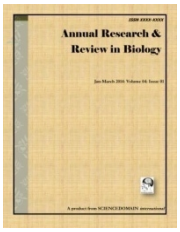

Annual Research & Review in Biology
4(6): 906-913, 2014

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Antimutagenicity and Anticancer Effects of *Biebersteinia multifida* DC

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MH, ME and FHD designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AG managed the plant material of the study. All authors read and approved the final manuscript.

Original Research Article

Received 27th September 2013
Accepted 26th November 2013
Published 12th December 2013

ABSTRACT

Aims: The purpose of this research is to examine antimutagenicity and anticancer effect of *Biebersteinia multifida.*
Study Design: Currently cancer is considered as one of the main factors of mortality globally. Many chemicals in our environment can cause genetic mutations and are potentially responsible for millions of cancer-related deaths. Nowadays the scientists are looking for food materials which can potentially prevent the cancer occurrence.
Place and Duration of Study: *Biebersteinia multifida* DC. dried roots were purchased from local market in Tehran bazar, the center of Tehran province, Iran, between June 2012 and January 2013.
Methodology: In this study human leukemia pre B-cells (Nalm-6) were cultured in RPMI 1640 [Sigma], supplemented with 10% fetal bovine serum (FBS), penicillin -streptomycin and L-glutamine. The cultures were incubated at 37ºC, 5% CO_2 and then inhibitory effect of ethanolic extract on their proliferation was measured by MTT assay. The ethanolic extract was evaluated in terms of antimutagenicity properties by a standard reverse mutation assay (Ames Test). This was performed with histidine auxotroph strain *of Salmonella*

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typhimurium (TA100).Thus, it requires histidine from a foreign supply to ensure its growth. The aforementioned strain gives rise to reverted colonies when expose to carcinogen substance (Sodium Azide) .
Results: The ethanolic extract prevented the reverted mutations and the hindrance percent 51.2% in antimutagenicity test. During MTT, human leukemia pre B-cells revealed to have a meaningful cell death when compared with controls (*P* = .01).
Conclusion: This study demonstrates the antimutagenicity effect of *Biebersteinia multifida DC*, and suggests that it may be potentially usefull as anticancer agent.

Keywords: Antimutagenicity; anticancer; Biebersteinia multifida DC; human leukemia pre B-cells;ames test.

1. INTRODUCTION

In recent years, the morbidity and mortality of cancer still reaches a high plateau and is a major public health problem worldwide [1,2].

Cancer is the major cause of human's death because of high incidence and mortality. The identification of new cytotoxic drug with low side effects on immune system has developed as important area in new studies of immunopharmacology [3].

Many studies report that a high diet in fruits and vegetables lowers the incidence of cancer [4,5]. Some of fruits and vegetables are considered as the main anticancer foods, because of their abundant antioxidants such as phenols, vitamin C, vitamin E, beta-carotene and licopene [6]. It has been reported that various fruit and vegetable extracts are capable of inhibiting the proteasome activity and this inhibition is associated with tumor cell apoptosis [7]. *Biebersteinia* is a genus of *Geraniaceae* family, including a herbaceous species called *B. multifida* growing in Iran. This species was found in Syria and Central Asia as well as Iran. In Persian this species is called *Adamak* [8,9]. All four species of Biebersteinia distributed geographically from central Asia to Greek in temperate mountain zones. Among these pharmacologically active species, only *B. multifida* and *B. orphanidis* have tuberous roots. In folk medicine, the tuberous roots of *B. multifida* have been used topically for the relief of inflammation and pain of musculoskeletal disorders [10] and orally in the treatment of nocturia in children and of phobia and anxiety in humans and domestic animals with no systematic approach to characterize the observed ethnopharmacological effects. Thus far, isolation of an alkaloid, vasicinone, and number of polysaccharides and peptide substances has been reported [11]. Flavonoids including 7-glucosides of apigenin, luteolin, and tricetin, as well as the 7-rutinoside of apigenin and luteolin have been isolated from its leaves which in part are responsible for antioxidant and antihemolytic activities [12-14]. Recently, essential oil composition of *B. multifida* was studied which exhibited that the main components were (E)-nerolidol, phytol, 6,10,14-trimethyl-2- pentadecanone and hexadecanoic acid [15]. Ethnopharmacological studies have revealed that the root extract of this plant has anti-inflammatory and analgesic activities that confirm the traditional use of *B. multifida* for the treatment of joint disturbances as well as in restoring bone fractures [16]. A bio-guided phytochemical analysis of *B. multifida* has not previously been reported. Although other reports have thus far identified a number of flavonoids, a few polysaccharides and one alkaloid (vasicinone) from root extract, in which vasicinone considered as the responsible molecule for observed pharmacological activities [17]. This research has been tried to consider antimutagenesis effect of *B. multifida* through Ames test [18,19] and the material is

used on cancerous cells in vitro. The purpose of this research is to examine antimutagenicity and anticancer effect of *Biebersteinia multifida.*

