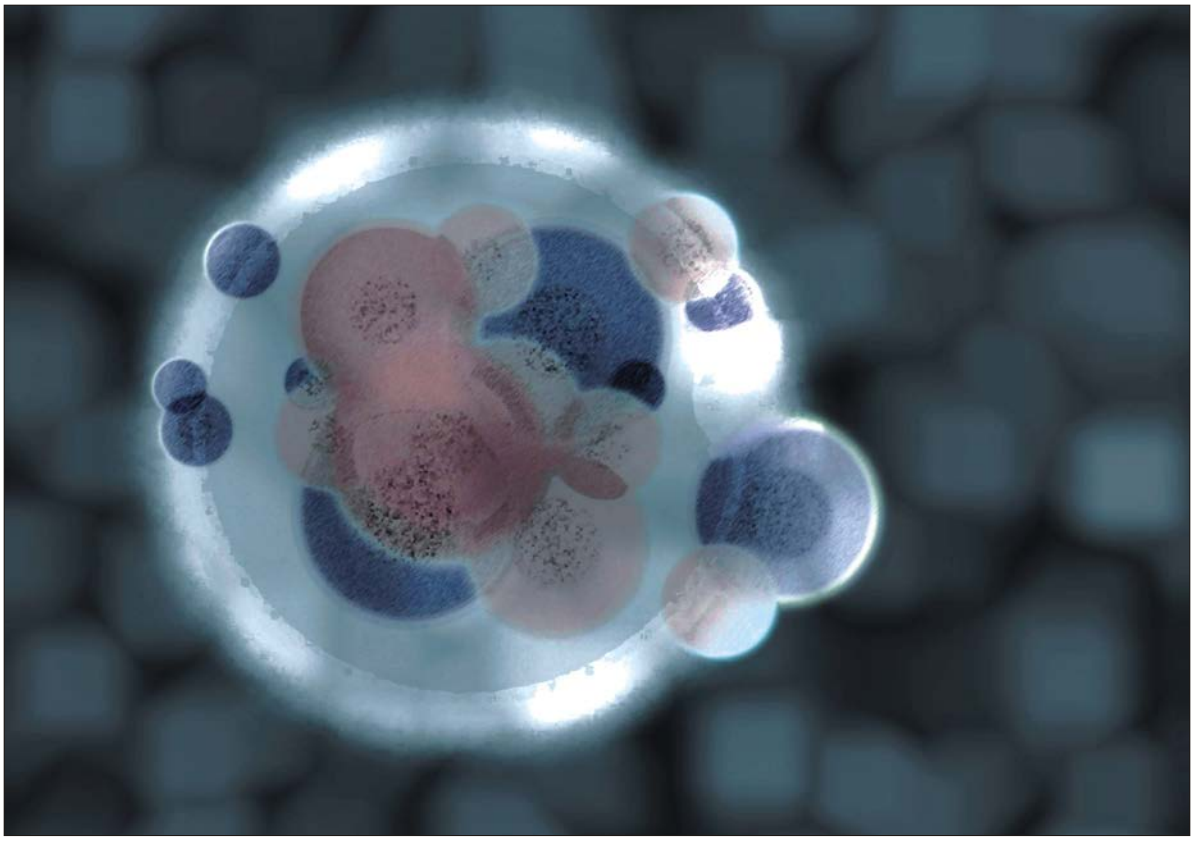

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2016.242.246

Research Article
Cytotoxic Effects of Aqueous Extracts of *Urtica urens* on Most Common Cancer Types in Saudi Arabia

Huda A. Al Doghaither, Ulfat M. Omar, Sawsan A. Rahimulddin and Ayat B. Al-Ghafari

Department of Biochemistry, Faculty of Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

