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In Vitro Anti-Neoplastic Properties of *Berberis vulgaris* L. var. asperma

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Abstract: Traditional herbal plants have played a major role in healing and preventive medicine for hundreds of years. *Berberis vulgaris* L. var. asperma is an indigenous seedless barberry fruit variety in Iran which is commonly utilized as additive in food and has been documented to possess several pharmacological properties of medical importance. This preliminary study investigated the anticancer activity of the seedless fruit ethanolic extract by determining anti-proliferative effects on human colon (HT-29) and breast cancer (MCF-7) cells via PrestoBlue® assay. Surprisingly, significant cytotoxic effects were observed with IC50 values of 7.5 and 10.7 mg/mL, respectively. No cytotoxic effects were observed on normal human fibroblasts suggesting biocompatibility. These results infer the promising pharmacognostic application of the seedless *B. vulgaris* fruit as potential source of novel chemopreventive and chemotherapeutic drugs.

Key Words: *Berberis vulgaris;* barberry, cytotoxicity, anticancer

1. INTRODUCTION

Pharmacognosy has recently been the trend in the search for antimicrobial, anti-cancer and other homeopathic molecules driven to normalize abnormalities in human health and physiology. There is a rising popularity and convincing evidence suggesting medicinal plants as unlimited reservoirs of potential drugs yet to be discovered which involve structural and biochemical diversity in their secondary metabolic products which may be capable of chemoprotective and chemotherapeutic activities. For this reason, scientists have broadened efforts toward the practical assessment and evaluation of traditional medicines, further cascading to novel discoveries of other plant species with potential medical applications.

The history of seedless barberry (*Berberis vulgaris* L. var. asperma) has encompassed the Persian empire as an ancient medicinal plant. Its red berries are popular and staple food additive

throughout Iranian history and cuisine. The fruits, called *zereshk*, are known to possess homoeopathic remedy for a myriad of illnesses which is also well-documented from other parts of the plant (Arayne et al., 2007). Traditional medicine in Iran reports several properties such as antibacterial, antipyretic, antipruritic and antiarrhythmic activities from different parts of the plant the mechanisms of which are completely unknown (Fatehi et al., 2005). Potential anticancer properties were also accounted sans the fruit with current research reportedly ongoing (Arayne et al., 2007).

This study deals with the preliminary assessment of the chemopreventive (anti-cancer) potential of dried *B. vulgaris* fruit. There are no existing reports among literature to date describing the use of barberry fruit as a potential anti-cancer, making this a pioneer in this pharmacognostic field.

2. METHODOLOGY

2.1 Plant specimen

Dried *B. vulgaris* L var. asperma fruits (zereshk) previously harvested and fungi free from Mashhad, Khorasan Province, Iran, were obtained and carefully kept in a dry and tight container to preserve freshness. Freshly dried fruits were the best option since staple Iranian dishes normally consume the berries in such form. Taxonomic identification was certified by the Mashhad University of Medical Sciences.

2.2 Human cell lines

Colon cancer (HT-29), breast cancer (MCF-7) and human dermal neonatal fibroblast (HDFn) cells were provided by the Cell and Tissue Culture Facility of the Molecular Science Unit Laboratory of De La Salle University. HDFn is a primary culture previously purchased from Invitrogen (USA) while HT-29 and MCF-7 were obtained from the American Type Culture Collection (ATCC).

2.3 Preparation of barberry fruit extract

Extraction procedure described by Rezaei et al. (2011) was followed. Thirty grams of dried barberry were powdered using liquid nitrogen and dissolved in 500 mL of 20% analytical grade ethanol resulting to 60 mg dry wt/mL. The suspension was continually mixed using a magnetic stirrer for 6 hours at room temperature, followed by filtration and centrifugation at 9000 rpm for 30 min to remove residual debris. The supernatant was carefully transferred and vacuum air-pumped (Rotavap) at room temperature until residual ethanol was completely removed. The resulting mixture was then passed through a 0.45 µm cellulose acetate membrane before further analysis.

