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SEEDS OF *TAMARINDUS INDICA* AS ANTI-CANCER IN SOME CELL LINE

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ABSTRACT
The present study was planned to evaluation the cytotoxicity effect of seeds methanolic extract of *Tamarindus indica* as anticancer at concentration (0.1-1000) µg/ml. Hence, this study has evaluated the *in vitro* effects of seeds methanolic extract on two cancer cell lines (Rhabdomyosarcoma cancer (RD) and Human Lymphoma cell line (SR)). The results of this study revealed that higher inhibition rate was occurred in 24 h incubation period at concentration (100 and 0.1) µg/ml for RD and SR cell lines respectively, so the higher inhibition rate was occurred in 72 h at concentration (1000 and 100) µg/ml for RD and SR cell lines respectively.The results suggest that seeds extract possess strong activities as anti-cancer functions.

KEY WORDS: *Tamarindus indica*, seeds extract, cancer cell lines.

INTRODUCTION

According to the WHO, More than 70% of all cancer deaths occurred in low- and middle-income countries, deaths from cancer worldwide are projected to continue rising, with an estimated 11.5 million deaths in 2030[1] . Herbal medicines remain the major source of health care for the world's population. WHO has recognized herbal medicine as an essential building block for primary health care? Tamarindus [*Tamarindus indica* , (*T. indica*)], belongs to the family Leguminosae (Fabaceae), commonly known as Tamarind tree, is one of the fruit tree species that is used as traditional medicine. Tamarind tree is found especially in the Indian subcontinent, Africa, Pakistan, Bangladesh, Nigeria and most of the tropical countries. It is preferred to be used for abdominal pain, diarrhea and dysentery, some bacterial infections and parasitic infestations, wound healing, constipation and inflammation[2].Traditionally seeds of *Tamarindus indica* are being used in asthma, bronchitis, leprosy, tuberculosis, wounds, ulcers, inflammation**,** stomach algia, diarrhea, dysentery, burning sensation, giddiness, vertigo, and diabetes[3]. It has been reported that seeds of *Tamarindus indica* are having antiulcer, anti-asthmatics, ant diabetic and antioxidant activity[4-5]. Also seeds of *Tamarindus indica* are rich in phenolic compounds, polymeric tannins, and fatty acids flavonoids, saponins, alkaloids, and glycosides[6]. Flavonoids, tannins, saponins and alkaloids are responsible for ant-inflammatory and analgesic activity [7]. Hence, this study was done to evaluation the cytotoxicity effect of *Tamarindus indica* methanolic extract as anti-cancer in two cell lines.

MATERIALS & METHODS

Preparation of extract

The *Tamarindus indica* were collected from local market of Baghdad city, the seeds were isolated and drying in room temperature for 10 days, after that, grounded to powder by using an electric blender. The powder was sieved by 40 mesh sieve. The extract was prepared by using maceration method. About 250 g of dried powder of the seeds were extracted with 1750 ml methanol (1:7) for 72 h. The extract was concentrated and dried (yield: 8% w/w). The dried methanolic extract was kept in airtight container in desiccator and used throughout the study[8].

Preparation the dilution

About 0.1 gram from dried methanolic extract solvent in 10 ml of distillated water as a stock, then a series of test tube to prepare five dilution (1000,100, 10, 1,0.1) µg/ml from the stock and solvent in free serum media (FSM).

Cell culture

Rhabdomyosarcoma (RD) cell line and Human lymphoma (SR) cell line were used in this study. RD cell line was derived from biopsy specimen of a pelvic rhabdomyo sarcoma of a 7 year old Caucasian girl[9-10]. SR cell line is a human lymphoma cell line originated in 1983 by Walter J. Urba and Dan L. Longo, from Caucasian male 11years old and it was taken from pleural effusion tissue. Cell lines were purchased from tissue culture unit/ Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR), The cells were cultured in 75 cm^2 tissue culture flasks under humidified 5% CO2 atmosphere at 37°C in RPMI-1640 medium (Sigma chemicals, England) with 10% fetal bovine serum (FBS), and penicillin- streptomycin (100 U/mL penicillin and 100 µg/mL streptomycin) During the course of the experiment[11].

Cytotoxicity Assay

Cells cultures in micro titer plate (96wells) were exposed to range of (seeds extracts, concentration during the log phase of growth and the effect determined after two incubation time (24 and 72 hours) , each well contain $1X10^4$cells/well, Serum calf medium 10% used for seeding. Plates were then incubated for 24hrs in 37C° for achieve cell attachment, then by using maintenance

medium (FSM), fivefold serial dilution were prepared starting from (1-10000 µg/ml) for methanolic extract of *Tamarindus indica* seeds. After incubation for 24 hrs., cells were exposed (four replicate at 200µl for each tested concentration), also 200 µl of only maintenance medium added to each well of control group, the times of exposure were 24 and 72 hrs. The plates were sealed with self-adhesive film then returned to incubator, after incubation periods were finished, cells were staining with MTT stain. The optical density of each well was read by using a micro-ELISA reader at a transmitting wavelength on 550 nm [11]. The inhibition rate was calculated according to [12] as follows:

IR% = ((A – B)/A) x 100

IR=inhibitor rate, A= the optical density of control, B= the optical density of test.