2. MATERIALS AND METHODS

2.1 Plant Material

Biebersteinia multifida DC.dried roots were purchased from local market in Tehran bazar, the center of Tehran province, Iran, and kept at the herbarium of faculty of pharmacy, Tehran University of Medicinal Sciences.Dried and powdered roots (800 g) were extracted successively with 2 L of ethanol 80% (v/v) by using a percolate extractor for 72 h at room temperature. The extracts were filtered using Whatman filter paper and then concentrated in vacuo at 40°C using a rotary evaporator. The elected concentrated solution was freeze-dried in a Christ Alpha 1-2D freeze-drier, to obtain the solid substance. After the obtaining, the solid extracts were kept in at 4°C until further tests.

2.2 Cell Culture

Nalm-6 cells were cultured in RPMI 1640 with 10% fetal bovine serum (FBS) and 1% penicilin-streptomycin at 37°C in 5% CO_2 incubator. Upon reaching appropriate confluence, the cells were passaged and incubated with IC_{50} with 0, 5, 10, 50, 75 and 100 µg/mL concentrations of *B. multifida* extract for a defined time. The cells were treated with ethanol at the same dose of that used in the maximum dose (100 microgram/ml) group too.

2.3 MTT Staining

In this technique, color effect of MTT (dimethylthiazol diphenyl tetrazolium bromide) on cells has been used in which alive cells, contained purple crystals as a result of color reduction by mitochondrial dehydrogenase of alive cells, would be countered and alive cells percentage would be determined by the following formula:

Viability= (alive cells number/ whole cells cultured) ×100

After 18 hours in order to full adherence of cells to the plate, different concentrations of the ethanolic extract have been added to cells and plates were incubated for 48 hours at 37°C and 5% CO_2.

MTT staining is on the basis of MTT reduction into an insoluble blue purple product (Formazan) by mitochondrial reductase in alive cells. Nalm-6 cells were seeded into 96-well plate ($5\text{-}7\times10^3$ cells per well). After 24 h incubation ethanolic extract in different concentrations were added to each well and incubated for 48 hours, followed by incubation with 5 mg/ml MTT for 4h. The supernatant was removed after centrifugation, finally 100 µL of DMSO was added to each well. 48 wells are used for the MTT assay. The absorbance of cells was measured at 570 nm with Eliza reader. Toxicity level was calculated by the following formula:

$$Cytotoxicity\% = 1 - \frac{Mean\ absorbance\ of\ \ toxicant}{Mean\ absorbance\ of\ \ negative\ control} \times 100$$

Viability % = 100 - Cytotoxicity %

To diminish test error level, MTT strain was added to some wells without cells and along with other wells, absorbance level was read and ultimately subtracted from whole the absorbance. The data were analyzed with Tukey Test measured by one way ANOVA.

2.4 IC_{50}Determination

The 50% inhibition concentration (IC_{50}) values of extract on Nalm6 cells at 48h were determined. IC_{50} was determined by probit analysis using the Pharm PCS (Pharmacologic Calculation System) statistical package (Springer- Verlag, USA)

2.5 Antimutagenesis Test

Salmonella typhimurium TA100 used forAmes test. Fresh bacterial culture should be used for test and incubation time of bacterial culture in nutrient broth should not be more than 16 hours. Appropriate bacterial concentration was considered 1-2×10^9 cells/ml. The 50% inhibition concentration (IC_{50} values of extract was added to test tube containing 0.5ml of the overnight fresh bacterial culture, 0.5ml of histidine and biotin solution (0.5 mMhistidin/0.5mMbiotin), 10 ml top agar(50 gr/Lit Agar + 50 gr/Lit NaCl), sodium azide as a carcinogene(1.5 µg/ml Sodium azide) and then content of this tube distributed on the surface of minimum medium of glucose agar (40% glucose), after 3 second shaking incubation was performed at 37°C for 48 hours. Each treatment was repeated 3 times. In the test after 48 h incubation at 37°C, reversed colonies were counted in control and test plates and after angular conversion, results were compared by analysis variance. Most materials in their original form are inactive in terms of carcinogenic effects and most materials to become metabolically are active to display mutagenesis properties. So it is necessary to add a microsomal sterile extract to mammalian tissue like rat. After 10 h starvation, livers of 10 male rats were separated. Starvation stimulates and enhances liver enzymes secretion. Livers homogenized in 0.15M potassium chloride and centrifuged for 10 min in 9000 rpm in at 4°C. Supernatant (S9 mixture) was removed and mixed with necessary cofactors including NADP and G6p (glucose 6 phosphate) and then 0.5ml of the solution was added to Top agar in order to consider anticancer effect. Also after the counting colonies in antimutagenesis test, prevention percentage or antioxidant activity has been calculated as follows (20):