2.3 PrestoBlue® cytotoxicity assay

HDFn previously cultured to confluence in DMEM with 10% FBS (Invitrogen, USA) were seeded into wells (1 x 10^3 cells/well) of a 96-well culture plate (Falcon, USA) and incubated at 37^{O}C in 5% CO_2 and 98% humidity for 24 hours. After cell attachment, 100 µL of the filter-sterilized barberry extracts were serially diluted (two-fold) into corresponding wells. Two-fold serial dilutions of Cytochalasin B (Sigma-Aldrich Chemicals, USA) were used as cytotoxic control. The plate was incubated for 4 days at 37°C in 5% CO_2, after which 10 µL of PrestoBlue® (Invitrogen Molecular Probes, 2010) were carefully added to each well. The plates will be further incubated for 30 min at 37°C in 5% CO_2. Absorbance measurement was carried out with Biotek ELx800 plate reader (Biotek ELx800, BioTek Instruments, USA) at 570 nm. Output was obtained as optical density readings from which the cytotoxicity index was computed. The same assay was independently performed on HT-29 and MCF-7, respectively, to determine the anticancer activity. All tests were conducted in triplicate. Statistical analysis between control (untreated) and experimental groups (treated) were carried out using paired t-test with $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

2.1 Biocompatibility on HDFn

Assessment of the biocompatibility of the ethanol barberry extract to human cells is a crucial step in considering its potential pharmacognostic application. A highly sensitive colorimetric assay was applied to investigate the survival and proliferation HDFn against two-fold serially-diluted concentrations of the ethanol extract. Cytotoxicity Index % (CI%) was calculated (Rezaei et al., 2011), with the resulting CI% plotted against serially-diluted concentrations of the ethanol extract (Figure 1).

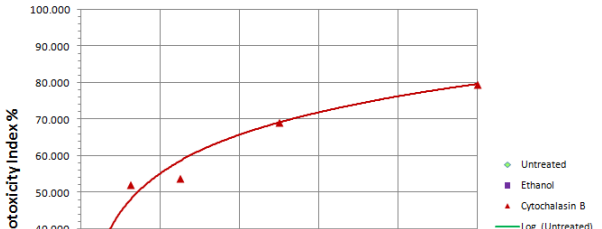


Fig. 1. Cytotoxicity graph (log regression) showing calculated CI% plotted vs. barberry ethanol extract concentrations. Higher regression profile corresponds to higher level of cytotoxicity while lower profiles show otherwise.

Based on the plot, ethanol extract-treated HDFn did not exhibit cytotoxic effect as evidenced by a low log regression profile compared to the high regression profile of the cytotoxic control cytochalasin B. Additionally, there is no computable IC50 (inhibitory concentration at 50%) for the ethanol extract since the profile did not reach the 50% mark. IC50 is a key parameter in determining the relative cytotoxicity of a test substance which is inferred as the relative amount of the extract that can kill 50% of the fibroblast population.

Consequentially, comparison of CI% values calculated from the highest extract concentration at 50% showed that the barberry ethanol extract's biocompatibility is not significantly different compared to the non-toxic untreated (p = 0.03034) shown in Figure 2, augmenting its non-cytotoxic property in vitro, whereas cytochalasin B showed highly significant toxicity compared with ethanol extract (p = 3.06E-07).

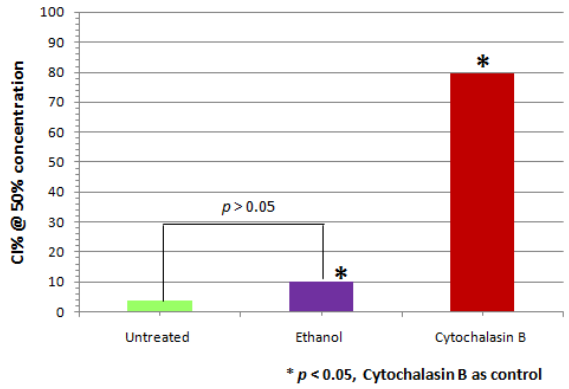
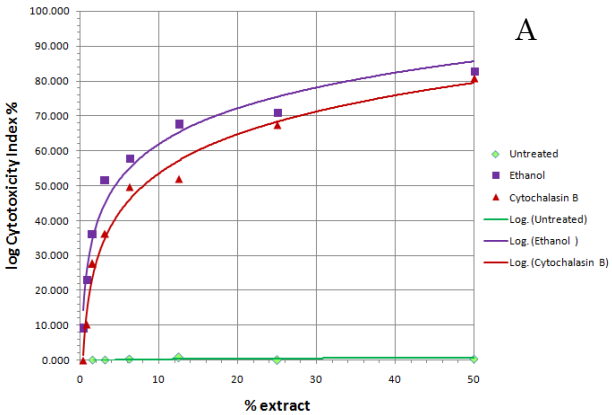


Fig. 2. Histogram showing mean CI% at the highest 50% concentration.

