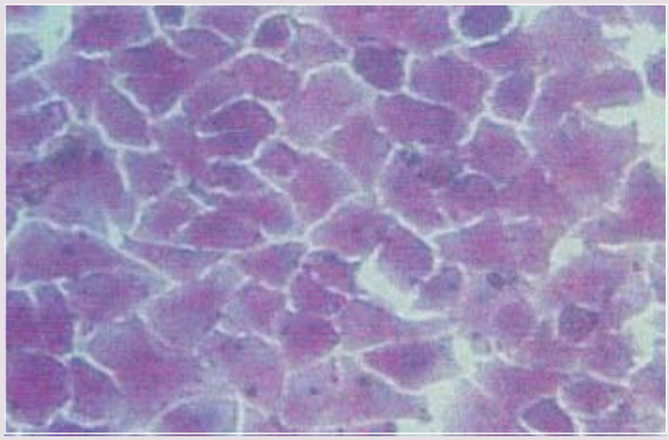
The comparison of relative tumor volumes of different groups revealed highly significant differences among all treated groups and those of untreated (control) group.

Cancer is the abnormal growth of cells in our bodies that can lead to death. Cancer cells usually invade and destroy normal cells. These cells are born due to imbalance in the body, and in order to correct this imbalance the cancer may be treated in different means. Billions of dollars have been spent on cancer research and yet, we do not understand exact nature of cancer (1). Every year, millions of people are diagnosed with cancer, leading to death. According to the American Cancer Society, deaths arising from cancer constitute 2–3% of the annual deaths recorded worldwide. Thus cancer kills about 3500 million people annually all over the world(2).

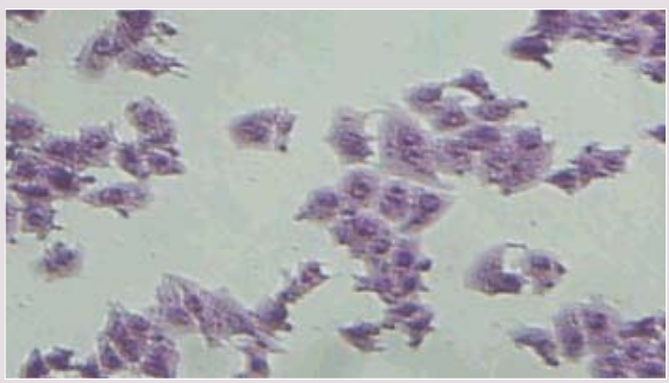
National Cancer Institute (NCI) of USA has found that the number of people die because cancer was more than 270,000 in USA (3). In Iraq, about 13,332 cancer cases have been documented in 2001; breast cancer was the most common form of cancer in women, while in men, bronchus and lung cancer were the most common (4).

Conventional cancer therapy is based on chemotherapy, radiotherapy, surgery or combinations of them. Because of high death rate associated with cancer and because of the serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and/or complementary methods of treatment. The important preventive methods for most of the cancer types include dietary changes, stopping the use of tobacco products, treating inflammatory diseases effectively and taking nutritional supplements that aid immune functions. Recent researches revolve round the urgency to evolve suitable chemotherapy consistent with new discoveries in cell biology for the treatment of cancer with no toxic effect (5).

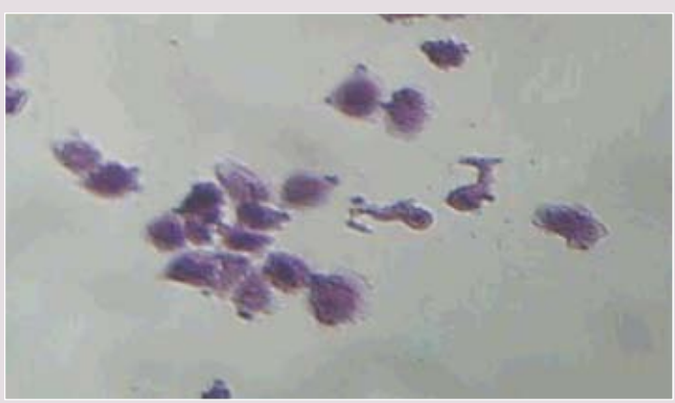
Chemotherapy, being a major treatment modality used for the control of advanced stages of malignancies and as a prophylactic against possible metastasis, exhibits severe toxicity on normal tissues (6,7). Plants have been used for treating various diseases of human beings and animals since time immemorial. They maintain the health and vitality of individuals, and also cure diseases, including cancer without causing toxicity. More than 50% of all modern drugs in



(a) Control (untreated).

