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Antimutagenic Properties of Nettle Leaf Aqueous Extract (*Urtica dioica* L.)

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ABSTRACT

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Cancer is a major public health burden in both developed and developing countries. Plant derived agents are being used for the treatment of cancer. Nettle (*Urtica dioica L.*) is widely grown in different parts of the world and has been used to promote health. In this study, aqueous extract of nettle leaf *(Urtica dioica* L.) was studied for antimutagenic properties against sodium azide and acrylic amide by Ames test in presence and absence of rat microsomal liver enzyme (S_9). The results showed that nettle leaf aqueous extracts can inhibit mutagenic agents of sodium azide and acrylic amide. Nettle leaf aqueous extracts with the inhibition of 40.39% sodium azide and 43.34%, acrylic amide showed high potential in decreasing mutagenic agents. Antimutagenic activity was increased significantly when there were liver microsome extract (S_9).

Key words: Nettle leaf, Ames test, Antimutagenic activity.

INTRODUCTION

Cancer is one of the main causes of death in the world and mutagens cause death in millions of patients. Noticing the side effects of the drugs used to treat cancer, scientists are looking for drugs with fewer side effects and more therapeutic effects. Accordingly, the number of studies in this field is rapidly increasing. An abundance of data indicates that diets high in fruits and vegetables are effective in protecting humans from cancer. The search for anticancer agents from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins[1-2]. Urtica dioica, nettle or stinging nettle belongs to family Urticaceae. It occurs as a perennial plant in temperate zones of Asia, America and Europe. It is of great medicinal value but the plant is undervalued by almost all of us [3]. Urtica dioica herbs are used to treat stomachache in Turkish folk medicine. In addition, this herb is used to treat rheumatic pain and for colds and cough and is used against liver insufficiency [4]. Each factor that causes removal, inhibition and inactivation of mutagen substances is rewarding. Today, bacteria are being used for the assessment of antimutagenic activities of different compounds in a short-time with excellent results[5]. One of the methods used for assessing the mutation prevention properties of a compound in bacteria is the Ames test. Ames test is a worldwide short-term bacterial reverse mutation test specifically designed for screening a variety of new chemical substances and drugs that can produce genetic damage that leads to gene mutations [6-7]. The Salmonella strains used in the test have different mutations in various genes in the histidine operon, each of these mutations is

designed to be responsive to mutagens that act via different mechanisms [7-8]. The aim of the present study was to investigate antimutagenic activity of nettle leaf aqueous extracts against sodium azide and acrylic amide by Ames test in presence and absence of S_9.

MATERIAL AND METHODS

Salmonella typhimurium TA100

Salmonella typhimurium TA100 was from Dr. Ames of the University of California, Berkeley, USA. *Salmonella typhimurium* TA100 was activated at 37°C for 48h on a plate containing minimal glucose agar supplemented with biotin, histidine and ampicillin. Tests of histidine requirement, rfa mutation, uvrB mutation and R- factor were carried out to confirm the genotypes of *S. typhimurium* TA100. Prior to each mutagenicity test, *S. typhimurium* TA100 was grown in Nutrient broth (Merck; Germany) at 37°C overnight[9].

Preparation Aqueous Extract of nettle leaf

Nettle leaves used in this study were collected in winter 2011 from Gilan province (Northern Iran). Then, nettle was left on a bench to dry. The dried sample was chopped into small parts with a blender. For water extraction, 20 g dried aerial parts of nettle ground into a fine powder in a mill and was mixed with 400 ml boiling water by magnetic stirrer during fifteen minutes. Finally, obtained solution was passed through filter[4].

Preparation of the rat microsomal liver enzyme (S_9) and mutagen substances

A broad range of carcinogenic agents require metabolic activation for recognition. In this investigation, 5 male rats (body weight~200g), were used. Rats were starved for 24 hours in order to get the titer of the liver enzymes to their highest levels. Animals were sacrificed by cervical dislocation and the livers were collected, homogenized in 0.15 M KCl. Livers were cut into pieces using sterile scissors and smashed prior to a 10 min centrifugation at 9000g. The supernatant (S_9) was stored at -80°C. The antimutagenic assay was performed in the presence and absence of S_9. Two chemical mutagen, sodium azide and acrylic amide were purchased from Merck company and dissolved in dimethyl sulfoxide (DMSO), a final concentration of 5 µg/ml [7, 8-10].

Ames test

Antimutagenic properties of of nettle leaf aqueous extracts against sodium azide and acrylic amide, were evaluated by a pre-incubation method of Maron and Ames (1983) in presence and absence of liver microsome extract (S_9) [8]. Each assay was performed in triplicates simultaneously and the percentage of inhibition was determined using the formula [1-T/M] ×100 [11].

