

Antimutagenesis Effects Of Naringin

Maliheh Entezari, and Fahimeh Ostadzadeh

Abstract—Cancer is one of the main cause of mortality in the world which appears by the effect of environmental physico-chemical mutagen and carcinogen agents. Chemotherapy and radiotherapy improve remission of the disease but some probability relaps is observed between 20-30% patients. A number of natural antitumor drugs exert their therapeutic effect by inducing or promoting apoptosis.

In the last years, many studies have been performed on the anticancer effects of flavonoids. In cancer therapies, natural compounds have been considered as effective inhibitor agents. The purpose of this research is to examine antimutagenicity and anticancer effect of Naringin. The Naringin was subsequently 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). In Ames Test the Naringin prevented the reverted mutations and the hindrance percent of Naringin was 91.4%. This is the first study that have revealed antimutagenicity effect of Naringin.

Keywords— Naringin,,Anticancer, Ames Test

I. 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] Very recently, identifying active components from food, vegetabeles, fruits with apoptosis inducing activity against cancer cell lines and has been emphasized that apoptosis is considered as a primary mechanism for chemoprevention of cancer. Thus, The studies devoting to assess that mechanisms of action of cancer cells appear to be remarkable importance. [3-6]

Many studies report that a high diet in fruits and vegetables lowers the incidence of cancer [7-9] It has been reported that various fruit and vegetable extracts, particularly grape extract are capable of inhibiting the protoasome activity and this inhibition is associated with tumor cell apoptosis [10]. This research has been tried to consider antimutagenesis effect of Naringin throughh Ames test. The Ames test uses mutant strains of *Salmonella typhimurium* that cannot grow in the absence of the amino acid histidine because a mutation has occurred in a gene that encodes one of the nine enzymes used in the pathway of histidine synthesis that prevents translation of a functional enzyme, and thus the conversion of the catabolic intermediate to histidine

cannot be completed. Therefore, the Ames mutants are auxotrophic and are called *histidine-dependent* or *his-* (pronounced hiss-minus) mutants because they can only grow if histidine is supplied in the growth medium.[11]

II. MATERIAL AND METHODS

Ames test has been used as a current method to assess antimutagenesis effect of *Citrus nobilis* on mutant bacteria, *Salmonella typhimurium*. *Salmonella typhimurium* TA100 used for Ames test. The mutant strain,in need of histidine, directly receipt from professor Ames. 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. According to Ames, *Citrus nobilis* was added to test tube containing 0.5ml of the overnight fresh bacterial culture, 0.5ml of histidine and biotin solution (0.5mMhistidin/0.5mMbiotin), 10 ml top agar(50 gr/lit Agar + 50 gr/lit NaCl), sodium azide as a carcinogene(1.5 µgr/ml Sadium 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 fruit juice 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 و 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 anticancer-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 and Naringin and M shows reversed colonies in petri dishes related to positive control(mutagen).

Maliheh Entezari, Department of Biology, Islamic Azad University, Tehran Medical Branch, Tehran, Iran, Email: entezarimali@yahoo.com

Fahimeh Ostadzadeh, Department of Biology, Islamic Azad University, Tehran Medical Branch, Tehran, Iran, Email: fahimeh84@yahoo.com

III. RESULTS

The results of colony counting in Ames test showed that there was a significant difference between Naringin antimutagenesis effect on colony growth with control (sodium azide) ($P < 0.01$).

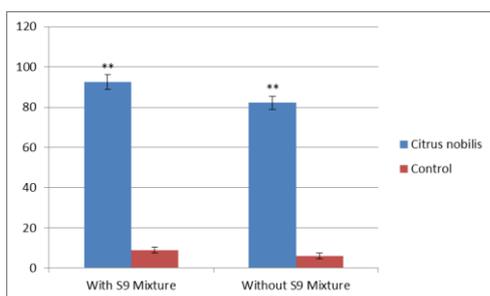


Fig. 1 Results of prevention percentage in Ames test of the Naringin in mutagenesis test

IV. DISCUSSION

Phenolic compounds constitute one of the main classes of secondary metabolites. [12] They also contribute to the nutritional qualities of fruits and vegetables. Among These compounds, flavonoids constitute one of the most ubiquitous groups of plant phenolics. [13] Flavonoids occur as aglycones, glycosides and methylated derivatives. [14] Flavonoids belong to a chemically heterogeneous group of small molecules with chemopreventive activity. [15] They exert specific cytotoxic activity towards cancer cells which has generated large interest in developing flavonoid based cytostatics for anti-cancer therapy. [16] Previous studies have demonstrated a significant anti-cancer activity in some natural flavonoids such as apigenin [17], genistein [18] quercetin [19] and luteolin [20]. In other studies, flavone, [21] luteolin, [22] genistein, [23] quercetin, [24] and fisetin [25] induced significant apoptosis in Bv-173 cells, while genistin and rutin did not. Obviously, it is important to have a rapid and inexpensive assay for testing chemicals we suspect are carcinogenic. 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. 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% [26]. To determine the number of revertants following exposure to a mutagen, and differentiate between the mutant strain (*his*- auxotrophs) and the new mutants we may generate (*his*+ revertants), the Ames test uses a chemically defined medium, in which the amounts of every ingredient are known. If a *his*- culture were placed on a chemically defined minimal agar lacking histidine, only those cells that have mutated to *his*+ (revertants) would grow and form colonies. In theory, the number of colonies that revert and grow is proportional to the mutagenicity of the test chemical. [27]

