Survey of Antimutagenicity and Anticancer effect of Phoenix dactylifera pollen grains

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ABSTRACT

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. The scientist are nowadays looking for food materials which can potentially prevent the cancer occurrence. The purpose of this research is to examine Antimutagenicity and Anticancer effect of Phoenix dactylifera pollen grains. The Phoenix dactylifera pollen grains was subsequently evaluated in terms of Antimutagenicity and Anticancer properties by a standard reverse mutation assay (Ames Test). For this test, the particularity of the strain of salmonella typhimurium chosen TA100 resides in the fact that undergone a specific mutation in the Histidine operon, and for this same reason 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), however, the prevents the reverted mutation by the carcinogen compound to occur and when compared to either the positive control (Sodium Azide) or the negative one (composed of distilled water), it determines the spontaneous mutation rate and this serves as a taken to prove the potential antioxidant effect of the above specimens showed antioxidant property to varying degrees. The average hindrance percent of Phoenix dactylifera pollen grains was 46% in Antimutagenicity test. The average hindrance percent of Phoenix dactylifera pollen grains was 49% and in Anticancer test. The present study is the first study that have revealed Antimutagenicity and Anticancer effect of Phoenix dactylifera pollen grains.

Key words: Phoenix dactylifera pollen grains, Anticancer, Antimutagenicity, Ames Test.

Introduction

Nowadays cancer is one of mortality factor in the world which takes place in result of different causes such as mutagenesis & carcinogen chemicals in the environment. Environmental agents which serves as mutagens are cancer factors. According to statistics almost more than 75% of cancers have an environmental origin [1,2]. Genetic damages and changes in DNA sequences and genes mutations and other changes in chromosomal structure play an important role in cancer [3]. Most of mutagenic and carcinogen agents display their destructive effects through free radicals including reactive oxygen’s species (ROS). So that antioxidants are able to reduce ROS. ROS have a role in etiology of diseases such as cancer, cardiocellular, nerves problems and senescence. So daily consumption of antioxidants enhances immunity of body against free radicals production and serves as anticancer [4,6]. 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 [7-9]. 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 effects on cultured cancerous cells in vitro. This research for the first time tried to consider anticancer effects of Phoenix dactylifera pollen grains through Ames test.

Materials and Methods

In this research Ames test has been used as a current method to assess anticancer and antimutagenesis effect of on mutant bacteria, salmonella typhimurium, and results have been assessed on the basis of bacterial colonies in selected conditions. Salmonella typhimurium TA100 used for Ames test. The mutant strain in need of histidin, directly receipt from professor Ames. Fresh bacterial culture should be used for test and incubation time for the overnight fresh bacterial culture in nutrient broth.
should not be more than 16 hours. Appropriate bacterial concentration has been considered as $1-2 \times 10^3$ cells at ml. Pollen grains has been added to test tube containing $0.5 ml$ of the overnight fresh bacterial culture, $0.5 ml$ of histidin and biotin solution ($0.5 m MHistidin / 0.5 m MBiotin$) and $10 ml$ top agar ($50 gr/lit Agar + 50 gr/lit Nacl$) and, sodium azide as a carcinogene ($1.5 \mu g/ml Sodium azide$) and then content of this tube distributed on the surface of minimum medium of glucose agar (%40 glucose) after 3 second shacking and incubated at $37^\circ C$ for 48 hours. Each treatment has been repeated 3 times. In the test after 48 h incubation at $37^\circ C$, reversed colonies counted in control and test plates and after angular conversion, results have been compared by variance analysis most of materials in their original form are inactive in terms of carcinogenic effects and most of materials have to become metabolic active to display mutagenesis properties. So it is necessary to add a microsomal sterile pollen grains from mammalian tissue like as rat. After 10 h starvation, livers of 10 male rat separated. Starvation stimulates and enhances liver enzymes secretion. Livers homogenized in potassium chloride0.15M and centrifuged for 10 min with 9000 cycles/min at $4^\circ C$. Supernatant (S9 mixture) removed and mixed with necessary cofactors NADP $3$ G-6p (glucose 6 phosphate) and then $0.5 ml$ of that added to Top agar in order to consider anticancer effect.

Also after counting colonies in anticancer-antimutagenesis test, prevention percentage or antioxidant activity has been calculated as follows [12]:

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

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

Results:

Results of anticancer and antimutagenesis effect of the pollen grains:

Comparison of results of colonies counting in Ames test of the pollen grains shows that there is a significant difference between antimutagenesis effects on colonies growth with controls (distilled water and sodium aside). ($P < 0.01$).

**Diagram 1:** Comparison of results of colonies counting in Ames.

**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 them or repress their cell division [13], 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 [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 lifelong by 60% [15]. During laboratory researches on poly metoxilated flavonoides including Tungertin, it has been revealed that these materials have anti oxidant and anticancer effects and preservative effect on neurons [16]. There is a study (2001) on limonins effect (flavonoids) on cell cycle which caused to changes in cell division and/or cell death (apoptosis) [17]. In 2005, by some examinations on Nobilin (flavonoid of citrus peels) it has been revealed that this substance has anticancer, anti virus and anti inflammation activity [18]. With regard to the fact that so far, anticancer and antimutagenesis effect of Phoenix dactylifera pollen grains has not been reported, in this regard, we used methods of vital capacity test and Ames test to consider its anticancer effect with special regard to the emphasizes on application of *salmonella typhimurium* to identify
antimutagenesis and anticancer level of chemicals. In this research, pollen grains displayed anticancer and antimutagenesis effect. 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 which the case is true to consider anticancer effect by adding S9 for metabolic activation [11,10].

This case is represented by considering prevention or antioxidant percent of that all kinds of have preservative effect and this effect pollen grains is very much. In this research, we have examined this pollen grains with rat liver extract (S9). Reason of adding S9 to the pollen grains is that some of anti cancer 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 this materials [19]. As shown, pollen grains with rat liver extract displays anticancer activity.

References