Abstract

Background and Objective: The use of traditional medicinal plants in the treatment of cancer has increased worldwide. The aim of the study is to illustrate the cytotoxic activity of aqueous extracts of dried and freeze-dried *Urtica urens* on MCF-7, HCT-116 and A549 cancer cell lines. **Materials and Methods:** The MTT assay was used to assess the cell viability of three cancer cell lines in comparison to normal human HSF cells. **Results:** Aqueous extracts of both dried and freeze-dried *Urtica urens* leaves, either at 5 or 10 μg mL^{-1}, have significantly reduced the cell viability of all cancer cell lines (MCF-7, HCT-116 and A549) with no harmful effect on normal HSF cells. **Conclusion:** Aqueous extracts of *Urtica urens* leaves, under certain concentrations and preparation conditions have significant cytotoxic effect on different types of cancer cell lines and thus, might be a promising natural anticancer agent.

Key words: *Urtica urens*, cytotoxicity, cancer cell lines, MTT, MCF-7, HCT-116, A549, freeze-dried, aqueous extracts

Received: August 26, 2016 Accepted: September 05, 2016 Published: September 15, 2016

Citation: Huda A. Al Doghaither, Ulfat M. Omar, Sawsan A. Rahimulddin and Ayat B. Al-Ghafari, 2016. Cytotoxic effects of aqueous extracts of *Urtica urens* on most common cancer types in Saudi Arabia. J. Biol. Sci., 16: 242-246.

Corresponding Author: Huda A. Al Doghaither, Department of Biochemistry, Faculty of Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cancer is a major public health problem worldwide. In 2008, it has accounted for 7.6 million deaths (around 13% of all deaths). Cancer-related deaths[1] are predicted to increase to over than 11 million in 2030. Lung cancer, along with colorectal (CRC) and breast cancers are the most prevalent cancers in the world[2]. Lung cancer has become one of the most leading cause of cancer-related death worldwide and within the kingdom of Saudi Arabia, due to the high incidence, rapid progression and poor prognosis[3,4]. The Saudi Cancer Registry (SCR) at King Faisal Specialist Hospital and Research Centre (KFSHRC) in Riyadh, reported that colorectal cancer was the second most common malignancy among Saudis for all ages (10.3%) and the number one malignancy in males (11.8%)[5]. On the other hand, breast cancer is the most common cancer among Saudi females and the number of breast cancer cases has increased considerably, especially among the younger age females[5]. Now a days, chemotherapy is one of the most effective treatment options available to treat different kinds of cancer including lung, colon and breast cancers. However, serious side effects have been reported for some anti-tumor chemotherapeutics. Therefore, exploration of an alternative treatment is becoming a purpose for many researchers[3].

Natural remedies and medicinal plants capable of curing a wide range of illnesses have been used throughout the ages. Plant-derived compounds have been an important source of several clinically useful anti-cancer agents[6]. *Urtica urens*, commonly known as small nettle, is a semi-woody plant belonging to genus *Urtica* that grows annually. Traditionally, people have been using this herb as the treatment for fever and skin inflammation[7]. Main constituents identified in the plant material are flavonoids, caffeoyl-esters, caffeic acid, scopoletin (cumarin), sitosterol (-3-O-glucoside), polysaccharides, fatty acids and minerals[8]. Due to the presence of flavonoids and other compounds with antioxidant properties, it is assumed that *Urtica urens* can be effective in reducing the growth of cancer cells.

Despite the wide spread use of *Urtica urens* in traditional plant medicines, there are no pharmacological data to support this, therefore the aim of the current study is to evaluate the cytotoxic effects of *Urtica urens* aqueous extract on different cancer cell lines. To the best of our knowledge, this is the first study investigating the cytotoxic activity of the *Urtica urens* water extract on cancer cell lines.

MATERIALS AND METHODS

Materials: High glucose Dulbecco's Modified Eagle's Medium powder (DMEM), foetal bovine serum (FBS) were purchased from Hyclone, USA. Trypsin/EDTA 0.25% (ethylene diamine tetraacetic acid) (1X), N-(2-hydroxyethyl) piperazine-N-(2-ethanesulfonic acid) (HEPES) buffer (1M), Antibiotic (penicillin/streptomycin) and 100X non-essential amino acids (NEAA) were purchased from Gibco, Canada. Phosphate buffered saline (PBS) tablets were purchased from Oxoid, Hampshire, UK. The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich chemical Co, Poole, UK.