The chemically defined medium used for the Ames test actually has a trace (growth limiting) amount of histidine. Trace amounts of histidine in the medium are necessary

because some mutagenic agents react preferentially with actively replicating DNA. When *his*- strains are plated on this medium, they grow until they run out of histidine (only 2-3 cell divisions lasting about one hour), and the result is a faint, nearly invisible lawn of growth within the overlay. Conversely, revertant bacteria should form large colonies because their growth is not limited because they can produce their own histidine. Each large colony represents one revertant bacterium and its offspring. By definition in the Ames test, a mutagen is any chemical agent that results in more than twice the number of mutants as occur spontaneously, and thus is potentially carcinogenic for humans.

Because the assay does not use a live animal model, it is inexpensive, easy, and fast. 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] This was found in the Naringin. The reverted mutations and the hindrance percent of Naringin was 91.4%. This is the first study that have revealed antimutagenicity effect of Naringin.

REFERENCES

- [1] D.W. Nicholson, "Caspase structure, proteolytic substrates, and function during apoptotic cell death," *Cell Death Differ.* Vol.6, pp. 1028-1042, 1999
<http://dx.doi.org/10.1038/sj.cdd.4400598>
- [2] T.K. Mao, J.J. Powell, J. van de Water, C.L. Keen, H.H. Schmitz, and M.E. Gershwin, "The influence of cocoa procyanidins on the transcription of interleukin-2 in peripheral blood mononuclear cells," *Int. J. Immunother.* Vol.15, pp. 23-29, 1999.
- [3] Y.C. Chen, S.C. Shen, J.M. Chow, C.H. Ko, and S.W. Tseng, "Flavone inhibition of tumor growth via apoptosis in vitro and in vivo," *Int. J. Oncol.* Vol. 25, pp. 661- 670, 2004.
- [4] E. Riboli, and T. Norat, "Epidemiologic evidence of the protective effect of fruit and vegetable consumption on cancer risk," *Am J Clin Nutr.* Vol.3(Suppl.), pp. 559S-69S, 2003.
- [5] K. Tadjalli-Mehr, N. Becker, M. Rahu, A. Stengrevics, J. Kurtinaitis, and M. Hakama, "Randomized trial with fruits and vegetables in prevention of cancer," *Acta Oncol.* Vol. 4, pp. 287-93, 2003.
<http://dx.doi.org/10.1080/02841860310011852>
- [6] N.J. Temple, and K.K. Gladwin, "Fruit, vegetables, and the prevention of cancer: research challenges," *Nutrition.* Vol.5, pp. 467-70, 2003.
[http://dx.doi.org/10.1016/S0899-9007\(02\)01037-7](http://dx.doi.org/10.1016/S0899-9007(02)01037-7)
- [7] M.S. Chen, D. Chen, and Q.P. Dou, "Inhibition of proteasome activity by various fruits and vegetables is associated with cancer cell death," *In Vivo.* Vol. 1, pp. 73-80, 2004.
- [8] M. Hashemi, M. Nouri Long, M. Entezari, Sh. Nafisi, and H. Nowroozi, "Anti-mutagenic and pro-apoptotic effects of apigenin on human chronic lymphocytic leukemia cells," *Acta Med Iran.* Vol. 48, pp. 283-8, 2010.
- [9] M.L. Traub, M. R.S. De Butte-Smith, Z. Zukin, and A.M. Etgen, "Oestradiol and insulin-like growth factor-1 reduce cell loss after global ischaemia in middle-aged female rats," *J. Neuroendocrinol.* Vol. 21, pp. 1038-1044, 2009.

