J. Appl. Environ. Biol. Sci., 7(12)91-99, 2017

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Efficacy of Aqueous Extracts of Some Common Saudi Spices as a Novel Anticancer Agents against Some Human Cancer Cell Lines

Heba Ali Nasr[1*] and Olfat Mahmoud Nassar[2,3]

[1]Department of Biology, Faculty of Science and Arts, Northern Border University (Rafha), Kingdom of Saudi Arabia.
[2]Department of Home Economics (Nutrition), Faculty of Science and Arts, Northern Border University (Rafha), Kingdom of Saudi Arabia.
[3]Nutrition and Food Sciences Department, Faculty of Home Economics, Minufiya University, P.O Box. 14442 Minufiya, Shibin El Kom, Egypt.

Received: August 20, 2017
Accepted: November 17, 2017

ABSTRACT

Cancer is the major cause of death worldwide and existing treatments like radiotherapy and chemotherapy have various side effects and also, are not found to be 100% successful. This has led to the need of alternate treatment options with minimum side effects. Spices significantly contribute to human health through their bioactivities. In this present study aqueous extracts of four common Saudi spices; Argel (*Solenostemmaargel*), Artemisia (*Artemisia cina*), Black seed (*Nigella sativa*) and Turmeric (*Curcurmadomestica*) were procured from local herbal markets from governorate of Rafha at the Northern Border region in Kingdom of Saudi Arabia and evaluated for anticancer activity against three human cancer cell lines by MTT assay method. The tested extracts showed cytotoxic activities against the tested cell lines with varied degrees. Among them, extract of Black seed was the highest cytotoxic one with IC_{50} 11.2, 34.5 &65 µg/ml, moderate cytotoxicity was recorded for extracts of both Argel and Artemisia with IC_{50} 23.6, 78.7 & 77.3 and 27.6, 52.2 & 81.8µg/ml forHEPG2, HCT116 and MCF7 cell lines respectively. While extract of Turmeric was the weakest one where it was only active against HEPG2 with IC_{50} 17.8 µg/ml but it was inactive against both HCT116 and MCF7. Aqueous extract of Black seed/Argel blend exhibited a synergistic effect where it exhibited good cytotoxic effect on the tested cancer cell lines with IC_{50}6.8, 15.2 and 27.5µg/ml for HEPG2, HCT116 &MCF7respectively. The obtained blend showed low cytotoxicity against human normal melanocyte cell line (HFB4) recording IC_{50} 2.3 µg/ml. Hence, aqueous extract of Black seed/Argel blend can be taken as a daily diet for treatment of different types of cancers. Our findings established that, using of traditional Saudi spices and their combined utilization as functional foods and dietary supplements had a synergistic effects against several types of cancers.
KEY WORDS: Aqueous extracts, Saudi spices, Anticancer, MTT assay, Cytotoxicity, Black seed/Argel blend.

INTRODUCTION

Cancer is defined by uncontrollable division of cells that destroy body tissue. The World Health Organization (WHO) stated that, cancer considers the major cause of morbidity and death for humans in our world[1]. Incidences of cancer are being observed to increase, recently and this is mainly attributed to urbanization, industrialization, lifestyle changes, increased population and increased elderly population[2].Globally, the number of cancer cases in 2012 was reached to 14.1 million and this number is in increase and may reach to 25 million in 2032. Further than the high incidence, cancer is also one of the leading causes of death where the number of cancer-related deaths in 2012 is recorded as 8.2 million which were attributed to many types of cancers such as liver, breast, cervical, lung, colorectal, gastric and prostatic cancer[3].

There are a number of normal cancer treatments includes chemotherapy, radiotherapy, surgery, angiogenesis inhibitors and targeted therapy with monoclonal antibodies. The increasing rate of mortality associated with cancer as well as the toxic side effects of such normal treatments led to searching for new natural anticancer agents especially from plants [4].

The plant kingdom contains a great source of new bioactive compounds which, due to their intrinsic biological properties, may be used in medicine as well as in other human health promoting areas [5]. Many commercially sold medicinal plants might contain chemical substances with potential mutagenic and/or carcinogenic properties [6,7] as well as with antitumor properties [8,9].

