International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2020; 8(5): 23-25
Received: 18-06-2020
Accepted: 11-07-2020

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Screening of *in vitro* cytotoxic activity of *Ephedra alata* used traditionally to treat cancer in Libya

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Abstract
Ephedra is one of the Libyan medicinal herbs that are traditionally involved in folk medicine to treatseveral diseases. *Ephedra* is known to have antioxidant and antibacterial properties. The goal of this study is to evaluate the cytotoxic activity of methanolic extract of *Ephedra alata* plant growing wild in the east of Libya against two human cancer cell lines HEPG2human liver cancer cell line and PC3human prostate cancer cell line. The results show that the plantmethanolic extract has a significant cytotoxic effect on both cell lines compared with doxorubicin as a positive control.

Keywords: *Ephedra alata,* human liver cancer cell line, human prostate cancer cell line and methanolic extract

1. Introduction
Research projects regarding cancer have evolved in the past decades since cancer is now the most common cause of death worldwide, making it of great concern among the world.
American Cancer Society has released global cancer statistics that by 2050 there is a prediction of 27 million new cancer cases and 17.5 million cancer deaths [1]. Various approaches have been developed to minimize the effect of cancer on the human body. In modern cancer treatment approaches, chemotherapy has become the leading acknowledged choice of therapy, along with other clinically available anti-cancer drugs, of synthetic or natural product origin, that are currently being utilized for the treatment of some types of cancer [2, 3].
In the Arab society, including the Libyan society, traditional herbal medicine has always been a way to cure a common minor illness such as a cough, constipation, diarrhea, stomach cramps, rheumatism, etc. Therefore, it has recently become a topic of interest in experimental fields and research. Quite a lot of studies have been conducted on herbs for their anti-cancer properties. For example, Hartwell has collected several data, approximately3, 000 plants, possessing anti-cancer properties, and later on used as potent cytotoxic drugs [4-6]. The secondary metabolites and their semi-synthetic derivatives obtained from the plants play an important role in anti-cancer drug therapy [7, 8]. *Ephedra* is one of these medicinal plants, belonging to the *Ephedra*ceae family. Different species of *Ephedra* are distributed around the world; among these are *Ephedra Lristanica*, *Ephedra strobiliacea*, *Ephedra sarcocarpa*, *Ephedra procera*, *Ephedra pachyclada* and, *Ephedra alata*, [9]. The herb grows abundantly in Libya, and used in traditional remedies for the treatment of chills, bronchial asthma, allergies, colds, edema, fever, coughs, flu, and headaches. This plant also shows anti-cancer properties [10-12]. Sympathomimetic alkaloids derived from phenylalanine such as ephedrine, pseudoephedrine, and other related compounds are the main components of *Ephedra alata* [13]. Several researches have proved that the aerial parts of this plant contain (−) ephedrineasthe main isomer (between 30% and 90%) [14]. Additionally, *Ephedra* is considered a source of phenolic compounds such as trans-cinnamic acid, catechin, syringin, epicatechin, symplocoside, kaempferol 3-O-rhamnoside 7-O-glucoside and isovitexin 2-O-rhamnoside, which all contribute significantly to the antioxidant activity of the plant and play important roles in disease resistance [15-17]. Phenolic compounds are also believed to display an essential role as health-protecting factors. Scientific evidence indicates that consumingdiets, which are rich in antioxidant compounds, will lower the risk of chronic diseases, including heart malfunction and cancer [18].
A universal property of tumor cells is the uncontrolled proliferation. Investigation of the cellular growth control mechanism has contributed to the understanding of carcinogenesis and the identification of compounds with specific anti-tumoral activity [19, 20].
Therefore, this study was designed to further insight and prove medical traditional uses of *Ephedra Alata* for anti-cancer remedies.

2. Material and methods

2.1 Plant material

The aerial parts of the *Ephedra Alata* were collected from Botraba area (about 100 km east of Benghazi, Libya) during August. Samples of the plant were sent to the Department of Pharmacognosy (Faculty of Pharmacy, University of Benghazi) for identification. The aerial parts were left to dry in open air. The dried plant materials were milled into a fine powder to be used for extraction.

2.2 Extraction preparation

The dried plant powder was extracted by cold maceration in 70% methanol. The solvent was then evaporated at a temperature of40°C under reduced pressure. The residuals were stored for further biological or chemical examination.