Plant material and extract preparation: *Urtica urens* leaves were obtained from a local market in Jeddah, Kingdom of Saudi Arabia. The leaves were washed and dried in the shade and coarsely pulverized. For the preparation of freeze-dried leaves, 50 g of the powdered leaves were macerated in boiling water (1.5 L) for 30 min at room temperature, with occasional shaking. The extract was filtered and the filtrate was freeze-dried and stored at -20°C until use. For the preparation of the aqueous extract of dried leaves of *Urtica urens*, 2 g of the dried leaves were macerated in 200 mL boiling water for 30 min at room temperature, with occasional shaking. The extract was filtered and the required concentrations were prepared directly before use. In the current study, two different concentrations of both aqueous extract preparations were used, 5 and 10 μg mL^{-1}.

Cell lines: Four human cell lines were used in this study, colon cancer (HCT-116), lung cancer (A549), breast adenocarcinoma (MCF-7) and normal skin fibroblasts cells (HSF). Breast MCF-7 and colon HCT-116 cell lines were obtained from Alzamil chair for cancer research in King Fahad Medical Research Center (KFMRC) at King Abdulaziz University (KAU), whereas lung A549 cell line was obtained from the immunology laboratory in King Fahad Medical Research Center (KFMRC) at King Abdulaziz University (KAU). The normal human skin fibroblasts cells (HSF) were obtained from foreskin circumcision operations, at King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia (KSA).

Determination of cell viability using MTT assay: The four cell lines were cultured under the same culture conditions. The high glucose DMEM was supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin/streptomycin and 1% non-essential amino acids. Cells were sub-cultured until

they become 70-90% confluent. Then, the growth medium was removed and treatments with two different concentrations of *Urtica urens* (5 and 10 μg mL^{-1}) were applied, to detect their cytotoxicity on these four cell lines.

For MTT assay, three independent experiments were performed. For the four cell lines, cells were seeded in a 96 well tissue culture plate at a concentration of 1×10^4 cells $well^{-1}$. After 24 h incubation, the cells were treated with two concentrations of dried and freeze-dried *Urtica urens* preparations (5 or 10 μg mL^{-1}) and incubated overnight in a 37°C/5% CO_2 incubator. Then, 20 μL of MTT dye was added and cells were incubated for 2 h at 37°C. The precipitates were dissolved with 200 μL of isopropyl and the color intensity was measured at 490 nm using a BioTek microplate reader.

Statistical analysis: The data from MTT assay were analyzed using Graph Pad Prism software version 6. The significant differences were evaluated using p-values of one-way analysis of variance (ANOVA) followed by Bonferroni's test correction. Significance was set at a cut-off level of $p\leq0.05$.

RESULTS

In order to measure the cytotoxic effect of two concentrations of aqueous extracts of dried and freeze-dried *Urtica urens* on HCT-116, MCF-7 and A549 cancer cell lines, cell viability was assessed by MTT assay. The cell viability was also assessed in a normal HSF cells to determine the selective cytotoxicity of *Urtica urens* on cancer cells. The results of the current study showed that exposing cancer cells for 24 h to the dried preparations of *Urtica urens*, either at 5 or 10 μg mL^{-1} had significant ($p\leq0.0001$) cytotoxic effects on the three cancer cell lines (HCT-116, MCF-7 and A549) with no harmful effect on normal HSF cells (Fig. 1a, b). At a concentration of 5 μg mL^{-1}, the cell viability percentage of MCF-7, HCT-116 and A549 was 79, 82 and 93%, respectively. On the other hand, at a concentration of 10 μg mL^{-1}, the cell viability percentage of MCF-7, HCT-116 and A549 was 72, 71 and 72%, respectively.