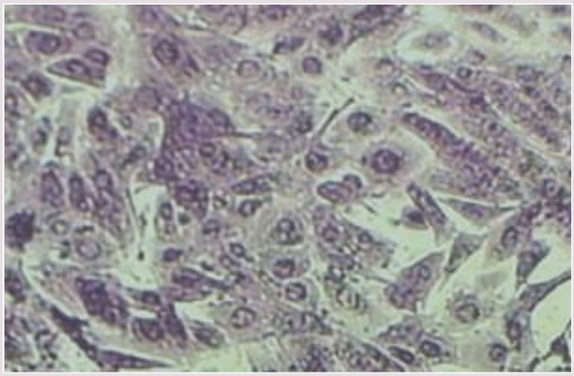


(b) Treated by aqueous extract of fruits.

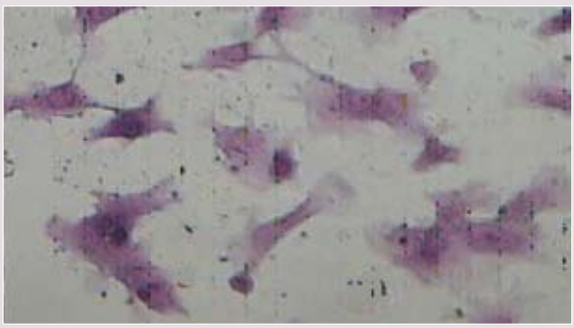


(c) Treated by ethanolic extract of pits.

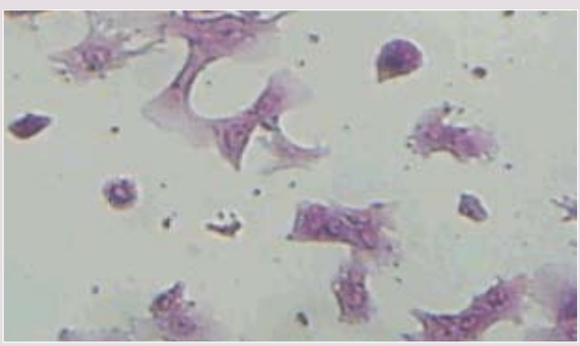
Fig. (1) Hep-2 cell line in control sample (a); and another samples (b) treated by 10000 µg/ml of aqueous extract of fruits and (c) treated with ethanolic extract of pits after 72 hrs of exposure (Crystal violet, 100 X).



(a) Control (untreated).



(b) Treated by aqueous extract of fruits.



(c) Treated by ethanolic extract of pits.

Fig.(2)AMN3 cell line in control sample(a); and another samples treated by 10000 µg/ml of aqueous extract of fruits (b); and ethanolic extract of pits (c) after 72 hrs of exposure (Crystal violet, 100 X).

clinical use are of natural products, many of which have the ability to control cancer cells (8).

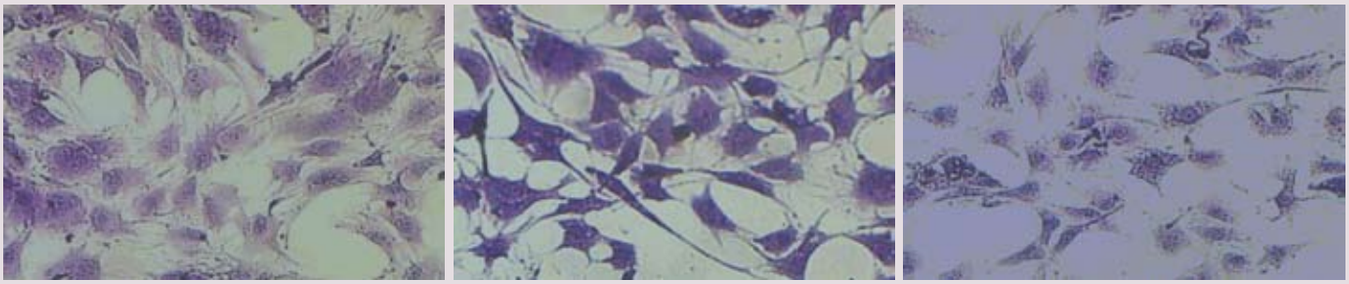
According to the estimates of the WHO, more than 80% of people in developing countries depend on traditional medicine for their primary health needs. A recent survey shows that more than 60% of cancer patients use

vitamins or herbs as therapy (9,10).