Herbal medicine is one of the oldest, if not the oldest, and probably remains to be of growing popularity. It is really intriguing that despite the great advancement in the fields of conventional medicine and drug discovery, the

*Corresponding Author: Dr. Heba Ali Nasr, Department of Biology, Faculty of Science and Arts, Northern Border University (Rafha), Kingdom of Saudi Arabia. Email: Heba770@yahoo.com

use of herbal formulations is still extremely widespread throughout the world, indicative of peoples' perception of the safety and therapeutic efficacy of such medicinal herbs. Although herbal medicine is more prevalent in Asia, Africa, and to a lesser extent in Europe, it is most likely the gentle, nourishing, efficacious, synergistic, cost-effective, and safe properties of medicinal herbs that make them an attractive option for many people as therapeutic agents [10,11]. It is known that, the dietary plant extracts, especially herbs and spices have a preventive or protective role against cancer which may due to its potency and lowest toxicity[12,13].

Due to the beneficial health effects of spices it used as traditional systems of medicine as well as it used to enhance the tasting or the sensory quality of foods. With the knowledge of the chemistry of the spice bioactive compounds, their beneficial health effects have been exhaustively investigated in recent decades [14,15]. Through their bioactivities, spices significantly contribute to human health and may be considered as the first ever nutraceutical. The multiple health beneficial of spices include some stimulating effects such as protection to the gastrointestinal tract, digestive stimulant action, hypolipidemic effect and antidiabetic influence as well as anti-inflammatory property, antioxidant potential and anticancer activity [16,17].

Through the history, spices were enjoyed with a rich traditional use due to their medicinal properties as well as their flavor enhancement characteristics. The increase in chronic diseases among the world also the rise in corresponding health care costs enhances the researchers and the public for discovering a multiple alternative health benefits related to foods, including those causes modification of tumor behavior and reduction in cancer risk [18]. Through the above mentioned reports, it was stated that, spices act as a promising anticancer activity. Hence, the present study was aimed to investigate the cytotoxic potency of aqueous extracts of four common Saudi spices against three human cancer cell lines to obtaining a new natural therapeutic product to be taken as a daily diet can be given to poor people in the rural areas to treat different types of cancers.

MATERIALS AND METHODS

Chemical and reagents

All the chemicals used in this study were of analytical grade. Dimethyl sulfoxide (DMSO), Cyclophosphamide, Dulbecco's Modified Eagle's Medium (DMEM), Phosphate Buffered Saline (PBS), Trypan blue, foetal bovine serum (FBS) & 3-(4,5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium bromide (MTT) were purchased from Sigma Company, USA.

Cell lines

The human liver carcinoma (HEPG2), the human colon carcinoma (HCT 116) and the human breast adenocarcinoma (MCF-7) cell lines were obtained from American Type Culture Collection (Rockville, MD) then maintained by serial sub-culturing at the National Cancer Institute (NCI), Cairo, Egypt.

Spices

Four common Saudi spices included; dry Argel (*Solenostemmaargel*) leaves, dry Artemisia (*Artemisia cina*) leaves, dry Black seed (*Nigella sativa*) and dry Turmeric (*Curcurmadomestica*)leaves were procured from local herbal markets at governorate of Rafha, then identified at the Pharmacognosy Department, Faculty of Pharmacy, Northern Border University, KSA.

Preparation of aqueous extracts of the selected spices

For preparing the aqueous extracts of the spices under investigation, 5g of each type were grinded in blender to fine powder then 1 g of each powder was soaked in 200 mL hot distilled water. The prepared extracts were filtered with Whatman No. 1 filter paper after standing for 24 h [19].

Anticancer activity

Maintenance of cell cultures

For maintenance the previously mentioned carcinoma cells, it were cultured in75 cm^2 cell culture flasks of RPMI -1640 medium containing 1% MEM non-essential amino acid solution, 10% fetal bovine serum and 1% penicillin-streptomycin solution in a humidified atmosphere of 95% air, 5% CO_2 at 35°C. The number of cells was maintained to be between 20 and 25. For studying the anticancer activity, the cells were cultured in plates of 96-well in a concentration 0.2 mL of cell solution/well then the cells were allowed to attach for 24 h before treatment with tested extracts. For determination the cytotoxicity of the tested materials, the cell monolayers were washed with PBS then the serially diluted materials were added and dispensed to the pre-cultured plates [20]. Anticancer activity was performed at the National Cancer Institute (NCI), Cairo, Egypt.