The same doses were also prepared by freeze-dry technique using 5 and 10 μg mL^{-1} aqueous extracts of freeze-dried leaves of *Uritca urens*. The effect of freeze-dried preparations of *Urtica urens* was slightly different on the cell viability of cancer cell lines compared to dried preparations. For breast MCF-7 and colon HCT-116 cells, the cell viability percentage with both concentrations (5 and 10 μg mL^{-1}) showed the same significant reduction as with the dried preparation ($p\leq0.0001$). Although, lung A549 cells showed a significant decrease in the cell viability, this reduction percentage indicated that A549 might has a slight resistance to *Urtica urens* in the freeze-dried preparation when compared to the dried preparation ($p\leq0.01$) (Fig. 1c, d). In contrast to cancer cells, the *Urtica urens* did not show any harmful effect on cell proliferation of HSF cells. At 5 μg mL^{-1}, the cell viability percentage of MCF-7, HCT-116 and A549 was 79, 81 and 93%, respectively, whereas at 10 μg mL^{-1}, the cell viability percentage of MCF-7, HCT-116 and A549 was 64, 60 and 74%, respectively, after 24 h of exposure to the aqueous extracts of the freeze-dried leaves of *Urtica urens*.

DISCUSSION

The use of herbal medicine among cancer patients has increased dramatically over the past 30 years. It is used to either treat cancer and/or to reduce the toxicity induced by chemotherapeutic drugs[9]. The purpose of this study is to illustrate the cytotoxic effects of different concentrations of aqueous extract of both dried and freeze-dried leaves of *Urtica urens* on different cancer cell lines. Cell cytotoxicity refers to the ability of cytotoxic agent to destroy living cells. By using a cytotoxic agent, normal cells can either be induced to undergo necrosis or apoptosis. It is well known that measuring cell cytotoxicity is quite indispensable in the process of developing therapeutic anti-cancer drugs. To the best of our knowledge, there is no documented report elucidating the effects of *Urtica urens* on cancer cell lines. The results of the current study revealed that the use of both dried and freeze-dried aqueous extract preparations showed a highly significant reduction in the cell viability of the three cancer cell lines without any effect on normal skin fibroblast (HSF) cells. For the dried leaves preparation, the results showed that both concentrations of the aqueous extracts have reduced the cell viability of cancer cells. For the freeze-dried preparation, at a concentration of 5 μg mL^{-1}, there was a slight resistance of lung A549 cells to *Urtica urens* when compared to the dried preparation. It has been reported previously that the leaves of the plant contain flavonoids and probably other substances, which might be affected by freeze-drying at certain concentrations. Various studies showed that freeze-drying has effects on the antioxidant activity. Shofian *et al.*[10] showed that some freeze-dried fruits have significantly lower ferric reducing antioxidant power value than fresh fruits while, in some other fruits the ferric reducing antioxidant power values of freeze-dried form remained unchanged. Interestingly, at the high concentration of the freeze-dried leaves, the cell viability was reduced significantly in the three types of cancer cells without resistance in lung