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans well as valuable components of seasoning, beverages, cosmetics, dyes and medicine. Most of this therapy involves the use of plant extracts or their active components (11). At least 250,000 species of plants do exist, out of which more than one thousand plants have been found to possess significant anticancer properties (12). The NCI collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity (13).

Many efforts have begun in the Iraqi Center for Cancer and Medical Genetics Research (ICCMGR), by its staff and graduate students, for finding alternative treatment of cancer diseases. These researches focus on local cancer cell lines and cancer inoculated animals for testing and evaluating the cytotoxic and genotoxic effects of medicinal herbs as alternative safe source of treatment. Crude plant extracts from Withania somnifera Dun, Eleettaria cardamomum (Cardamom), Cyperus rotundus L., Zingiber officinal, Cucurbita maxima, Cucurbita pepe, Peganum harmala, Saliva triloba, Artemisia herba alba, Nerium oleander, Vinca rosea, Olea europea L., Rheum ribes (Rhubarb), Thymus syriacus (thyme), Urtica dioica, Capparis spinosa L., Apium graveolens, Silybum marianum L., Punica granatum, Vitis vinifera, Lactuca serriola L. and Camellia sinesis have shown significant cytotoxic effects against malignant cell lines in vitro, some of which have shown antitumor effects in vivo (14).

For centuries, Phoenix dactylifera (Palmae) has been used in the Middle East as a staple food. No fewer than 800 uses are recorded for the date palm (15). The Palm family is a symbol of prosperity and love to Muslims and its legend dates back to Judeo-Christian mythology. In local medicinal practices, dates are considered a tonic. Some consider it to be an aphrodisiac. The flower of the plant is used as a purgative (16).



(a) Control (untreated). (b) Treated by aqueous extract of fruits. (c) Treated by ethanolic extract of pits.

Fig. (3) REF cell line in control sample (a) and another samples (b) treated by 10000 µg/ml of aqueous extract of fruits and (c) ethanolic extract of pits after 72 hrs of exposure (Crystal violet, 100 X).

Experimentally, antioxidant and antimutagenic activity in date fruit is quite potent and implicates the presence of compounds with potent free-radical-scavenging activity (17). The aqueous extracts of the flesh and pits of dates have ameliorative activity on carbon tetrachloride (CCl4)-induced hepatotoxicity, and Neuroprotective effects on focal cerebral ischemia in rats (18,19). Date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle-stimulating hormone and luteinizing hormone in rats (15). The pollen grains of date palm have been used by Egyptians to improve fertility in women (20). Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of estrogens or testosterone (21,22).

Many Middle Easterners believe that consumption of dates, particularly in the morning on an empty stomach, can reverse the actions of any toxic material that the subject may have been exposed to (23). Therefore, we sought to assess the anticancer activity of date flesh and pits on some malignant cell lines and to find out whether they have any antitumor effect on transplanted murine mammary adenocarcinoma in mice.

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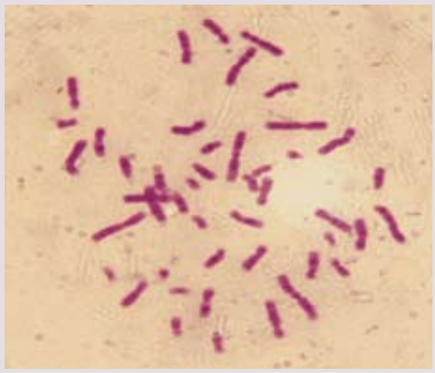

Fig. (4) Chromosomes of peripheral human lymphocyte (male) which appear normal without any detected structural or numerical chromosomal aberrations after 72 hrs of exposure with 10000 µg/ml of ethanolic extract of pits (Giemsa stain, 1000X).

adenocarcinoma-AMN3) and one normal cell line (rat embryo fibroblast-REF). The study also includes evaluation of the effect of these extracts on several cytogenetic parameters such as mitotic index (MI%), blast index (BI%) and chromosomal aberrations (CA) after in vitro culture of peripheral blood lymphocytes. This work also includes a study of the therapeutic potential of two extracts, one from fruits and the other from pits in the treatment of transplanted murine mammary adenocarcinoma in mice.