$$\text{Prevention percent} = (1 - \frac{T}{M}) \times 100$$

T is reversed colonies in each Petri dish under carcinogen + extract and M is reversed colonies in petri dishes related to positive control (mutagen).

3. RESULTS AND DISCUSSION

The results of MTT test on cancerous cells under various concentrations of extract has been shown in Fig. 1. There was a significant difference between extract effect on growth depression of cancerous cells (P = .01). The IC_{50} after 48h was calculated $51.3 \pm 0.62 \mu g/ml$ (P = .01).

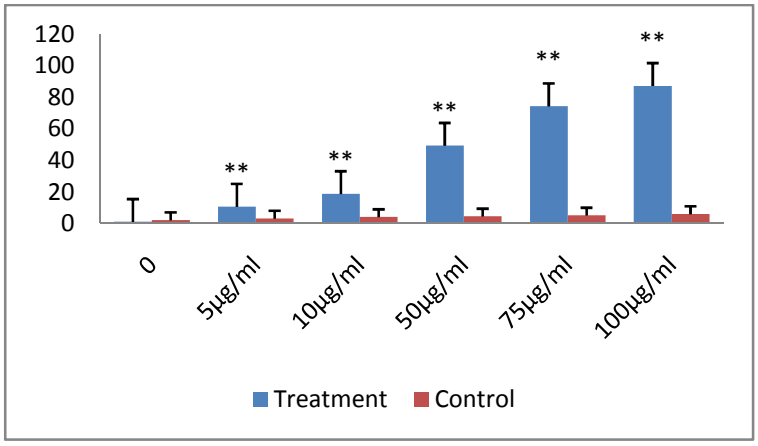

Fig. 1. Results of MTT test on cancerous cells under various concentrations of *B. multifida* extract (Treatment) and ethanol (Control)

The results of colony counting in Ames test under 50 µg/ml ofthe ethanolic extract(with regard to the results of vital capacity test) showed that there was a significant difference on colony growth of *Salmonella typhimurium* TA100 with controls (Fig. 2) (*P* = .01).The hindrance percent was 51.2% in antimutagenicity test.

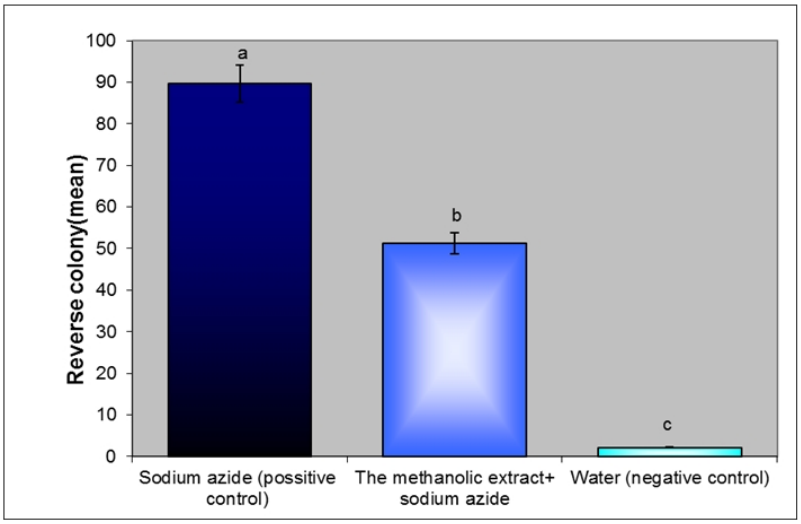

Fig. 2. Results of colony counting in Ames test under 25 µg/ml of the ethanolic extractin mutagenesis test

3. DISCUSSION

Since usual methods on cancer treatment (surgery, chemical treatment, radiotherapy) have an effect on natural dividing cells, in addition to tumor cell, and kill or arrest their cell division [21]. In recent years, herbals found widespread use in prevention and treatment of cancer which in this procedure, tumor cells are controlled while natural cells remain intact [22]. The

effect of diverse antioxidant foods on cancer and cardiovascular disease has been proved and it has been revealed that these materials cause to enhance long life by 60% [23]. During laboratory researches on poly metoxilated flavonoides including tungertin, it has been revealed that these materials have antioxidant and anticancer effects and preservative effect on neurons [24].