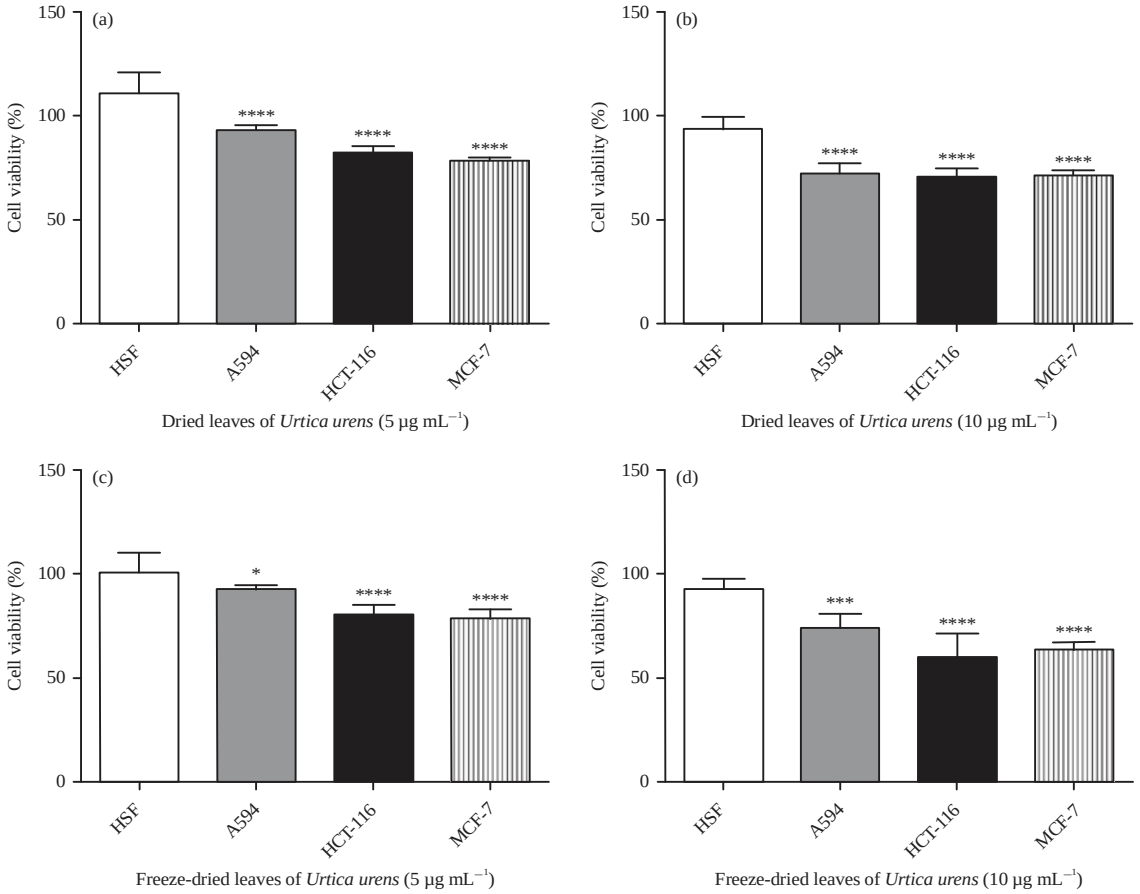

Fig. 1(a-d): Determination of the cytotoxic effects of *Urtica urens* on cancer cells. The MTT assay was performed on three cancer cell lines (lung A549, colon HCT-116 and breast MCF7). The data represent the mean of three independent experiments (n = 3 ±SEM). Comparisons of means were made using one-way ANOVA followed by Bonferroni's test, *$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$, (a) Cells treated with 5 µg mL^{-1} of dried aqueous extracts of *Urtica urens* leaves compared to normal HSF cells, (b) Cells treated with 10 µg mL^{-1} of dried aqueous extracts of *Urtica urens*, (c) Cells treated with 5 µg mL^{-1} of freeze-dried aqueous extracts of *Urtica urens* and (d) Cells treated with 10 µg mL^{-1} of freeze-dried aqueous extracts of *Urtica urens*. The results showed that both preparations of *Urtica urens* at both concentrations have cytotoxic effects on the three cancer cell lines. Interestingly, in freeze-dried preparation at 5 µg mL^{-1}, lung A549 cells were slightly resistant to the extract preparations compared to the other two cancer cells (colon HCT-116 and breast MCF-7) cells

cancer cell line. Although, the concentration was higher, the HSF cells were not affected by the treatment. The findings of the current study suggest that freeze-drying is more effective when high concentration of *Urtica urens* extract was used in the treatment of cancer cells. However, it is not known which component exactly was affected by freeze-drying at low concentrations.