Aqueous and ethanolic extraction of the date palm fruits gave extracts with a yield of 24.33% and 14.2% respectively, while the pits gave extracts with a yield of 7.4% and 13.6% from aqueous and ethanolic extraction respectively. The hexanic extract of pits was pale yellowish-green oil with a pleasant odor with a yield of 4.1 ml/100 gm. The extraction of fruits by hexane did not give any yield.

The chemical tests of aqueous extracts of date palm fruits and pits detected flavonoids, glycosides, resins, tannins and terpenes. Moreover, ethanolic extracts of fruits and pits were found to contain alkaloids. The hexanic extract of pits gave positive tests with steroids and terpenes only.

The in vitro cell growth assay showed that there was time- and concentration-dependent cytotoxic effects of crude extracts of date palm fruits and pits on Hep2 and AMN3 cell lines. The highest significant effect of these extracts was achieved after 72 hrs of exposure with the highest concentration (10000 µg/ml). Both aqueous extract of fruits (AF) and ethanolic extract of pits (EP) caused growth inhibition percentage (76.3% , 89.4%) for Hep2 and (84.1% , 93.4%) for AMN3 respectively. However, 72 hrs exposure to crude extracts of fruits and pits at concentration of 10000 µg/ml caused slight inhibitory effect on REF cell line, reaching 21.1% and 17.7% for AF and EP respectively.

In general, the percentage of cell viability of all cell lines was inhibited after exposure to all crude extracts in a time- and concentration-dependent manner, although, the lowest concentration of aqueous extract of pits showed significant increment in the cell viability of Hep2 after 24 and 48 hrs exposure indicating a hormetic effect.

On the other hand, all crude extracts of fruits and pits caused significant reduction in the mitotic index and blast index of peripheral human lymphocytes, but without any structural or numerical chromosomal aberrations. Also these extracts neither replaced phytohemagglutinin (PHA) as mitogenic agent, nor colcemide as mitotic arresting agent at metaphase.

The therapeutic doses of both AF and EP were determined according to LD50 in mice. The results indicated high effectiveness of both extracts in a dose- and time-dependent manner. The highest therapeutic doses of AF and EP (1.2 and 1 gm/kg B.wt. respectively) showed the best therapeutic effect by reducing the tumor volume in mice to about 73.9% and 83.8% respectively.

The comparison of relative tumor volumes of different groups revealed highly significant differences among all treated groups and those of untreated (control) group.

Conclusion

Medicinal plants maintain the health and vitality of individuals, and also cure various diseases, including cancer without causing toxicity. The results of this study revealed that AF and EP possessed high cytotoxic effect on some cancerous cell lines and slight inhibitory effect on normal cell line; indicating the specificity of both extracts against malignant cells. Both extracts also revealed wide safety range

Fig. (5) Untreated mouse (control) at the end of the experiment, the tumour surface is complicated by superficial ulcer and clot formation.

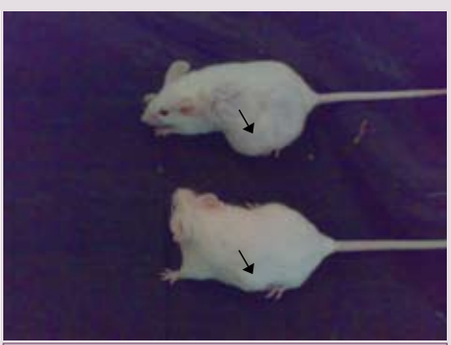

Fig. (6) Comparison of tumor volumes between untreated mouse (control, up) and one of the other group treated by ethanolic extract of pits (down).

in healthy mice and high antitumor effect when used in treatment of transplanted tumor in mice.

Further studies on the effect of extracts obtained from fruits, pits, or other parts of the date palm tree, may reveal valuable information as to the importance of date palm in cancer therapy as well as some other medicinal aspects of "The Blessed Tree".