Oxidative stress by free radicals is an important event in the cell that can cause aging and human degenerative diseases including cancer, heart diseases, multiple sclerosis, Parkinson's disease, autoimmune disease and senile dementia. Stresses, physical damage, viral infection, cytotoxic or carcinogenic compounds as a consequence of chemical or biological aggression may cause peroxidation of polyunsaturated fatty acids of cell membranes and liberation of toxic substances such as free radicals. Studies concerning the relationship between the morbidity due to cancer and heart diseases and the consumption of fruits and vegetables indicated that polyphenols present in large amount in fruits and vegetables have a significant impact on the morbidity decrease from these diseases [25-27]. Recently,attention has been focused on antioxidant products of natural sources isolated of plant products. Polyphenolic compounds are found mainly in fruits and vegetables as secondary plant metabolites. Many polyphenols such as kaempferol, quercetin, luteolin, myricetin and catechin express strong antioxidative, antiinflamatory, antiallergic and antineoplasic properties [28]. The high antioxidant activity of plant phenolic compounds attractive to the food industry, prompting their use as replacements for synthetic antioxidants and also as nutraceuticals, playing a role in preventing many diseases. Reactive oxygen species such as hydroxyl, superoxide and peroxyl radicals are formed in human tissue cells result in extensive oxidative damage that leads to age-related degenerative conditions, cancer and wide range of other human diseases [28-30]. Antioxidants from natural sources increase the shelf-life of foods [30]. Therefore, consumption of antioxidant and addition of antioxidant in food materials protect the body as well as foods against these events. Antioxidative properties of the essential oils and various extracts from many plants are of great interest in both academia and the food industry, since their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones.

In the present study vital capacity test and Ames test were used to consider its anticancer effect with special emphasizes on application of *Salmonella typhimurium* (TA100) to identify antimutagenesis and anticancer level of chemicals. In this research According to the Ames theory which presented in 1982, in case the number of colonies on positive control medium (contained carcinogen) is two times more than test sample, the substance will be considered as an antimutagenesis and anticancer. According to the Ames theory, when prevention percent ranges between 25-40%, mutagenesis effect in this test sample is assumed medium and when prevention percent is more than 40, mutagenesis effect of the test sample is strong and in case prevention percent is less than 25, mutagenesis effect is negative [18,19] This value for the ethanolic extract was medium.

In this research, we have examined this extract with rat liver extract (S9). Reason of adding S9 to the ethanolic extract is that some of anticancer substances remain inactive and can not attach to DNA till enter into an being with electrophilic enzymes. Bacteria lack this system, so liver extract S9 is applied as active system of cytochrome P-450/P-448 for activation of the materials [19]. Findings from the present study indicate that the *B. multifida* extract is highly cytotoxic to human leukemia cells.

4. CONCLUSION

This study demonstrates the antimutagenicity effect of *Biebersteinia multifida DC*, and suggests that it may be potentially usefull as anticancer agent.

