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Anticancer activity of *Phoenix dactylifera* seeds extract: *In vitro* and *in vivo* studies

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ABSTRACT

Date (*Phonix dactylifera*) has great interest in biomedical applications. This study was conducted to evaluate the phytochemicals constitute, antioxidants activity, in vitro and in vivo anticancer activity of P. dactylifera seeds extract (PDSE). Phytochemicals analysis (qualitative and quantitative), two different human cancer cell lines and murine Ehrlich ascetic carcinoma (EAC) bearing mice were used. The phytochemical analysis showed that the total phenolics, flavonoids, saponin and anthocyanin contents were 1479, 219, 11600 µg/ml and 212 µg/g, respectively. Total antioxidant capacity, DPPH scavenging percentages were 98 µg/ml, and 84 %, respectively. *In vitro* inhibition concentration (IC_{50}) of PDSE against breast (MCF-7) and hepatic (HepG-2) cancer cell lines were 25.2 and 40.5 µg/ml, respectively. Treatment with PDSE (100 mg/Kg) decreased the total volume and counts of tumor. Biochemically, PDSE treatment ameliorated the hematological, hepatic, renal and oxidative stress post tumor challenge. Collectively, PDSE have promising natural cancer-fighting phytochemicals.

1. Introduction

Cancer is a serious disease. Several traditional and new approaches have been applied for cancer treatment including chemotherapy, radiotherapy, immunotherapy, and gene therapy. Even though, chemotherapy is the best choice to treat cancer, its side effects still one of the most restricted reasons to use it as chemotherapy (Nurgali *et al.*, 2018; Al-Mahayri *et al.*, 2020). Several side effects have been reported after chemotherapy because of increasing the free radical and oxidative stress agents (Huang *et al.*, 2016), cardiotoxicity (Florescu *et al.*, 2013), renal and hepatic failure (El-Nagger *et al.*, 2015) represent the most side effects of chemotherapy. Efforts to find new therapeutic agents from natural products as anticancer agents have been reported (Iqbal *et al.*, 2017). Different plants have been used to ameliorate the severity of side effects of chemotherapy because of its content of several phytochemicals constitute (Scheck *et al.*, 2006; El-Naggar *et al.*, 2018).

Date *(Phonix dactylifera)* is one of the most important fruit in many countries all over the world (Al-Harrasi *et al.*, 2014). *P. dactylifera* by-products (seeds) is a waste that approximately 10-15 % of dates weight, which used for animal feed and in making of non-caffeinated coffee (Al-Farsi *et al.*, 2007). *P. dactylifera* seeds have potential bioactive materials, for instance, phenolic, flavonoid and fiber (Hamada *et al.*, 2002; Habib and Ibrahim, 2011b). *P. dactylifera* seeds have been used in traditional medicine for management of chronic disease such as diabetes and liver diseases (Duke, 1992). *P. dactylifera* seeds contain high content of minerals (Al-Shahib and Marshall, 2003). Bouhlali et al. (2015) stated that *P. dactylifera* seeds contain high content of phenolic and flavonoid contents. Few studies have reported the anticancer efficacy of *P. dactylifera* seeds. Therefore, the current study was conducted to assess the phytochemicals constitute and the efficacy of *P. dactylifera* seeds extract (PDSE) *in vitro* on different human cancer cell lines and *in vivo* on tumor experimental mouse model.

2. Materials and Methods

2.1. Chemicals

Cisplatin (Cis-diamminedichloroplatinum II) was obtained from Sigma-Aldrich (St Quentin Fallavier, France). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were determined by kits that purchased from Bio-diagnostic Company, Egypt.

2.2. Preparation of plant seeds extract

P. dactylifera fruits were obtained from a market in Tanta city, Egypt. The seeds were isolated, dried in shade and crushed to powder. 50 g of seeds powder was mixed vigorously with 500 ml 70% (V/V) ethanol for preparing hydro-ethanolic extract. Prepared extract was filtered then the solvent was air-dried, and the extract was weighed and suspended in 0.9% sterile saline for further processing.

