STUDY OF ANTICANCER EFFECTS OF ALCOHOLIC AND AQUEOUS EXTRACT OF CITRUS AURANTIUM FRUIT THROUGH LIVER MICROSOMES AND SALMONELLA TYPHIMURIUM TA100 BACTERIUM

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Abstract

Background and Purpose: As we know, there are many problems in the treatment of many different types of tumors and cancers. Using antioxidant compounds could play a key role in the prevention of cancer. Researchers are exploring and finding natural substances that can prevent cancer. In this regard, in this study, they investigated the antioxidant and anti-cancer properties of fruit extract of citrus against a mutagenic materials of sodium azide by using liver microsomes and Ames test.

Methods: In this experimental study after aqueous, ethanol, methanol extraction of citrus, anti-mutagenic power determination test was performed based on the Ames method and by using mutant strains of Salmonella and carcinogen substance of sodium azide in the presence of rat liver microsomes.

Results: The results showed that the numbers of mutant colonies were reduced in the presence of all three types of extracts. In such a way that aqueous, ethanol extracts respectively by 52 and 88 percent have the lowest and most anti-ejection impact. The differences between the average numbers of bounced colonies per plate in relation to the Mutagenic substance were analyzed by using SPSS software and one-way analysis of variance.

Conclusion: In this study, the anti-cancer effects of different extracts of citrus were indicated that can be appear due to the presence of flavonoids with antioxidant compounds like vitamin C and A.

Keywords: Anti-cancer, Citrus, Salmonella Typhimurium.

Introduction

Most of the mutagenic and carcinogenic substances through free radicals such as free radicals of oxygen (Reactive Oxygen Species- ROS) show their destructive effect (1). In many cases, different inhibitory mechanisms in the body can not apply the full protection of aid and requires a combination of antioxidants, especially food. Some of natural
and synthetic combinations have antioxidant properties that play an important role and protect the liver against harmful factors. Actually, antioxidants are molecules or compounds that act as free radicals and can reduce the harmful effects of ROS (2), the damages cause by the reaction of oxidative to proteins, DNA and other molecules can worsen diseases such as cancer, aging, atherosclerosis, and cataract. The daily intake of antioxidants would increase immune defense of body against production of free radicals and thus these compounds can act as an anti-cancer factor(3). Due to the adverse effects of free radicals and oxidative stress reactions in the presence of antioxidant combination seem to be necessary that can protect the body from damage caused by oxidative stress. Antioxidants play an important role in the prevention and treatment of biological and medical sciences. The researchers are always looking for compounds that are known as antioxidants (4). Over the years, the plant has always been an important source of health and pharmaceuticals, some plants due to large amounts of beta-carotene and Lipotin and anthraquinone compounds, flavonoids, vitamins E, C, A are the main sources of anti-cancer (5 ).

Orange tree of Citrus aurantium is related to citrus genus and Citrus family (Rutaceae) and appears to be a hybrid between two species of orange which are obtained from C.maxima (pomelo) and C.reticulata (Mandarin), evergreen trees of orange have height averaging around 3-9 meters. The flowers of these trees (orange) are one of the most fragrant citrus flowers which are mostly white and bisexual and they can be pollinated automatically or with the help of insect. The different parts of various samples of citrus such as fruits leaves skin and roots can be used in different ways because various Citrus genus are containing active combinations and ingredients like flavonoids, Monoterpenoids, Acid Malick, Acid Citrica and Sugars, Proteins and Salts, Vitamin C organic minerals, protein and minerals. Orange is a macro medicinal plant and it is native to Iran country and is known assour orange.

Methods

This study is conducted with the aim of investigating the anti-mutation effects of citrus (orange fruit) extracts based on the dismutase test of Salmonella / microsomes which is described by Maroon Vimes and also the alternative test which was changed by Mortelmans and Zeiger (11). In this regard, a mixture should be prepared entitled by S9 (livermicrosomes). To prepare S9, mice by theapproximateweighingof25 ± 2were starved for 24 hours randomly in Animal House of Payam Noor University in Birjand so in this order, liver enzymes secretion stimulated and increased by hunger. Then the animals were killed and sacrificed and animals’ livers with sterile forceps were removed from the livers in cold sterilized potassium chloride 0.15 M and freshly prepared, were washed for several times. After washing, sterilizing livers in a sterile porcelain mortar were totally crushed with scissors, 3cc of potassium
chloride per each grams of rat liver (0.15M) were added to the livers and when the homogenized mixture was prepared, it was distributed in sterile centrifuge tube and for 10 minutes at around of 8700 rpm (900 gr) and centrifuged at 4 ° C. were oriented, in this regard, red blood cells are separated and the milky supernatant was used and isolated supernatant (mix S9) blended with the necessary cofactors (NADP and 6P G- glucose-6 Phosphate). In other hand, it was used of the Salmonella typhimurium bacteria of TA \textsubscript{100} for the mutant substance which is related to Histidine for Ames test (Ames). New bacteria cultivation should be used for the test and incubation time in the fresh overnight cultivation of bacteria in nutrient broth environment should not exceed from 16 hours. The proper concentrations of bacteria were considered 1-2 ×10^9 cells per ml. To perform this test, 3 tubes containing 50 ml fresh overnight culture and TA \textsubscript{100} 0.5 ml of Histidine and biotin solution /5Mhistidin/./5Mbiotin and ml 10 Top agar (50g / lit Agar + 50 gr / lit NaCl) and carcinogen of azide Sodium (1/5 Mgr / ml Sodium azide) were prepared then added to each tubes and pipes 0.5 ml of mixture of S9 \textsubscript{9} respectively each material was mixed with 100 ml of water extract (Group 1), ethanol (group 2) and methanol (Group 3) sterilize orange. In addition, one group was considered as a negative control group that did not receive mutagenic substances and sodium azide and only extracted and distilled water was added to the other contents of the tube. And to the positive control group was not added any extracts, and this group was only received mutagenic substances and sodium azide and other contents of the test tube. And then the contents of the tube after 3 seconds of shaking was distributed at the average glucose level of at least 40% glucose agar and for 48 hours at 37 ° C were placed in incubator. After 48 hours, the numbers of returned colonies were counted on each platform. The difference between the average numbers of returned colonies per plate in connection with mutagens was performed by using SPSS software and one-way ANOVA analysis. To be ensuring about the integrity of the results, three-way plates were considered for each material. Mutagenic effects of sodium azide in the absence of test specimens were conducted for preventing of 100 percent or zero percent cases. Percentage of inhibition was calculated according to the following equation:

$$S = \left(1 - \frac{T}{M}\right) \times 100$$

In which, T is the number of returned colonies in each plates and in the presence of anti-mutant factor, S is the percentage of inhibition of mutation and M is the number of returned colonies in each plate of positive control. The number of returned colonies spontaneously in the negative control must be decreased from the numerator and denominator that based on research of Ang, when the percentage is equal or less than 25 percent, anti-mutagenic
activities of weak inhibition between 25 and 40 percent, was average and as long as the percent inhibition is more than 40%, the dismutase effect of sample gets stronger (12).

Preparation of Plant Extracts: In this study, was used of orange fruit (citrus) which is provided from the Nowferest region of South Khorasan province and its species was confirmed by the Botanical experts of South Khorasan PNU. In order to prepare this extraction: plant fruit was dried in suitable conditions and away from direct sunlight and then was powdered and in equal proportions with 80% ethanol, 80% methanol and soaked in distilled water for 48 hours and every 8 hours for 10 minutes was on the shakers machine. After filtration with the round of 4500 were centrifuged for 8 minutes to separate suspended particles in it. The obtained liquid was placed in the oven at 70 °C so the water and alcohol evaporated and dried completely (percolation method). The extraction provided by the saline was diluted. Each of the extracts in this study due to previous research conducted on the effects of medicinal orange plant was considered 6% (13).

Results and Discussion of the Results:
The results clearly showed that all aqueous, ethanol and methanol extracts of bergamot plant has strong antibacterial effects against sodium azide. The ethanol extract with 75% has the most effective anti-ejection and aqueous extract with 54% has the lowest among the three extracts with anti-mutagenic activities, and positive and negative control group did not show any mutation effect (Table 1).

Table 1: Evaluation of the dismutase effect of citrus extract on sodium azide in laboratory conditions by using strains of Salmonella typhimurium in the presence of rat liver extract*** \( p \leq 0.5 \).

<table>
<thead>
<tr>
<th>The experimental groups</th>
<th>Returns the number of colonies</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>22 ± 2</td>
<td>Without percentage</td>
</tr>
<tr>
<td>Positive control</td>
<td>138± 15.62</td>
<td>Without percentage</td>
</tr>
<tr>
<td>Group 1 (extract)</td>
<td>76± 7.21</td>
<td>54% ***</td>
</tr>
<tr>
<td>Group No. 2 (with ethanol extract)</td>
<td>52± 12.71</td>
<td>75% ***</td>
</tr>
<tr>
<td>Group 3 (with methanol extract)</td>
<td>65± 16.9</td>
<td>63% ***</td>
</tr>
</tbody>
</table>

According to the research of Ang, when the percentage of mutant inhibition is more than 40%, the sample dismutase effect is strong (12). In this study, all the citrus extracts showed high percentage that reflects the inhibitory effect of anti-mutation and the presence of antioxidant compounds in it.
The researchers showed that upper values of Flavonoids cause containment of DNA damage in the presence of free Radicals. In addition to that, antioxidants vitamins, such as vitamin C have been demonstrated by other researchers (14). Since the researchers have managed eight kinds of Flavonoids from citrus extraction (15, 16) and on the other hand, the presence of Protein materials and minerals of vitamin C, in citrus fruits have been proven (9,10) so we can attribute some parts of an anti-ejection properties to antioxidant properties.

In Ames test which was carried out in this research, all tested extracts showed the ability of inhibition mutations of the sodium azide mutation in the presence of liver mutagenic microsomes. Strong anti-mutagenic activities of extracts, especially ethanol extract of citrus showed the effective antioxidant compounds in the extracts and better dissolving of them in ethanol.

**Conclusion**

In this study, all aqueous, ethanol and methanol extracts of citrus fruits by containing inhibition of high mutation with a percentage of 40%, showed a strong antioxidant effect and all the tested extracts, showed that ethanol extract with 75% had the greatest anti-mutagenic impact and aqueous extract with 54% had the lowest anti-mutagenic impact among the tested extracts and since so far no poisoning in injectable dose of citrus in this study have not been reported in different previous tests so it can be argued that orange consumption can be somewhat prevent from the genetic mutations, however, it should be noted that the genetic damage is created form different mechanism and ways and none of these tests alone cannot provide and study all possible mechanisms and ways and this requires further study and wider molecular mechanisms, so as to achieve a good strategy for the prevention of DNA damage.

**References**


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