ACKNOWLEDGEMENTS

This investigation was supported by Research Institue for Islamic and Complementary Medicine, Iran University of Medical Sciences, Tehran, Iran.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hashemi M, Nouri Long M, Entezari M, Nafisi S, Nowroozii H. Anti-mutagenic and pro-apoptotic effects of apigenin on human chronic lymphocytic leukemia cells. Acta Med Iran. 2010;48283-8.
2. Padhye S, Banerjee S, Ahmad A,Mohammad R, and Sarkar FH: From here to eternity—the secret of pharaohs: therapeutic potential of black cumin seeds and beyond. Cancer Ther. 2008;6:495–510.
3. Entezari M, Majd A, Falahian F, Mehrabian S, Hashemi M, Ardeshiry Lajim A. Antimutagenicity and Anticancer Effects of Citrus Medica Fruit Juice. Acta Med Iran. 2009;47:373-7.
4. Ghasemian A, Mehrabian S, Majd A.Peel Extracts of two Iranian cultivars of Pomegranate (*Punica granatum*) have antioxidant and antimutagenic activities. Pakistan Journal Biological Sci. 2006;7:1402-1405
5. Banerjee S, Padhye S, Azmi A, Wang Z, Philip PA, Kucuk O, Sarkar FH, MohammadM.Review on molecular and therapeutic potential of thymoquinone in c ancer. Nutr Cancer. 2010;62:938-46.
6. Cragg GM, Newman DJ. Plants as a source of anticancer agents. Journal of Ethnopharmacology. 2005;100:72–79.
7. Chen D, Landis-Piwowar KR, Chen MS, Dou QP. Inhibition of proteasome activity by the dietary flavonoid apigenin is associated with growth inhibition in cultured breast cancer cells and xenografts. Breast Cancer Res. 2007;9(6):R80.
8. Mozaffarian V. A dictionary of Iranian plant names. Tehran; 1996.
9. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoids antioxidants: chemistry, metabolism and structure–activity relationships. J Nutr Biochem. 2002;13:572-84.
10. Amirghofran Z. Medicinal plants as immunosuppressive agents in traditional Iranian medicine. Iran J Immunol. 2010;7:65–73.
11. Arifkhodzhaev AO, Rakhimov DA Polysaccharides of saponin-bearing plants. V. Structural investigation of glucans A, B, and C and their oligosaccharides from Biebersteinia multifida plants. Chem Nat Compd. 1994;30:655–60.
12. Greenham J, Vassiliades DD, Harborne JB, Williams CA, Eagles J, Grayer RJ, Veitch NC. A distinctive flavonoid chemistry for the anomalous genus Biebersteinia. Phytochemistry. 2001;56:87–91.
13. Omurkamzinova VB, Maurel ND, Bikbulatova TN. Flavonoids of Biebersteinia multifida. Chem Nat Compd. 1991;27:636–637.

14. Nabavi SF, Ebrahimzadeh MA, Nabavi SM, Eslami B, Dehpour A. Antihemolytic and antioxidant activities of Biebersteinia multifida. Eur Rev Med Pharmacol Sci. 2010;14:823–830.
15. Javidnia K, Miri R, Soltani M, Khosravi AR: Essential oil composition of biebersteinia multifida DC. (Biebersteiniaceae) from Iran. J Essent Oil Res 2010;22:611–12.
16. Farsam H, Amanlou M, Dehpour AR, Jahaniani F: Anti-inflammatory and analgesic activity of Biebersteinia multifida DC. root extract. J Ethnopharmacol. 2000;71:443–447
17. Monsef-Esfahani HR, Amini M, Goodarzi N, Saiedmohammadi F, Hajiaghaee R, Faramarzi MA, Tofighi Z, Ghahremani MH. Coumarin compounds of Biebersteinia multifida roots show potential anxiolytic effects in mice. Daru. 2013 Jun 27;21(1):51
18. Ames BN. Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test. Mutat Res.1976;31:347-349
19. Ames BN, Durston WE, Yamasaki E, Lee FD. Carcinoges are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection.Proc,Natl.Acad.Sci. 1973;70:2281-2285
20. Ong T, Wong WZ, Stewart JD, Brockman HE. Chlorophyllin: A potent antimutagen against environmental and dietary complex mixture. Mutation Res. 1986;173:111-115
21. Chabner BA, Friedman MA. Progress against rare and not so-rare cancer. New Engl J Med. 1992;236:564-68
22. Franks LM, Teich NM. Introduction to the cellular and molecular biology of cancer. New York: Oxford university press; 1997.
23. Sunj J, Chu YF, Wu X. Antioxidant and antiproliferative activities of common fruits. J Agric Food Chem. 2002;25:7449-54
24. Bennett JP, Gompert S, Wollenweber E. Inhibitory Effects of natural inflavonoids on secretion from mast cells and neutrophils. Arzneimittel forschung. 1981;31:433-7
25. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoids antioxidants: chemistry, metabolism and structure–activity relationships. J Nutr Biochem. 2002;13:572-84.
26. Hertog MGL, Hollman PCH, Van de Putte B. Content of potentially anticarcinogenic flavonoids of teainfusions, wines and fruit juices. J Agri Food Chem. 1993;41**:**1242-6
27. R ice-Evans CA. Flavonoid antioxidants, Curr Med Chem. 2001;8:797-807.
28. Reaven PD, Witzum JL. Oxidised LDL in atherogenesis. Role of dietary modification. Ann Rev Nutr. 1996;16:51-71.
29. Aruoma IO. Antioxidant action of plant foods. Use of oxidative DNA damage, as a tool for studying antioxidant efficacy. Free Radical Res. 1999;30:419-27.
30. Schwarz K, Bertelsen G, Nissen LR, Gardnu PT, Heinonen NI, Hopia A, et al. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging. Lipid oxidation and analysis of the principal antioxidant compounds. Eur Food Res Technol. 2001;212:319-28.

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