- <http://dx.doi.org/10.1111/j.1365-2826.2009.01927.x>
- [10] T. W. Wu, L. H. Zeng, J. Wu, and K. P. Fung, "Morin: a wood pigment that protects three types of human cells in the cardiovascular system against oxyradical damage," *Biochemical Pharmacology* Volume: 47, Issue: 6, Pages: 1994;1099-1103.
- [11] B.N. Ames, "Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test," *utat Res*, Vol. 3, pp. 347-349, 1976.
- [12] J.B. Harborne, *The flavonoids- Advances in Research Since 1980*, 1st ed. London, UK.: Chapman and Hall, 2009.
- [13] H. Chang, M. Mi, W. Ling, J. Zhu, Q. Zhang, N. Wei, Y. Zhou, Y. Tang, and J. Yuan, "Structurally Related Cytotoxic Effects of Flavonoids on Human Cancer Cells in Vitro," *Arch Pharm Res*, Vol 31, pp. 1137-1144, 2008.
<http://dx.doi.org/10.1007/s12272-001-1280-8>
- [14] K.Brusselmans, R. Vrolix, G. Verhoeven, and J. V.Swinnen, "Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity," *J. Biol. Chem.*, Vol., 280, pp. 5636-5645, 2005.
<http://dx.doi.org/10.1074/jbc.M408177200>
- [15] S. I.Choi, C. S. Jeong,, S. Y. Cho., and Y. S. Lee, "Mechanism of apoptosis induced by apigenin in HepG2 human hepatoma cells: involvement of reactive oxygen species generated by NADPH oxidase," *Arch. Pharm. Res*, Vol. 30, pp. 1328-1335, 2007.
<http://dx.doi.org/10.1007/BF02980274>
- [16] M. A.Jeune, J. D., Kumi, and J.Brown, "Anticancer activities of pomegranate extracts and genistein in human breast cancer cells," *J. Med. Food*, Vol. 8, pp. 469-475, 2005.
<http://dx.doi.org/10.1089/jmf.2005.8.469>
- [17] M. R.Vijayababu, P. Kanagaraj, A.Arunkumar, R.Ilangovan, A.Dharmarajan, and J.Arunakaran, "Quercetin induces p53-independent apoptosis in human prostate cancer cells," *Oncol Res*, Vol.16, pp. 67-74, 2006.
- [18] M.R. Vijayababu, P. Kanagaraj, A. Arunkumar, R. Ilangovan, A. Dharmarajan, and J. Arunakaran, "Quercetin induces p53-independent apoptosis in human prostate cancer cells by modulating Bcl-2-related proteins: a possible mediation by IGFBP-3," *Oncol. Res*, Vol. 16, pp. 67-74, 2006.
- [19] Y.Lim do, Y.J eong, A. L.Tyner, and J. H.Park, "Induction of cell cycle arrest and apoptosis in HT-29 human colon cancer cells by the dietary compound luteolin," *Am. J. Physiol. Gastrointest. Liver. Physiol*, Vol. 292, pp. G66-75, 2007.
- [20] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *J. Immunol. Methods*, Vol. 65, pp. 55-63, 1983.
[http://dx.doi.org/10.1016/0022-1759\(83\)90303-4](http://dx.doi.org/10.1016/0022-1759(83)90303-4)
- [21] R. Strick, P.L. Strissel, S. Borgers, S.L. Smith, and J.D. Rowley, "Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukaemia," *Proc Natl Acad Sci*, Vol. 97, pp. 4790-5, 2010.
<http://dx.doi.org/10.1073/pnas.070061297>
- [22] F. Casagrande, and J.M. Darbon, "Effects of structurally related flavonoids on cell cycle progression of human melanoma cells: regulation of cyclin-dependent kinases CDK2 and CDK1," *Biochem Pharmacol.*, Vol. 61, pp. 1205-15, 2010.
[http://dx.doi.org/10.1016/S0006-2952\(01\)00583-4](http://dx.doi.org/10.1016/S0006-2952(01)00583-4)
- [23] D.H. Han, M.S. Denison, H. Tachibana, and Yamada K, "Relationship between estrogen receptor-binding and estrogenic activities of environmental estrogens and suppression by flavonoids," *Biosci Biotechnol Biochem*, Vol. 66, pp. 1479-87, 2009.
<http://dx.doi.org/10.1271/bbb.66.1479>
- [24] D.W. Lamson, and M.S. Brignall, "Antioxidants and cancer, part 3: Quercetin," *Altern Med Rev*, Vol. 5, pp. 196-208, 2000.
- [25] C.A. Lipinski, F. Lombardo, B.W. Dominy, and P.J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings". *Adv. Drug. Del. Rev*, Vol. 23, pp. 3-25, 2005.
[http://dx.doi.org/10.1016/S0169-409X\(96\)00423-1](http://dx.doi.org/10.1016/S0169-409X(96)00423-1)
- [26] J. Sunj, Y.F. Chu, and X. Wu, "Antioxidant and antiproliferative activities of common fruits," *J Agric Food Chem*, Vol 25, pp. 7449-54, 2002.
- [27] M. Entezari, M. Hashemi, M. Ashki, S. Ebrahimian, M. Bayat, and A.R. Azizi Saraji ., "Studying the effect Echinophora platyloba extract on bactira (Staphilococcus aureus and Pseudomonas aeroginosa) and fungi (Candidia albicans, Aspergillus flavus and Aspergillus niger) invitro," *World Journal of Medical Sciences*, Vol. 4, pp. 89-92, 2009.