2.3. Phytochemicals analysis

Total phenolic content of PDSE was evaluated and expressed as (mg) of gallic acid equivalents (GAE) per (g) of extract using GAE calibration curve using the Folin-Ciocalteau reagent according to the methods of Singleton et al. (1999). Total flavonoid content was determined according to the methods of Zhishen et al. (1999), using the aluminum chloride colorimetric method expressed as (mg) quercetin equivalent per gram of extract from a calibration curve of quercetin. Method that described by Prieto et al. (1999) was used to determine the total antioxidant capacities (TAC) that expressed as ascorbic acid equivalent. DPPH free radical scavenging capacity was evaluated spectrophotometrically as described by to Blois et al. (1999). The scavenging activity on the DPPH radical was expressed as inhibition percentage that equal [(AC –AS)/(AC)] ×100. Saponin and anthocyanin contents of PDSE were determined according to the methods of Ebrahimzadeh and Niknam (1998) and Padmavati et al. (1997), respectively.

2.4. Cell lines culture and MTT assay

The human breast cancer (MCF-7) and hepatocellular carcinoma (HepG-2) cell lines were obtained from VACSERA Tissue Culture Unit (Cairo, Egypt). The cells were cultured in DMEM medium (GIBCO, New York, USA) supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin/streptomycin and 2% L-glutamine at 37 °C for under 5% CO_2, 95% air. The seeds extract was diluted with DMEM to different concentrations (from 5 to 500 µg/ml) and applied to the MCF-7 and HepG2 cells in triplicate, then, 10 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-terazolium bromide (MTT) was added and incubated at 37 °C for 4 h. The purple formazan crystal formed was dissolved by using dimethyl sulphoxide. Cisplatin (Cis) was used as a positive standard. The absorbance was read at 570 nm using ELIZA reader. The concentration of the extracts that inhibit 50 % of cells (IC_{50}) was calculated from the sigmoidal curve.

2.5. Mice and Ehrlich ascites carcinoma (EAC) tumor cells inoculation

Female Swiss albino mice (20 ± 2 g) were obtained from National Research Center (NRC, Cairo, Egypt). Animals were housed in cages (5/cage), in 12 h/12 h dark/light cycle under laboratory condition of temperature and humidity. Mice were kept for a week before starting the experiment for acclimatization. Handling of mice according to the ethical guidelines approved by the animal care and use committee, Faculty of Science, Tanta University (ACUC-SCI-TU), Egypt. The EAC cells were collected from the tumor bearing mice purchased from the National Cancer Institute (NCI, Cairo, Egypt). The viable and dead cells were counted using trypan blue method, tumor cells were adjusted at 2×106 cells/mouse for intraperitoneal (i.p) inoculation.

2.6. Experimental design

Forty (40) female Swiss albino mice were divided randomly into five groups (n = 10/group). The first group (Gp1) was used as a negative control. From the 2^{nd} to the 4^{th} groups of mice were inoculated i.p with 2×10^6 EAC cells/mouse. After one day of tumor cells inoculation, the 2^{nd}, 3^{rd}, and $4^{th,}$ groups of mice were injected i.p daily for 6 consecutive days with 200 µl of PBS, Cis (2 mg/kg), PDSE (100 mg/kg). At day 14, all groups of mice were weighted at the beginning (initial b.wt) and at the end of the experiment (final b.wt). The percentage of the change in the total body weight (T.B.W %) was calculated as follows: (final b.wt – initial b.wt / initial b.wt) × 100. All mice were sacrificed and the total tumor volume, count, live, and dead tumor cells were detected.

2.8. Statistical analysis

Data were presented as mean ± SD and were analyzed using one–way analysis of variance (ANOVA) followed by Dunnet test and $p < 0.05$ or $p < 0.01$ were statistically significant.

3. Results

3.1. Phytochemical analysis of PDSE

The phytochemical analysis revealed that the total phenolic and saponin contents were 1479 µg/ml and 11600 µg/g, respectively. Further investigation showed that the contents of the total flavonoids and anthocyanin were 219 µg/ml and 11600 µg/g, respectively. While the total antioxidant capacity (TAC), DPPH scavenging percentage and the median inhibition concentration (IC_{50}) were 98 µg/ml, 84 % and 59 µg/ml, respectively (Table 1).

Table 1. Phytochemical analysis of PDSE

Phytochemical analysis	PDSE
Total phenolic (µg/ml)	1479
Total flavonoids (µg/ml)	219
TAC (µg/ml)	98
DPPH scavenging%	84 %
IC_{50} of DPPH (mg/ml)	59
Saponin (µg/g)	11600
Anthocyanin (µg/ml)	212

3.2. *In vitro* antitumor activity of PDSE

Breast cancer (MCF-7) and hepatic caner (HepG-2) cell lines were used to evaluate the *in vitro* anticancer activity of PDSE. The results showed that the in vitro IC_{50} of PDSE against MCF-7 and HepG-2 were 25.2 and 40.5 µg/ml, respectively (Figure 1).