Moreover, *Urtica dioica* (stinging nettle) is also a member of the Urticaceae family are considered therapeutically interchangeable. Numerous studied have reported the effect of *Urtica dioica* on the cytotoxic activity of cancer cell lines[11]. In agreement with these results, a previous study performed by Fattahi *et al.*[12] proved the cytotoxic activity of *Urtica dioica* on BT-474 and Hela cell lines. Another study also reported the cytotoxic effect of *Urtica dioica* on human prostate carcinoma LNCaP cells[11]. Also, it was demonstrated that aqueous extracts of *Urtica dioica* leaves can decrease MCF-7 cell line proliferation[13,14].

CONCLUSION

In conclusion, aqueous extracts of *Urtica urens* leaves have cytotoxic effects on cancer cell lines, under certain concentrations and preparation conditions and thus might be

a promising anticancer agent. Further researches are needed in order to elucidate the mechanism of action of this plant on cancer cells.

REFERENCES

1. WHO., 2010. World Health Statistics 2010. World Health Organization, Geneva, Switzerland, ISBN: 978-92-4-1563987, Pages: 177.
2. Labianca, R., G.D. Beretta, B. Kildani, L. Milesi and F. Merlin *et al*., 2010. Colon cancer. Crit. Rev. Oncol. Hematol., 74: 106-133.
3. Wei, Y., Y. Xu, X. Han, Y. Qi and L. Xu *et al*., 2013. Anti-cancer effects of dioscin on three kinds of human lung cancer cell lines through inducing DNA damage and activating mitochondrial signal pathway. Food Chem. Toxicol., 59: 118-128.
4. Ferlay, J., 2001. GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide. IARC Press, Lyon.
5. Al-Eid, H.S., S. Bazarbashi and A. Al-Zahrani, 2011. Cancer incidence report Saudi Arabia 2005. Saudi Cancer Registry, Saudi Arabia.
6. Cragg, G.M. and D.J. Newman, 2005. Plants as a source of anti-cancer agents. J. Ethnoharmacol., 100: 72-79.
7. Anonymous, 2007. Urtica dioica: Urtica urens (nettle). Altern. Med. Rev., 12: 280-284.
8. Doukkali, Z., K. Taghzouti, E.H. Bouidida, M. Nadjmouddine, Y. Cherrah and K. Alaoui, 2015. Evaluation of anxiolytic activity of methanolic extract of Urtica urens in a mice model. Behav. Brain Funct., Vol. 11. 10.1186/s12993-015-0063-y.
9. Yin, S.Y., W.C. Wei, F.Y. Jian and N.S. Yang, 2013. Therapeutic applications of herbal medicines for cancer patients. Evid. Based Complement. Altern. Med., Vol. 2013. 10.1155/2013/302426.
10. Shofian, N.M., A.A. Hamid, A. Osman, N. Saari, F. Anwar, M.S. Dek and M.R. Hairuddin, 2011. Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. Int. J. Mol. Sci., 12: 4678-4692.
11. Levy, A., D. Sivanesan, R. Murugan, J. Jornadal, Y. Quinonez, M. Jaffe and A. Rathinavelu, 2014. Urtica dioica induces cytotoxicity in human prostate carcinoma LNCaP cells: Involvement of oxidative stress, mitochondrial depolarization and apoptosis. Trop. J. Pharm. Res., 13: 711-717.
12. Fattahi, S., E. Zabihi, Z. Abedian, R. Pourbagher, A.M. Ardekani, A. Mostafazadeh and H. Akhavan-Niaki, 2014. Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in vitro* antiproliferative effect on hela and BT-474 Cell lines. Int. J. Mol. Cell. Med., 3: 102-107.
13. Fattahi, S., A.M. Ardekani, E. Zabihi, Z. Abedian, A. Mostafazadeh, R. Pourbagher and H. Akhavan-Niaki, 2013. Antioxidant and apoptotic effects of an aqueous extract of *Urtica dioica* on the MCF-7 human breast cancer cell line. Asian Pac. J. Cancer Prev., 14: 5317-5323.
14. Guler, E.R., 2013. Investigation of chemopreventif properties of *Urtica dioica* L., in MCF-7 and MDA 231 breast cancer cell lines. New J. Med., 30: 50-53.