Antimutagenicity and anticancer effects of Biebersteinia multifida DC

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 and human leukemia pre B-cells(Nalm-6) were cultured in RPMI 1640[Sigma], supplemented with 10% fetal bovine serum(FBS), penecilin-streptomycin and L-glutamine. The cultures were incubated at 37ºC, 5% CO2 and then inhibitory effect of methanolic extract on their proliferation was measured by MTT assay. The methanolic 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 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 methanolic 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 has a potential as an 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 world wide [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 lipotene[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]. Ames test is one of the most current test to assay anticancer and antimutagenesis effects using bacteria with special mutants [10,11] and the material is used on cancerous cells in vitro.

2. MATERIAL 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% penecilin-streptomycin at 37°C in 5% CO₂ incubator. Upon reaching appropriate confluence, the cells were passaged. The cells were incubated with morin in different concentrations were added to each well and incubated for 48 hours, and then stained with MTT.

2.3 MTT staining

In this technique, color effect of MTT 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:

\[
\text{Viability} = \left( \frac{\text{alive cells number}}{\text{whole cells cultured}} \right) \times 100
\]

After 18 hours in order to full adherence of cells to the plate, different concentrations of the methanolic extract have been added to cells and plates were incubated for 48 hours at 37°C and 5% CO₂.

MTT staining is on the basis of MTT(dimethylthiazol diphenyl tetrazolium bromide) reduction into an insoluble blue-purple product (Formazan) by mitochondrial reductase in alive cells. Nalm-6 cells were seeded into 96-well plate (5-7x10³ cells per well). After 24 h incubation, morin 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. The absorbance of cells was measured at 570 nm with Eliza reader. Toxicity level was calculated by the following formula:

\[
\text{Cytotoxicity} = 1 - \frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}} \times 100
\]

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 IC50 determination

The 50% inhibition concentration (IC50) values of extract on Nalm6 cells at 24h were determined. IC50 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 for Ames 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 $1 - 2 \times 10^9$ cells / ml. The 50% inhibition concentration (IC50) values of extract was added to test tube containing 0.5ml of the overnight fresh bacterial culture, 0.5ml of histidine and biotin solution ($0.5mM\text{histidin}/0.5mM\text{biotin}$), 10 ml top agar(50 gr/lit Agar + 50 gr/lit NaCl), sodium azide as a carcinogene(1.5 μgr/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 mins in 9000 rpm in at 4°C. Supernatant (S9 mixture) was removed and mixed with necessary cofactors including NADP and G-6p(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(12):

\[
\text{prevention percent} = \left(1 - \frac{T}{M}\right) \times 100
\]

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 figure 1. There was a significant difference between extract effect on growth depression of cancerous ($P = .01$). The IC50 after 24h was calculated $51.3 \pm 0.62\mu g/ml$ ($P = .05$).

![Cytotoxicity effects on Nalm6 cell line%](image1.png)

Fig1: Results of MTT test on cancerous cells under various concentrations of *B. multifida* extract

The results of colony counting in Ames test under 50 μl/ml of the methanolic extract (with regard to the results of vital capacity test) showed that there was a significant difference on colony growth with controls (figure 2) ($P = .01$). The hindrance percent was 51.2% in antimutagenicity test.

![Reverse colony(mean)](image2.png)

Fig2: Results of colony counting in Ames test under 25 μl/ml of the methanolic extract in mutagenesis test
Since usual methods on cancer treatment (surgery, chemical treatment, radiotherapy) have
effect on natural dividing cells, in addition to tumor cell, and kill or arrest their cell
division[13]. In recent years, herbs found widespread use in prevention and treatment of
cancer which in this procedure, tumor cells are controlled while natural cells remain
intact[14]. 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% [15]. During laboratory researches on poly metoxilated flavonoids including tungertin,
it has been revealed that these materials have antioxidant and anticancer effects and
preservative effect on neurons[16].
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 [17-19].
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, quercitin, luteolin,
myricetin and catechin express strong antioxidative, antiflamatory, antiallergic and
antineoplasic properties [20]. 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, super oxide 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 [20-22]. Antioxidants from natural sources
increase the shelf-life of foods[22]. 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 emphasis on application of salmonella typhimurium to identify
antimutagenesis and anticancer level of chemicals. In this research, half-ripe and ripe fruit
juice displayed anticancer and antimutagenesis effect which half-ripe fruit juice was more
effective than ripe fruit juice. 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 [11,10] This value for the methanolic extract
was medium.
In this research, we have examined this extract with rat liver extract (S9). Reason of adding
S9 to the methanolic 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, supporting its use as an effective therapeutic agent in the management of human Leukemia cells.

4. CONCLUSION

This study demonstrates the antimutagenicity effect of Biebersteinia multifida DC, and suggests that it has a potential as an anticancer agent.

ACKNOWLEDGEMENTS

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS’ CONTRIBUTIONS

All authors read and approved the final manuscript.

REFERENCES

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
10. Ames BN. Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test. Mutat Res. 1976;31:347-349