Statistical Analysis

The Statistical Analysis System [13] was used to identify the effect of different factors in study parameters. Least significant difference –LSD test was used to compare between means in this study significantly, all experiments were performed in the Iraqi center for cancer and medical genetic research.

RESULTS & DISCUSSION

Cytotoxicity study on RD cell line

The results revealed that maximum cytotoxic effect (68.0 ±2.79 and 61.8 ±2.98) occur at (100 and 1000) µg/ml respectively in incubation period 24 and 72 hours while the minimum cytotoxic effect (49.8 ±2.42 and 40.55 ±1.73) occur at concentration (1 and 0.1) µg/ml respectively in incubation period 24 and 72 hours. Highly significant between concentration in the same period at level (P<0.01), as well as between two incubation period at the concentration 0.1µg/ml, also there is significant variation at concentration (10 and 100) µg/ml in the same period at level (P<0.05) except at the concentration (1 and 1000) µg/ml, in which, there is no- significant.

TABLE 1: Effect of *Tamarindus indica* seeds extract on RD cell line

Concentration (µg/ml)	24 h	72 h	LSD value	P-value
0.1	65.8 ± 2.95	40.55 ± 1.73	6.302 **	0.00751
1	**49**.8 ± 2.42	49.6 ± 2.04	3.567 NS	0.755
10	59.0 ± 3.07	49.7 ± 1.84	5.183 *	0.0346
100	68.0 ± 2.79	61.3 ± 3.26	5.963 *	0.0377
1000	58.0 ± 2.35	61.8 ± 2.98	4.841 NS	0.149
LDS value	6.273 **	7.644 **	---	---
P-value	0.00226	0.00728	---	---
* (P<0.05), ** (P<0.01).				

Cytotoxicity study on SR cell line

In incubation period 24 and 72 hours while the minimum cytotoxic effect (21.3± 0.85 and 14.1± 0.52) occur at concentration (10) µg/ml respectively in incubation period 24 and 72hours.Highly significant between concentration in the incubation period 24hours at level (P<0.01) and significant difference between concentration in incubation period 72 hours at level (P<0.05). For the effect of time on inhibition growth, there is a significant variation between two incubation period at the concentration (0.1, 10 and 1000) µg/ml in the same period at level (P<0.05) except at the concentration (1 and 100) µg/ml, in which, there is non -significant.

TABLE 2: Effect of *Tamarindus indica* seed extract on SR cell line

Concentration (µ/ml)	24 h	**72** h	LSD value	P-value
0.1	39.5 ± 2.38	31.5 ± 1.55	5.209 *	0.0462
1	23.2 ± 1.40	24.5 ± 0.96	4.172 NS	0.522
10	21.3 ± 0.85	14.1 ± 0.52	5.976 *	0.0371
100	31.8 ± 1.24	32.6 ± 1.61	3.257 NS	0.494
1000	31.4 ± 1.63	24.2 ± 1.09	5.117 *	0.041
LDS value	5.509 **	5.713 *	---	---
P-value	0.00969	0.0072	---	---
* (P<0.05), ** (P<0.01).				

A wide range of biologically activity is isolated from higher plants and a number of them are reported to have antitumor and immunomodulatory activities. The mechanisms that mediate the biological activity of these plants are still not clearly understood. Plant extract from natural resources usually do not attack cancer cells directly but produce their antitumor effects by activating different immune responses in the host. For the past several decades, attempts have been made to search for safer immunomodulation agents, and one of the special focuses has been on the biological response modifiers (BRMs) derived from natural products[14].

The result of this study revealed that seeds extract has more effect on RD cell line than SR cell line. So the anticancer activity of seeds extract on RD and SR cell lines have been not evaluated with increasing incubation time, this mean that 24 incubation time is enough to kill the cancer cells which indicated to time independent effect as well as dose independent effect in two cell lines except in RD cell line, in which the effect was increased with

concentration increasing. These effects were occurred due to the active material which found in seeds of *T indica* such as oil and poly saccharide which lead to cancer cells killing. In addition, the presence of caffeic acid together with other polyphenols in *T. indica* seeds can enhance the antioxidant activities of treated cancer cells which can provide protection against oxidative damage [15]. Addition to that, some seeds composition can effect on cell viability, cell cycle, migration, phosphorylation and gene expression [16].From the present study and the earlier reports, it was suggested that seeds methanolic extract of *Tamarindus indica* could be developed as an adjuvant for cancer management after conducting clinical trials in vivo and human subjects.

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