Cytotoxic activity using MTT assay

The cytotoxic activity of the prepared aqueous extracts against the selected human cancer cell lines was studied by MTT assay according to the method of Mossmann [21]. Briefly, the tested human cancer cells were incubated for 4 h in serum free medium containing 0.8 mg of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT). The cells were washed with 1 ml of phosphate buffer saline (PBS) followed by addition of 1 ml dimethyl sulfoxide (DMSO) then the solution was shaken gently for 10 min until the complete dissolution. 96-well plates were inoculated with aliquots (200 µL) of the prepared solution, the absorbance was recorded using the microplate spectrophotometer system at 560 nm. The obtained results were analyzed using the software of Soft max pro (version 2.2.1) as percentage of the control value. For obtaining the survival curve, the relationship between the extract concentration and surviving fraction was plotted and IC_{50} (the concentration required for 50% inhibition of cell viability) was calculated. The highest active extracts were selected and mixed in equal concentrations (1:1) and reevaluated against the same tested cancer cell lines in order to compare their blend effect.

Evaluation of the cytotoxic activity against human normal melanocyte cell line (HFB4)

The selected Black seed/Argel blend was evaluated for cytotoxic activity against human normal melanocyte cell line (HFB4); to assess their selectivity to cancer cells.

Statistical analysis

The results were recorded as mean values and the statistical significance was calculated using ANOVA (one-way analysis of variance), the differences were considered significant when p-value< 0.05. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Cancer is among the leading causes of morbidity and mortality worldwide. To developing new anticancer drugs it was estimated that, cost over $5,000,000 and 7 to 10 years are required. This cost includes, specimens collection, crude extracts evaluation, purification and identification, synthesis of the active substance as well as testing the drug on preclinical and clinical studies [5].

The common techniques which were used for cancer treatment became unfavorable because of its serious side effects in addition to the growing resistance toward anticancer effects that worsens the future of such treatments. Thus, the focus toward the natural products such as spices became urgent for developing new anticancer agents and to save the future of cancer treatment [22-24].

The cytotoxicity effects of spices are differing with their constituents of the bioactive compound, therefore, the present study was conducted in order to compare the cytotoxic effects of aqueous extracts of four common Saudi spices for cytotoxic activity against three human cancer cell lines. The use of naturally-occurring agents to regulate tumorigenesis is on the rise. Several herbal extracts, pure plant-derived active constituents, and food additives have been reported to possess potent anti-cancer properties and cancer-ameliorating effects [25].

Generally, the results of the cytotoxic activity of the four tested extracts showed that, all of the tested extracts have cytotoxic activities against the tested cell lines with varied degrees. It is well known that many drugs of those commonly used for remedy are of herbal origin [26-28].

Among these extracts, Black seed (*Nigella sativa*) extract was the highest cytotoxic one with IC_{50} 11.2, 34.5 & 65 µg/ml against human liver carcinoma (HEPG2), the human colon carcinoma (HCT 116) and the human breast adenocarcinoma (MCF-7) cell lines respectively (Figure 1). The traditional using of the seeds, oils and extracts of *N. sativa* as an anticancer agent in different countries is due to thousands of years. The thymoquinone (TQ) crude oil extracted from the black seed is effective against many diseases as well as it have anticancer against various types of cancers such as liver, breast, lung, etc. with much safety [29].

Aqueous and alcohol extracts of *N. sativa* were found to be effective *in vitro* in inactivating MCF-7 breast cancer cells [30]. The volatile oil of *N. sativa* has anticancer activity against the colon carcinoma cells of rats with no adverse side effects [31]. Thabrew et al. [32] tested the cytotoxic activity of *N. sativa* seed with different concentrations (0–50 mg/ml) against human hepatoma HepG2 cell lineand88% inhibitory effect on HepG2 was recorded after 24-h incubation. Banerjee et al. [33] reported that, *Nigella sativa* have a strong effects on HCT-116 with cell inhibition 69.21%. The wide-range anti-cancer effects of *Nigella sativa*, also known as black seed or black cumin, have been extensively studied using different in vitro and in vivo models [25].

Extract of dry Argel (*Solenostemmaargel*) leaves was the second highest cytotoxic one where it showed moderate cytotoxic activity with IC_{50} 23.6, 78.7 & 77.3 µg/ml against human liver carcinoma (HEPG2), the human colon carcinoma (HCT 116) and the human breast adenocarcinoma (MCF-7) cell lines respectively (Figure 2). As a

medicinal plant, *S. argel* is used in folkloric medicine against a wide range of diseases and health problems. It was used as anti-inflammatory [34,35]and anti- oxidant [36]and anticancer [37,38].