Procedure in presence of liver microsome (S_9)

In this assay 0.5 ml of nettle leaf aqueous extract is mixed 0.1 ml of the overnight culture *S. Typhimurium* TA100 and 0.1 ml of our mutagenic substances including sodium azide and acrylic amide in test-tube containing 3ml top agar. Then, 0.1ml of histidine and biotin 0.5 mM solution and 0.5ml of liver microsome extract (S_9) were added. After were poured on glucose minimal medium and incubated for 24h at 37°C.

Positive control

The mixture of 0.1 ml of overnight cultured *S. typhimurium* TA100, 0.1 ml of mutagenic substances including sodium azide and acrylic amide were prepared and were poured in test-tube containing 3ml top agar. Then, 0.1ml of histidine and biotine 0.5 mM solution and 0.5ml of liver microsome extract (S_9) were added, after shaking for 3 minutes, the test-tube contents was poured on glucose minimal medium and incubated for 24h at 37°C.

Negative control

The mixture of 0.1ml of overnight cultured *S. typhimurium* TA100, 0.1ml of DMSO, 0.1 ml of histidine and biotine 0.5 mM solution and 0.5 ml of liver microsome extract (S_9) were added to 3ml of top agar. After shaking for 3 minutes, it was poured on glucose minimal medium and incubated for 24h at 37°C.

Procedure in absence of liver extract (S_9)

All the steps in this stage are the same as previous part. But, here, it was not used from liver microsome extract (S_9).

RESULTS AND DISCUSSION

Strain TA100 identity

In accordance with the *Salmonella typhimurium* TA100 strain genotype, the presence of colony in biotin-histidine medium and absence one in control biotin medium show that these strains are dependent to histidine. The existence of inhibitory zone around the disk indicates that the bacteria do not grow and the Rfa mutation was occurred. This mutation can causes relative decreasing of lipopolysaccharide barriers and then, increase cell wall permeability for bigger molecules. If the inhibitory zone is not presence around the disk, the bacterium has R-factor plasmid and also, lack of growth in radiated culture region indicates that uvr B mutation was ocurred.

Determination antimutagenic activity

The results showed that nettle leaf aqueous extracts can inhibit mutagenic agents of sodium azide and acrylic amide (Table 1, 2). Antimutagenic activity was increased significantly when there were S_9. Olive leaf extract with the inhibition of 40.39% sodium azide and 43.34% acrylic amide showed high potential in decreasing mutagenic agents.

Cancer is the second leading cause of death in the United States, where one in four deaths is due to cancer. Plants have long been used in the treatment of cancer [12]. The use of antimutagens and anticarcinogens in everyday life is the most effective procedure for preventing human cancer and genetic disease [13]. Ruan *et al*, reported that antimutagenic

Table 1: Antimutagenic effect of nettle leaf aqueous extracts against sodium azide

Revertant colony	*S.typhimurium* / S_9^-		*S.typhimurium* / S_9^+	
Sampels	Revertants (CFU/plate)	Inhibition (%)	Revertants (CFU/plate)	Inhibition (%)
Positive control(sodium azide)	392	-	463	-
Negative contorol(DMSO)	53	-	75	-
Nettle leaf aqueous extract	250	36.22%	276	40.39%

Table 2: Antimutagenic effect of nettle leaf aqueous extracts against acrylic amide

Revertant colony	*S.typhimurium* / S_9^-		*S.typhimurium* / S_9^+	
Sampels	Revertants (CFU/plate)	Inhibition (%)	Revertants (CFU/plate)	Inhibition (%)
Positive control (acrylic amide)	368	-	473	-
Negative contorol (DMSO)	53	-	75	-
Nettle leaf aqueous extract	226	38.59%	268	43.34%

substances may prevent cancer because they can destroy mutagens both inside and outside body cells, and block mutagens that damage DNA and cause mutations in cells [14]. Natural products discovered from medicinal plants have played an important role in the treatment of cancer [12]. Nettle has great medicinal potential. Nettle is nutritionally high in vitamins A, C and D, also minerals iron, manganese, potassium and calcium. It is also beneficial during pregnancy [3]. Clinical evidence shows that freeze-dried extracts of nettle reduce allergy symptoms [15]. The study undertaken by Gulcin *et al*, showed that nettle water extract had an appropriate antioxidant power so that the its inhibitor power of free radicals in DPPH test [16]. Also, the investigations on other plants antimutagenic properties [17,18-19]. Ames test was used to determine antimutagenic of olive leaf. This method is very fast

and economical and used to identify antimutagenic and mutagenicity of agents. In this study, consideration of antimutagenic and anti-carcinogenic of nettle leaf aqueous extracts compared with positive controls (sodium azide and acrylic amide) indicates good antimutagenic properties nettle leaf extracts. It can be concluded that nettle leaf should have more effective place in treatment because of antimutagenic and anti-carcinogenic. Of course, more comprehensive researches are needed for indicating scope and exact mechanism of this function. Extract of this plant can be purified and used for food and pharmaceutical industries.

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