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Effect of *Rosa multiflora* extract on chemical mutagens using Ames Assay

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Abstract

Antimutagenicity of flower pleats of *Rosa multiflora* extract was checked by using "Ames Assay" for direct acting mutagens and those requiring metabolic activation. Two strains of *Salmonella typhimurium* – TA 98 and TA1535 were used. These strains are histidine requiring mutant strains. When mutagen is added to the culture, the strain is mutated back, there by losing the histidine dependence for its growth. The addition flower extract it prevents the strains to be mutated back to the non-histidine dependence for its growth. For direct acting mutagens sodium azide, ethidium bromide and hydroxyl amine with 1 mg rose flower extract gives 93%, 98%, 95% inhibition in revertant colonies were observed. For mutagen requiring activation, nicotine in tobacco and cigarette gives 97% and 94% inhibition of revertant colonies. These above results indicate that rose flower extract could inhibit the mutagenicity induced by direct acting mutagens as well as mutagen needing activation. These results indicate possible antimutagenic activity of compounds present in rose flower.

Key words: *Rosa multiflora*, sodium azide, ethidium bromide, hydroxyl amine and histidine.

Introduction

Large numbers of plant species are a source of biologically active compounds which include polyphenols, different types of carotenoids such as α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene and zeaxanthin [1, 2]. Many phenolic compounds present in food and vegetable posses potent and desirable biological activities against cancer. Various natural carotenoids, besides β-carotene were proven to have anticarcinogenic activity and some of them showed more potent activity than β-carotene. Lutein is the major carotenoids present in human blood. It is

covalently bound to one or more fatty acids present in some fruits and flowers. Lutein improves visual function in some patients with retinal degeneration [3]. Compounds from plant could act as protective agent with respect to human carcinogenesis, acting against the initiation, promotion or progression stages of the process [4, 5]. Invitro and in vivo studies have shown that carotenoid supplementation is associated with decreased DNA damage. Among these lutein is the most important carotenoid. It has specific biological functions in decreasing cancer development [6] enhancing immune function and protecting against ate related macular degeneration. Dietary lutein also reduced the mammary tumor growth and development. Infection with bacteria and viruses that may develop into cancer. Important mechanisms by which infectious agent may include carcinogenesis include the production of chronic inflammation, the transformation of cells by insertion of oncogenes and inhibition of tumor suppressors, and the induction of immunosuppression [7].

The chemical origin of human malignancies was recognized by observations of unusual cancer induction in persons in certain occupational groups. The capacity of the chemicals to cause cancer has been studied in animals. Environmental and life style expenses can modify cancer risk. Individual genetic factor also influence cancer risk. Since mutations are largely responsible for activating protoongenes and inactivating tumor suppressor genes, the mutational spectra of chemical and physical carcinogens are of interest to define endogenous and exogenous mutational mechanisms [8]. Several chemicals have been implicated in cancer causation. Some of these are sodium azide, ethidium bromide, hydroxyl amine, MNNG, NPD etc.

In this study we have critically evaluated the antimutagenic activities of rose flower extract using *Salmonella typhimurium* strains (Ames test). Carotenoids and polyphenols have been shown to have significant antioxidant activity and have been shown to be useful in reducing the oxidative stress induced by UV radiation and other chemicals. It protects the macular and skin. Many carcinogenic agents have been shown to induce mutation through interaction with material. In this investigation, we have studied the effect of rose flower extract on reversing the mutation with azide, ethidium bromide and hydroxyl amine as well as nicotine content in tobacco leaf and cigarette in *Salmonella typhimurium* strains TA 98 and TA 1535.

Results and Discussion

Genotype testing

Histidine requirement **-** Growth was observed only on Histidine/ Biotin plates for tester strains TA98 and TA1535 after 24 hours incubation at 37ºC. Control plates did not show growth indicating the absolute requirement of histidine/ biotin for the strains to grow.

rfa mutation **-** The tester strains TA 98 and TA 1535 showed a clear zone of inhibition appeared around the crystal violet disc after incubation at 37ºC for 24 hrs. The clear zone indicated the presence of rfa mutation, which permitted large molecules such as crystal violet to enter and kill the bacteria that confirmed rfa mutation.