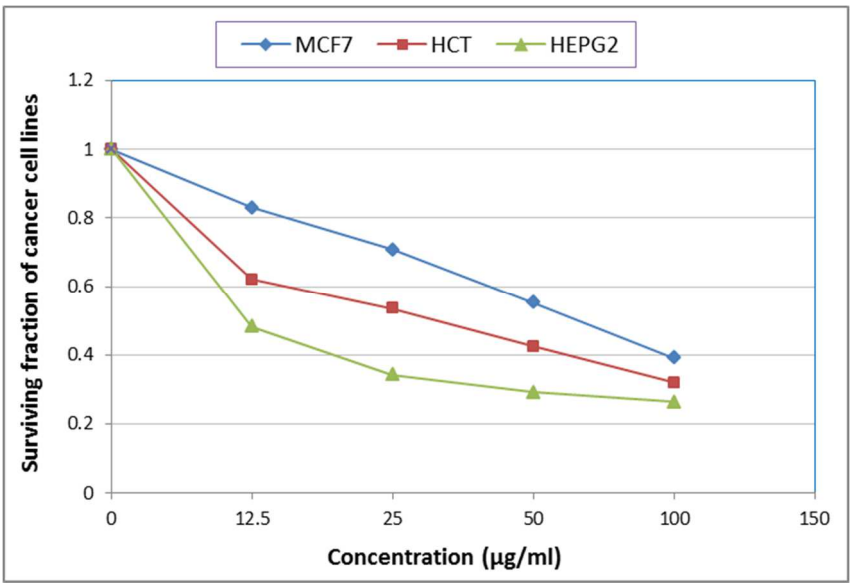

Figure 1: Cytotoxic activity of Black seed against the different human cancer cell lines.

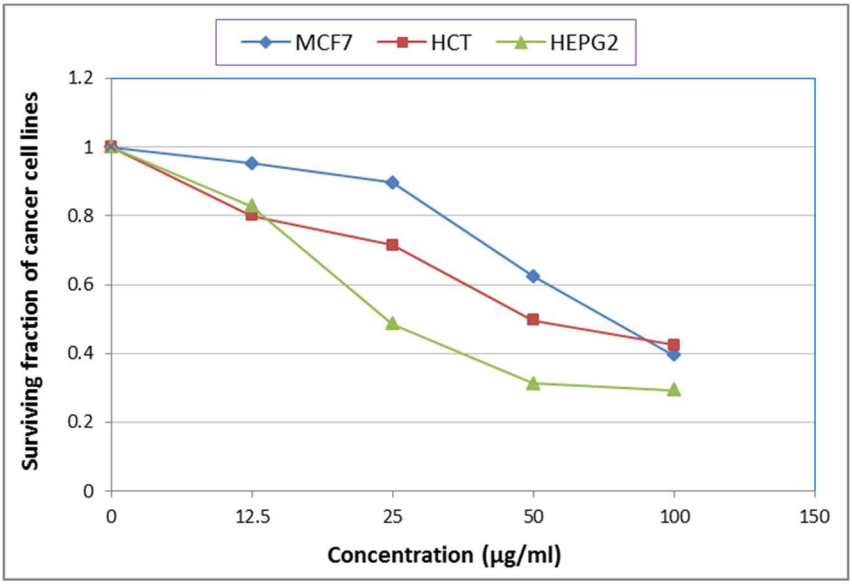

Figure 2: Cytotoxic activity of Argel against the different human cancer cell lines.

Moderate cytotoxic activity was recorded for the extract of dry *Artemisia* (*Artemisia cina*) leaves where it showed IC_{50} 23.6, 78.7 & 77.3 µg/ml against human liver carcinoma (HEPG2), the human colon carcinoma (HCT 116) and the human breast adenocarcinoma (MCF-7) cell lines respectively (Figure 3). In the middle Ages, *Artemisia* is regarded as a broad remedy for all diseases owing to its curative medical powers. Antimalarial and anticancer activities are the most well-known biological effects accounted for different species of the genus *Artemisia* [39-41].

The weakest cytotoxic activity was recorded for extract of dry Turmeric (*Curcurmadomestica*) leaves where it was only active against human liver carcinoma (HEPG2) with IC_{50} 17.8 µg/ml but it failed to inhibit 50% of cell viability at 100 µg/ml of its concentration thus its IC_{50} was recorded as non-active (NA) against both the human

colon carcinoma (HCT 116) and the human breast adenocarcinoma (MCF-7) (Figure 4). The active constituents of turmeric are curcumin and lectin; curcumin initiates apoptosis of cancerous cells without affecting the normal cells[42]. According to previous reports, turmeric is a good source of natural flavonoids, which have been shown to have antioxidant activity, free radical-scavenging capacity and anticancer activities [43].