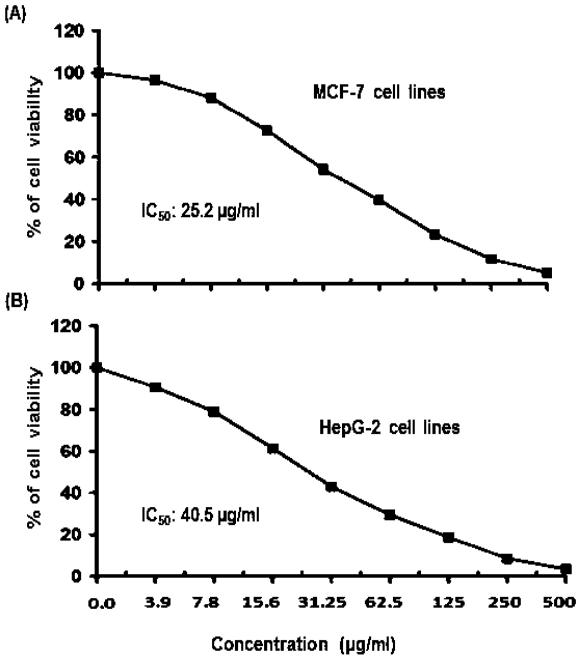

Figure 1. *In vitro* median inhibition concentrations (IC_{50}) of PDSE on breast cancer cell line (MCF-7) (A) and hepatic cancer cell line (HepG-2) (B) after 24 h of treatment.

3.3. *In vivo* antitumor activity of PDSE

The study extended further to address the impact of the efficacy of PDSE in EAC-bearing mice in vivo. The results showed that the administration of PDSE (100 mg/Kg) for 6 consecutive days decreased the total tumor volume when compared to their EAC-bearing mice alone (Table 2). The results also showed that the number of live tumor cells was decreased concomitant with an increase in the number of dead EAC-cells in groups of mice which had treated with cisplatin alone or PDSE (Gp3 and 4) when compared to their numbers in EAC-bearing mice alone (Table 2). The total body weight in the groups of mice (Gp3 and 4) was not increased significantly after 14 days of the tumor inoculation. However, the total body weight increased significantly in the group of mice which only had inoculated with EAC-cells alone (Gp2) (Figure 2).

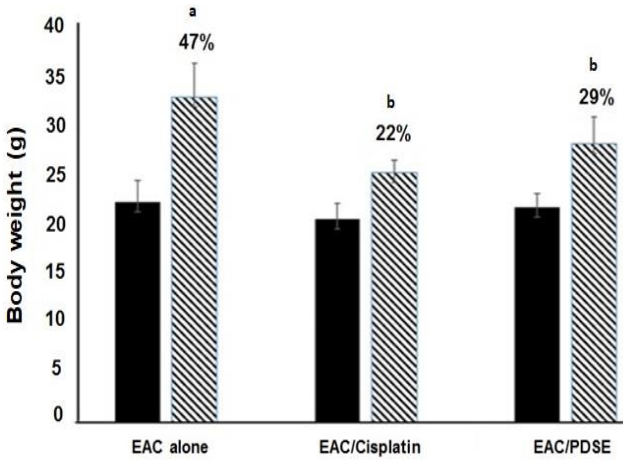

Figure 2. The initial, final and changes of body weights of mice in different groups post 14 days of EAC-inoculations and treatments.

Table 2. The tumor volume, tumor cells count, live and dead cancer cells in different group mice under the study

Groups	Total tumor Volume (ml)		Total cells count ($\times 10^6$) /mouse		Total live cells count ($\times 10^6$) /mouse		Total dead cells count ($\times 10^6$)/mouse	
	M ± SD	r%	M ± SD	r%	M ± SD	r%	M ± SD	r%
Gp2	$11^a \pm 1.53$	-	$653^a \pm 19$	-	$623^a \pm 36$	-	$30^a \pm 1.10$	-
Gp3	$1.0^b \pm 0.87$	90%	$120^b \pm 15$	81%	$20^b \pm 3.4$	96%	$100^b \pm 9.23$	- 1.3%
Gp4	$3.5^c \pm 1.7$	68%	$190^c \pm 16$	71%	$120^c \pm 10.3$	81%	$70^c \pm 9.37$	- 2.3%

Gp2: EAC alone, Gp3: Cisplatin (2 mg/kg/6); Gp4: *P. dactylifera* seeds extract, r%: the percentage of reduction, T.V.: total tumor volume, T.C.C.: total tumor cell count, L.C.: live cells, D.C.: dead cells.