References

1-Estrogen and cancer website, 2006; www.womenshealth.com, www.amazon.com

2- American Cancer Society, A biotechnology company dedicated to cancer treatment, viewed on 25 January 2006; www.cancervax. com/info/index. htm

3- Ahmedin, J.; Rebecca, S.; Elizabeth, W.; Taylor, M.; Jiaquan, X. and Michael, J. (2007). Cancer Statistics. American Cancer Society. CA. Cancer. J. Clin., 57: 43-66.

4- Ministry of Health (2003). Results of Iraqi Cancer Registry 1999-2001, Iraqi Cancer Board, Baghdad, Iraq.

5- Madhuri, S. and Pandey, G.(2009). Some anticancer medicinal plants of foreign origin. Current Science, 96(6), 25:779-783.

6- Somkumar, A. P.(2003) Studies on anticancer effects of Ocimum sanctum and Withania somnifera on experimentally induced cancer in mice. Ph.D. thesis, JNKVV, Jabalpur, India.

7- Pandey, G. and Madhuri, S.(2006). Medicinal plants: better remedy for neoplasm. Indian Drugs, 43: 869–874.

8- Rosangkima, G. and Prasad, S. B.(2004). Antitumour activity of some plants from Meghalaya and Mizoram against murine ascites Dolton's lymphoma. Indian J. Exp. Biol., 42: 981–988.

9- Madhuri, S. and Pandey, G.(2008). Some dietary agricultural plants with anticancer properties. Plant Arch., 8:13–16.

10- Sivalokanathan, S., Ilayaraja, M. and Balasubramanium, M. P.(2005) Efficacy of Terminalia arjuna (Roxb.) on N-nitrosodiethylamine induced hepatocellular carcinoma in rats. Indian J. Exp. Biol., 43: 264–267.

11- Craig, W.J. (1999). Health-promoting properties of common herbs. Am. J. Clin. Nutr., 70: 491S-499S.

12- Mukherjee, A.K. ; Basu, S. ; Sarkar, N. and Ghosha, A.C. (2001). Advances in cancer therapy with plant based natural products. Current Medicinal Chemistry, 8:1467-1486 .

13- Shoeb, M.(2006). Anticancer agents from medicinal plants. Bangladesh J. Pharmacol, 1: 35-41.

14- Yaseen, N.Y.; Hussein, S.M.; Saleh, F.S. and Mohammad, M.H.(2008). Plant and biological extracts in cancer therapy. Iraqi Center for Cancer and Medical Genetics Research(ICCMGR), Baghdad, Iraq.

15- El-Mougy, S.A.; Abdel-Aziz, S.A.; Al-Shanawany, M. and Omar, A.(1991). The gonadotropic activity of Palmae in mature male rats. Alexandria J. Pharmac. Sci., 5:156–159.

16- Zohget, M. and Elsheikh, A.(2000). Wild Plants in the Region of Riyadh. Riyadh, Saudi Arabia; King Saud University Press; p.185–186.

17- Vayalil, P.K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (Phoenix dactylifera L. Aracaceae). J. Agric. Food Chem., 50:610-617 .

18- AlQarawi A.A.; Mousa, H.M.; Abdel-Rahman H.A. and ElMougy S.A.(2004) Protective Effect of Extracts from Dates (Phoenix dactylifera L.) on Carbon Tetrachloride–Induced Hepatotoxicity in Rats. Intern. J. Appl. Res. Vet. Med., 3(2):176-180.

19- Majid, A.S.; Marzieh, P.; Shahriar, D.; Zahed, S.K. and Pari, K.T.(2008). Neuroprotective effects of aqueous date fruit extract on focal cerebral ischemia in rats. Pak. J. Med. Sci.,24(5):661-65.

20- Amin, E.S.; Awad, O.; Abdel-Samad, M. and Iskander, M.N.(1969) Isolation of estrone from Moghat roots and from pollen grains of Egyptian date palm. Phytochem., 9:295–297.

21- Elgasim, E.A.; Alyousif, Y.A. and Homeida, A.M.(1995). Possible hormonal activity of date pits and flesh fed to meat animals. Food Chem., 52:149–150.

22- Ali, B.H.; Bashir, A.K. and Al-Hadrami, G.(1999). Reproductive hormonal status of rats treated with date pits. Food Chem., 66:437–441.

23- AlQarawi, A.A.; Abdel-Rahman H.A. and ElMougy, S.A.(2001) Hepatoprotective activity of licorice in rat liver injury models. J. Herbs Spices Med. Plants, 8:7–14.