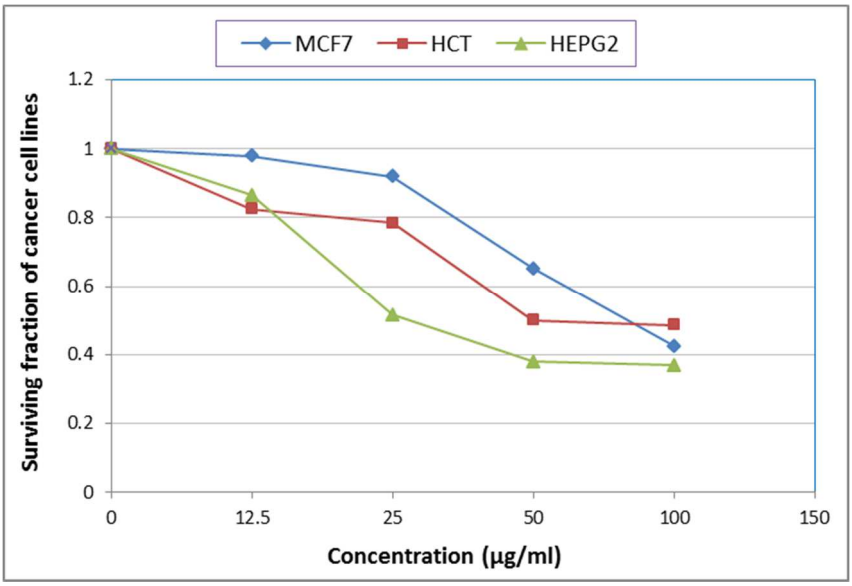

Figure 3: Cytotoxic activity of Artemisia against the different human cancer cell lines.

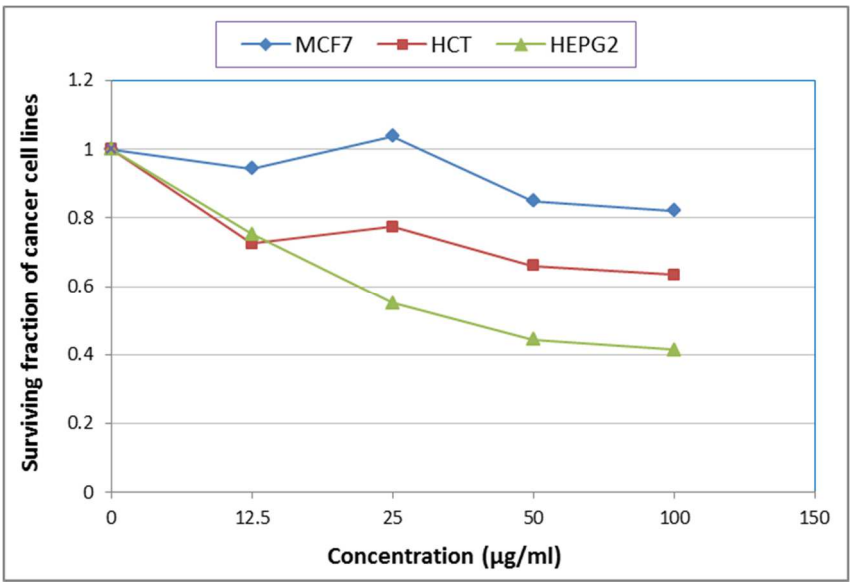

Figure 4: Cytotoxic activity of Turmeric against the different human cancer cell lines.

From the above mentioned results it was noticed that, the lowest IC_{50} values were recorded for the aqueous extracts of both the Black seed and Argel in comparison with IC_{50} of the other tested spices. Thus, they were selected as the highest active spices and mixed in equal concentrations (1/1) then further evaluated for cytotoxic activity against the same cancer cell lines. It was found that, the aqueous extract of Black seed/Argel blend showed excellent cytotoxic effect on the tested cancer cell lines with IC_{50}6.8, 15.23 and 27.5 µg/ml for HEPG2, HCT116 & MCF7 respectively (Figure 5).