2.3 Cytotoxic effect assessment

Cell viability was evaluated according to (Mosmann, 1983) through the mitochondrial-dependent reduction of the color of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) from yellow to purple [21].

The entire procedure was carried out in a sterile place using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, and Sanford, ME, USA). The cells were suspended in DMEM medium, 1% antibiotic-antimycotic mixture (10,000µg/ml Streptomycin Sulfate, 10,000U/ml Potassium Penicillin, and 25µg/ml Amphotericin B) and 1% L-glutamine at a temperature of 37ºC under 5% carbon dioxide.

The cells were batch cultured for ten days, then seeded in fresh complete growth medium in 96-well microtiter plastic plates at a concentration of $10x10^3$ cells/well at 37ºC for one day under 5% CO2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). The media was aspirated, fresh medium free of serum was added, the cells were incubated with different concentrations of the sample (100, 50, 25, 12.5, 6.25, 3.125, and 1.56ug/ml).Cells were incubated alone as a negative control. After two days of incubation, the medium was aspirated, 40ul MTT salt (2.5µg/ml) was added to each well and incubated for additional four hours at temperature of 37ºC under 5% carbon dioxide. 200µL of 10% Sodium dodecyl sulphatein deionized water was added to each well and incubated at 37ºC overnight to eliminate the reaction and dissolve of the formed crystals. A positive control composed of 100µg/mldoxorubicin was used as a standard cytotoxic natural agent that exerts 100% lethality under the same experimental conditions. The absorbance was measured at 595nm and a reference wavelength of 620nm by a micro plate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) [22-24].

SPSS 11 program was used to run independent t-test to evaluate the statistical significance between the negative control (cells with vehicle) and samples. DMSO (dimethyl sulfoxide) was used for the dissolution of the plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated using the following formula:

((Reading of extract/ Reading of negative control) -1) x 100

3. Results and Discussion

The following table shows the cytotoxic activity of the plant extract against HepG2human liver cancer cell line and PC3 human prostate cancer cell line.

Table 1: Cytotoxic Activity of the methanolic extract of the *Ephedra alata* (IC_{50} µg/ml)*.

Drug name	HepG2 IC_{50}	PC3 IC_{50}
Ephedra alata	32.9	30.4
Doxorubicin	21.6	23.8

*Significance different from standard (Doxorubicin) $P<0.05$ n=3

Cytotoxic effects of the extract of *Ephedra alata* was expressed as IC_{50} values. According to the table, there was significant cytotoxicity on HepG2 and PC3 with IC_{50}; 32.9 and 30.4, respectively.

It is of interest that the extract of the plant showed cytotoxicity against the cancer cell lines, and if there would to be corresponding results *in vivo*, then traditional healers would have some sort of scientific support for treating cancer patients.

Pathological examination of *E. pachyclada*, a different species from the same plant, showed that hepatic injuries, such as inflammation, necrosis and hepatitis, are partially healed with the plant extracts due to its hepato-protective mechanism to inhibit oxidative stress and inflammation [25].

The extracts of *Ephedra* significantly alleviate hepatocyte apoptosis and inflammatory factor infiltration by lowering of serum alanine aminotransferase and total bilirubin (T. Bil) activity, it also reduces the levels of TNF-α and the activities of caspase-3, 8, and 9 [26, 27].

A study has reported that there are anti-proliferative and analgesic effects in herbacetin, the aglycon of herbacetin 7-O-neohesperidoside isolated from *Ephedra* plants [28].

4. Conclusion

Several plant species rich in flavonoids are reported to have disease preventive and therapeutic properties. This observation is of particular significance since flavonoids are considered ingredients found in many fruits and vegetables, explaining the association of fruit and vegetable consumption with reduced cancer risk that have been reported in previous studies [29-31]. Cytotoxic activity recorded in this study collate with these findings since the phytochemical evaluation indicated the presence of flavonoids in the plant [16].From the result, it is clear that the extract has potent *in vitro* cytotoxic activity against both cell lines. Further studies are also in a process to evaluate the most potent fraction of the active plant.

Furthermore, other parts of the *Ephedra* plant are also important to be further examined for their antimicrobial, antioxidant and cytotoxic effect.

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