UVrB mutation **-** TA 1535 and TA 98 showed growth only on unradiated side of the plates. The irradiated side of the plates did not show any growth. This indicates the UV sensitivity of the organism due to UVrB deletion.

R Factor- Growth was observed in the ampicillin plates along the streaks made with TA98 and it is consider as R factor. This strain possessed PKM 101, DNA essential for ampicillin resistance. No growth was seen on TA1535 as it had no R-factor and it was tested as a control for ampicillin sensitivity. The R-factor served as a convenient marker that made it possible to test for the presence of plasmid.

Toxicity **-** We have tested the toxicity of Rose flower extract by plating different concentration of flower extract such as 100µg- 1µg/ plate. Addition of flower extract did not inhibit the growth of the organism indicating that rose extract did not show any toxicity to these organisms.

Antimutagenic activity of Rose extracts using direct acting mutagen **-** Activity of the extract was dependent on concentration of the mutagen used, nature of mutagen, type of Salmonella strain used.

Antimutagenic activity of rose extracts using mutagens needing activation **-** Mutagenicity produced by nicotine present in the tobacco leaf and cigarette (5mg/plate) to *Salmonella* strain TA98 after its activation using S9 fraction was found to be inhibited by Rose flower extracts. There were 97, 88, 73, and 61% inhibition in the number of revertant colonies formed when nicotine in the tobacco leaf was used to induce mutagenicity by rose flower extracts at 1mg, 500µg, 250µg, and100µg respectively. In the case of cigarette, inhibition of revertant colony formation was 94, 81, 69, and 54 % at 1mg, 500µg, 250µg, and 100µg respectively.

The above results indicate that the rose flower extracts could inhibit the mutagenicity induced by direct acting mutagens as well as mutagens needing activation in the *Salmonella typhimurium* strains. It showed most significant inhibition to the mutagenicity induced to TA 1535 the direct acting mutagens such as NaN_3 and EtBr. It also showed significant inhibition to the mutagenicity induced to TA 98 by direct acting mutagen hydroxyl amine and nicotine present in tobacco leaf and cigarette which was activated by mammalian liver S9 fraction. These results indicated possible antimutagenic activity of the compounds present in Rose flower.

It has been suggested that the use of antimutagen in daily life will be the most effective procedure for preventing human cancer and genetic disease. These compounds interfere with mutagen metabolism or they may act as mutagen scavengers [13]. They may also inhibit either the initiation or promotion phase of the carcinogenic process [14, 15 and 16].

Cancer is a broad term used for identifying a large number of diseases. It is one of the major diseases that affect nearly 25% of the population in developed countries and nearly 50 %of these deaths are due to this disease. Somatic gene mutations are the basic events for the conversion of a normal cell to a mutant cell. This mutant cell converted to malignant cell through several genetic changes [17]. Specific inhibition of these steps can significantly inhibit the cancer causation. Apoptosis is also an important mechanism of cellular defense in reducing the risks, so

the natural products which can induce the apoptosis may inhibit the malignant cell formation [18].

Cancer cells are quickly adapted to the toxic environment, keep dividing and forming more cells without a control mechanism. These factors make cancer an extremely difficult disease to treat. The anticancer drugs destroy cells by stopping them from growing or dividing at one or more points in their growth cycle. But the chemotherapy kills the healthy normal cells also. To avoid that herbal therapies are followed. There are several plant products which have been shown to prevent the development of cancer in animals and some of them are in clinical trials. These drugs are called chemopreventive agents which include carotenoids, polyphenols, and flavanoids and so on. More than thousand natural chemopreventive agents have been identified. Chemotherapy drugs work by varying mechanisms to induce cellular death. Some chemotherapy drugs kill cells by inflicting massive free- radical damage, while other chemotherapy drugs interfere with different cellular metabolic process in order to eradicate cancer cells. Depending on the type of cytotoxic drugs used, however, antioxidants may confer protection to cancer cells during active chemotherapy [19].