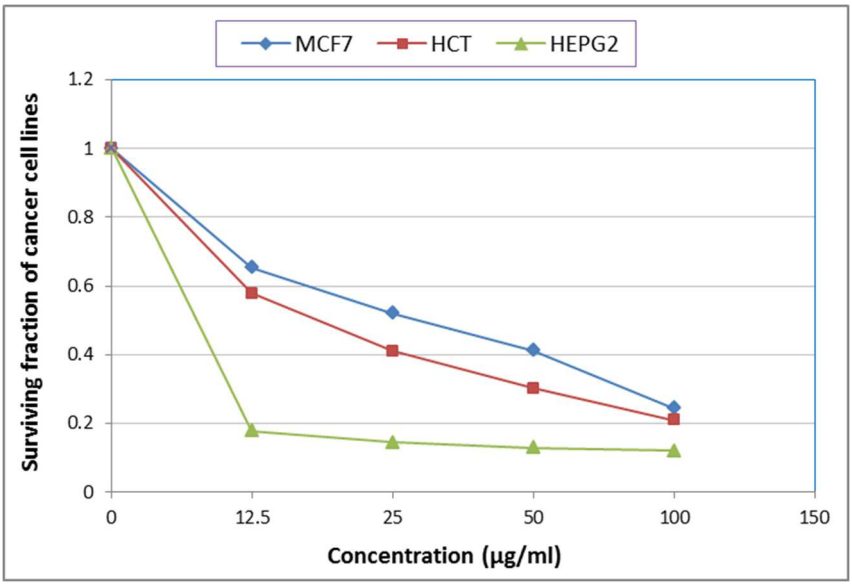

Figure 5: Cytotoxic activity of Black seed/Argelblend against the different human cancer cell lines.

Comparing $IC_{50}$of the individual effect for each Black seed and Argel extracts with $IC_{50}$of the combined action (Table 1) demonstrated a promising synergistic effect and strong cytotoxic activity against the all tested cancer lines, where $IC_{50}$of the blend was decreased greatly to be less than IC_{50} of the lowest one and this is depend on the active constituent present in each plant.

To assess the selectivity to cancer cells the selected Black seed/Argel blend was evaluated for cytotoxic activity against human normal melanocyte cell line (HFB4) and it was found that, the blend showed a relatively low cytotoxicity to normal cells with IC_{50} 2.3 µg/ml (Table 1). Clinically, several anticancer drugs are effective for cancer treatment, but it is common for these chemotherapeutic agents to have serious side-effects as a result of their nonselective cytotoxic effect on both cancer and normal cells; thus, compounds with selective antitumor activity that do not have side-effects are urgently required for clinical use.

Table 1: IC_{50} of individual and combined effect of Black seed and Argel extracts.

Cell lines	IC_{50} (µg/ml)		
Extracts	Black seed	Argel	Black seed/Argel
HEPG2	11.2	23.6	6.8
HCT116	34.5	78.7	15.2
MCF7	65	77.3	27.5
HFB4	NT	NT	2.3

NT=not tested.

To the best of our knowledge, there is a few reports discussed the combined effect of the different spices for their anticancer activity whether on the same used spices or others. Generally, all these studies recorded a synergistic cytotoxic effect for the combined action comparing with the individual effect. The aqueous extract of cinnamon/clove blend showed strong anticancer activity with synergistic effect on liver cancer cell line Hep G2[44]. Also, El-Aziz et al. [45] reported that, extract of the mixture black seed/melatonin and retinoic acid has a strong anticancer activity in mammary carcinoma of rats.

CONCLUSION

The results obtained in this study established that, the aqueous extract of the blend black seed/Argel showed synergistic effect where it had highly selective cytotoxicity to the human; liver (HEPG2), colon (HCT116) and breast (MCF7) carcinoma cells, but the lowest activity against human normal melanocyte cells (HFB4). Thus we can conclude using of traditional spices in combination is effective in tumor prevention and therapy.

RECOMMENDATIONS

Resent findings have not yet been able to explain the exact mechanism of anticancer effects of this blend thus; we suggest that black seed/Argel blend's anti-cancer activity needs to be further studied on animal models for studying the biology and genetics of human cancers as well as for preclinical investigation of anti-cancer therapeutics and cancer prevention.

ACKNOWLEDGEMENT

Heba A. Nasr and Olfat M. Nassar gratefully acknowledge the approval and support of this research study by the grant No. 7120-SAR-2017-1-7-F from Deanship of Scientific Research, Northern Border University, Arar, KSA.

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