2.2 Anticancer potential of extract

Figures 3A & B respectively show the viability of HT-29 and MCF-7 cells after four days of incubation with barberry fruit extract. There is apparent dose dependent significant reduction on the viability of the cells based on calculated CI% values. Calculated IC50 values for HT-29 and MCF-7 were established to be at 12.51 % (7.5 mg/mL) and 17.83% (10.7 mg/mL), respectively, both of which were surprisingly significantly more effective than the positive control. Calculated p values were 7.85069E-10 for HT-29 and 0.00247 for MCF-7 in comparison with Cytochalasin B.

The cytotoxic ability might be linked to the antioxidant activity and content of the extract. Antioxidants have been previously shown to prevent some level of carcinogenesis. In this study, the alkaloids present and identified were berberine, columbamine and isocorydine. Berberine is already a proven phytochemical with active anticancer ability by mechanisms reported to suppress anti-apoptotic genes and potentiation of apoptosis in cancer cells in vitro (Pandey et al., 2008). Columbamine has recently been reported to suppress proliferation and metastasis in osteocarcinoma with low cytotoxicity (Bao et al., 2012). While isocorydine was also found to have potential anti-proliferative effects particularly in hepatocellular carcinoma via G2/M cell cycle arrest and apoptosis (Sun et al., 2012). With regard to the flavonoid rutin, Zhao et al. (2007) reported both immunomodulatory and anticancer activities, while the acid phenol cholorogenate is classically said to have strong antioxidant and antitumor activity (Jung et al., 1999).



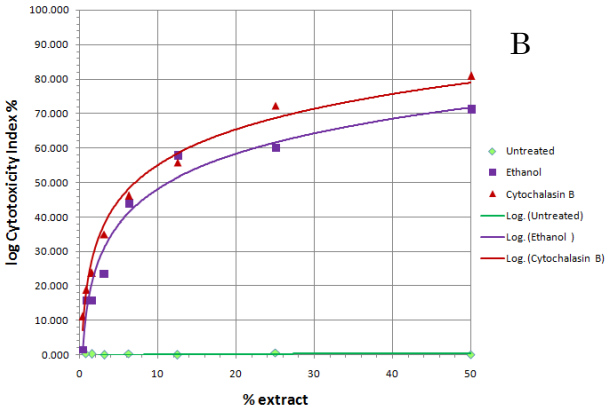


Fig. 3. (A) HT-29 and (B) MCF-7 cytotoxicity profiles (log regression) showing calculated CI% plotted vs. serially diluted barberry ethanol extract concentrations.

4. CONCLUSIONS

This research dealt about the preliminary phytochemical analysis of the seedless *B. vulgaris* fruit ethanolic extract including the in vitro assessment of antioxidant and anticancer activities in human colon and breast cancer cells. Previous investigations from available literature discussed anticancer properties from different parts of the plant except the fruit making this study, so far, the first attempt.

The fruit extract seemed to be highly cytotoxic by effectively suppressing the proliferation in both human colon and breast cancer cells in vitro, with calculated IC50 values significantly higher than the positive control Cytochalasin B. Moreover, the extract may indeed be a very promising anticancer primarily because of its non-cytotoxic property or biocompatibility with normal human fibroblast cells, selectively killing only cancer cells in vitro.

For further and future studies, it is recommended to investigate the cytoprotective capability of the extract against stress secondary to free radicals. This experiment is vital in finding out the cellular effectiveness of its antioxidant activity. Moreover, cancer cell lines other than colon and breast should be included in the study to determine the broadness of its effectivity within the vast range of human carcinomas.

5. ACKNOWLEDGMENTS

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