4. Discussion

Cancer is spreading worldwide and expected to increase due to the increase of pollution sources and changes in the human lifestyle. Radiation, chemotherapy and surgery are the conventional protocols for cancer treatments. Treatment with chemotherapy led to the induction of apoptosis of tumor cells however, it has adverse effects on normal healthy cells (El-Naggar *et al.,* 2015, 2016). Natural compounds in some medicinal plants have a potent antioxidant, anticancer and could ameliorate the side effects of chemotherapy (El-Naggar *et al.,* 2018; Choi, 2019). Previous study showed that *P. dactylifera* fruit has bioactive compounds with antioxidant and anti-tumor effects (Rahmani *et al.,* 2014). Interestingly, *P. dactylifera* seed which is a waste by-product of the date fruit that associated with favorable nutritional properties because of high content of phenolic compounds (Habib and Ibrahim, 2011). Therefore, the current study, was performed to detect the phytochemicals constitute of PDSE by quantitatively methods and GC-MS analysis. Furthermore, this study was extended to assess the anticancer efficacy of PDSE *in vitro* using MCF-7, HepG-2 cell lines and *in vivo* by EAC-bearing mice model, respectively. Secondary metabolites of metabolites such as phenols, flavonoids were characterized by GC-MS analysis (Lewis and Ausubel, 2006). Our results revealed that PDSE contains several bioactive compounds including octasiloxane, linoleate, palmitate and pregnane. Such these compounds could have potential effects as antibacterial, antioxidant and anticancer agents (Tungmunnithum *et al.,* 2018).

The total antioxidant capacity (TAC) and DPPH scavenging activity was found to be related to the total phenolic and flavonoid contents (Maqsood *et al.,* 2015). Based on our findings, PDSE was found to be rich with some phytochemicals constitute which could be potential candidates as anticancer agents. This finding was in agreement with previous study by Bouhlali et al. (2015).

In vitro and *in vivo* antitumor efficacy of PDSE showed potent antitumor activity against MCF-7 and HepG-2 cell lines and against EAC-bearing mice. The potential antitumor effect of PDSE could be due to their phytochemicals constitute and the presence of bioactive compounds. Our finding was in agreement with previous study by Platat et al. (2015) who mentioned that PDSE has protective effect against oxidative damage in rats through their antioxidant potential.

Presence of bioactive molecules such as phenolic contents (gallic, syringic and catechin), saponine and flavonoids in PDSE could have potent antioxidant and anticancer properties (Devi *et al.,* 2015; Wani and Kumar, 2018; Al Juhaimi *et al.,* 2018). Treatment of tumor bearing mice with PDSE improved the levels of liver enzymes (ALT and AST) as well as kidney function parameters (urea and creatinine) when compared with tumor bearing mice treated with Cis alone. These findings were in line with Al-Meqbaali et al. (2017) who confirmed that treatment with PDSE reduced the elevation of liver enzymes in date seed powder treated rats. Interestingly, the antioxidant enzymes (SOD and CAT) were increased in PDSE treated groups. On the other hand, the level of lipid peroxidation end product (MDA) was decreased as compared to Cis-treated group of mice. These effects could be due to the presence of many bioactive components which act as antioxidants able to scavenge free radicals resulted from the growing of tumor cells in mice (Al-Meqbaali *et al.,* 2017). In conclusion, the extract of *P. dactylifera* seeds showed potential anticancer activity *in vitro* and *in vivo* due to the presence of high contents of potent bioactive compounds.

Conflict of interest

All authors declare that they have no conflict of interest.

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Author contributions
SR conceptualized the study, performed the experiments, analysis date and wrote the draft. The author read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations
Ethics approval and consent to participate All experimental procedures were conducted in accordance with the ethical standards and were approved by the Institutional Animal Care and Use Committee (IACUC) at National Organization for Drug Control and Research (NODCAR) (approval no. NODCAR/III/41/2019).

Consent for publication
Not applicable.

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