In the present investigation we have checked the potential activity of rose flower extracts for its antimutagenicity using *Salmonella typhimurium* assay. This is mainly based on the reversion of mutant cells by mutagenic agents that are Ames test. It was found that the Rose flower extract would inhibit the revertant formation produced by direct acting mutagens such as sodium azide, ethidium bromide, and hydroxyl amine. The rose extracts showed more than 95 % inhibition of mutagenicity at concentration of 1 mg/plate and the activity decreased with decreased concentration. It could also produce significant inhibition of mutagenicity produced by nicotine present in tobacco leaf and cigarette which gets activated by P450 enzyme. These results are highly significant. More research works are needed to find out the potential of the compounds present in rose extracts as a chemopreventive agent to fight against cancer.

For direct acting mutagens sodium azide, ethidium bromide, and hydroxyl amine with 1 mg Rose flower extract gives 93%, 98%, 95% inhibition in revertant colonies were observed. For mutagen requiring activation, nicotine in tobacco and cigarette gives 97% and 94% inhibition of revertant colonies. These above results indicate that rose flower extract could inhibit the mutagenicity induced by direct acting mutagens as well as mutagen needing activation.

Materials and Methods

Mutagens- Sodium azide, Ethidium bromide, Hydroxyl amine
Antibiotics- Ampicillin
Animal-Wister rat for the preparation of S9 fraction

***Salmonella* mutagenicity test (Ames test) -** *Salmonella typhimurium* strains TA1535 and TA98 were. The strain TA1535 detected mutagens that caused base pair substitutions; TA98 detected various frameshift mutagens. Frozen cultures of the tester strains were stored at -20ºC.

Rose flower extract- In different concentrations the rose flower extracts was prepared.

Confirming genotypes of the *Salmonella* strain

Histidine requirement - The histidine character of the tester strains was confirmed by demonstrating the histidine requirement for growth on selective agar plates such as histidine/biotin plate and biotin control plate. Biotin was also required by all of the tester strains because of the UVrB deletion which extended through the bio-gene. Cotton swab was dipped in the 12 hour broth culture and a single sweep was made across the histidine/biotin plate. Then the plates were incubated overnight at 37ºC and the growth was examined on the next day.

rfa mutation -Strains having the deep rough (rfa) character were tested for crystal violet sensitivity [9]. 0.1 ml of fresh overnight culture of the tester strains (TA98 and TA1535) was added to a tube containing 2 ml of molten agar at 45ºC. The top agar tubes were vortexed for 3 sec at low speed and poured on nutrient agar plate without histidine and biotin. The plates were tilted and rotated for the even distribution of the top agar on the plates. The plates were placed on a leveled surface and allowed several times for agar to become firm.10µl of 1 mg/ml solution of crystal violet was pipetted to the center of sterile paper disc (1/4 inch) and discs were transferred to each of the inoculated plates using sterile forceps. The discs were lightly pressed with forceps to embed it slightly in the overlay. The plates were incubated at 37ºC and observed for crystal violet sensitivity.

UVrB mutation – The UvrB mutation was confirmed by demonstrating UV sensitivity in strains that contain this mutation [10]. The R-factor strains TA98 and non R-factor strains TA1535 were streaked in parallel stripes with sterile swabs across the nutrient agar plate. A piece of cardboard was placed over the uncovered plate so that half of each of bacterial streak was covered. The plates were irradiated with a 15W germicidal lamp approximately at a distance of 35 cm and were irradiated for 8 sec. T he irradiated plates were incubated at 37º C for 12-24 hours.

R factor – The R-factor strains TA98 were tested for the presence of the ampicillin resistance factor. To test for ampicillin resistance, the cultures were streaked across of an ampicillin plate using the procedure as described for confirming the histidine requirement. The non R-factor strain, TA1535 was tested on the same plate as a control for ampicillin activity.

Extraction of compounds from Rose flower - The extraction procedure was done using the Soxhlet extractor. The Rose petals were dried and powdered. 40 grams of the rose powder was run through the Soxhlet extractor using 200 ml of acetone as the solvent. The extraction was continued for 4-5 hours and was collected in separate petridishes. Then the petridishes were kept opened for few hours for the complete evaporation of the solvent. After that the extract was collected in pasty form by adding petroleum ether.

Antimutagenicity assay

Antimutagenicity of flower was tested in *S .typhimurium* strains TA98 and TA1535 using direct acting mutagens and those needing activation by Ames test [11].

1. Determination of antimutagenicity against direct acting mutagens - Plate incorporation method was done for antimutagenicity assay without microsomal activation. Fresh bacterial cultures of *S. typhimurium* strains TA 1535 and TA 98 (1-2x10^9cells/ml) were mixed with

2ml of molten agar containing 0.5 mm histidine/biotin solution, different concentration of flower extract (0.1-1 mg/plate) and direct acting mutagens such as sodium azide (2.5µg/plate), ethidium bromide (2.5µg/plate) or hydroxyl amine (1µg/plate). Further it was spread over minimal glucose agar plates. Plates were incubated for 48 hours at 37ºC and the revertant colonies were counted.

Table 1: Antimutagenicity of flower pleats of *Rosa multiflora* extract against Ethidium bromide & hydroxyl amine on *Salmonella typhimurium* strain TA 1535

Group	Average no. of colonies present		% inhibition	
	Ethidium bromide	Hydroxyl amine	Ethidium bromide	Hydroxyl amine
Control	255	288	-	-
1 mg	5	14	98	95
500 µg	46	58	82	80
250 µg	74	110	71	62
100 µg	89	149	65	48

2. Determination of antimutagenicity against mutagens needing activation - Antimutagenicity of Rose flower extract was tested in *S. typhimurium* strain TA98 against mutagen needing microsomal activation, nicotine using cigarette and tobacco leaf (5 mg/plate) according to the method of plate pre-incubation [12]. The contents in the cigarette were powdered and are used as the mutagen. Similarly the tobacco leaves were dried and powdered and used it as the mutagen. 0.5 ml of S9 mix was incubated with these mutagens, 0.1 ml of bacterial culture (1-2 x 10^9 cells/ml) and various concentrations of flower extract and incubated for 30 minutes at 37ºC. Further it was overlaid on minimal glucose agar plates and incubated for 48 hours at 37ºC and revertant colonies were counted.

Table 2: Antimutagenicity of flower pleats of *Rosa multiflora* extract against Nicotine present in Tobacco leaf & Nicotine present in cigarette on *S. typhimurium* strain TA98

Group	Average no. of colonies present		% inhibition	
	Nicotine present in Tobacco leaf	Nicotine present in cigarette	Nicotine present in Tobacco leaf	Nicotine present in cigarette
Control	191	294	-	-
1 mg	7	18	97	94
500 µg	23	56	97	81
250 µg	51	92	73	69
100 µg	74	136	61	54

Conclusion

The antimutagenicity of rose flower extract was tested with the *Salmonella* strains by applying Ames assay. The strains are mutated by both direct acting mutagens and also mutagen needing activation. The addition of flower extract prevents the strains to be mutated back to the non-histidine dependence for its growth. For mutagen requiring activation, nicotine in tobacco and cigarette gives 97% and 94% inhibition of revertant colonies. The results indicate that rose flower extract could inhibit the mutagenicity in the strains. This is a novel approach which can be used instead of other antimutagenic plants and their extracts. This study shows that compounds which are present in rose flower have the antimutagenic activity.

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Abbreviations Used: MNNG, NPD, UV, TA, UVrB, deep rough (rfa) character, R-factor