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Short communication

Antimutagenic and Potential Anticarcinogenic Activities of Aloe-Vera Gel and Aqueous Garlic Extract in the Bacterial Reverse Mutation Test (Ames Assay)

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ABSTRACT

The study was carried out to verify the potential anticarcinogenic and antimutagenic activity of garlic and aloe-vera. The ability of aqueous garlic extract and Aloe-Vera gel to inhibit mutation in tester strain of *Escherichia coli* WP2 uvrA was determined in this study. (The tester *E. coli* tryptophan auxotroph strain was obtained from Yale University U.S.A.). The spontaneous mutation rate of *E. coli* WP 2 uvrA was determined in the Ames assay to be 2.2×10^{-7} . The acridine mutagen showed 333.8% increase of spontaneously reverting colonies of the tester strain. Different concentrations of aqueous garlic extract and aloe-vera gel conferred varying degrees of antimutagenic activities on the tester *E. coli* WP2 uvrA. Aqueous garlic extract was found to have its highest antimutagenic activity at a concentration of $0.5\text{g}/\text{cm}^3$ (the highest concentration tested) with 81.02% reduction in revertant colonies were observed. $0.1\text{g}/\text{cm}^3$ and $0.25\text{g}/\text{cm}^3$ garlic extract produced 72.16% and 74.82% reduction of revertant colonies respectively. Aloe-Vera gel produced 43.6%, 37.2% and 33.68% reduction in revertant colonies at concentration of 0.5ml, 0.2ml and 0.1ml per plate of the *E. coli* respectively. From the result obtained in this study, garlic has tremendous potential antimutagenic and anticarcinogenic substances..

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Key words: - *Oreochromis niloticus*, infection, *Clinostomum tilapiae*, histopathology

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INTRODUCTION

Cancer (Latin Cancer, Crab) is a disease or disorder in which a single normal body cell undergoes genetic transformation into a cancer cell and will begin to reproduce uncontrollable (Varmus and Weinberg, 1993). There is a high correlation between carcinogenicity and mutagenicity. Ninety percent (i.e., 156/174) of the carcinogens tested were found to be mutagenic in the Ames assay (McCann *et. al.*, 1975b). Recently, considerable attention has been focused on identifying naturally occurring chemo-preventing substances capable of inhibiting, retarding or reversing the multistage carcinogenesis (usually initiated by mutation) (Surh, 1999).

Garlic (*Allium sativum*) is one of the most popular spices in the world and it has been reported to contain a powerful natural antibiotic stronger than penicillin and tetracycline (Adetumbi and Lau, 1983). Aloe vera (*Aloe barbadensis* mill) has been reported to provide unique benefit to human body. Some of these benefits are its deep penetration of the body tissue, antiseptic effects, stimulation of cell growth, nerve settling and detoxification of the human body cell (Haller, 1990). Since a wealth of the advantages mentioned above occur in garlic and Aloe vera gel, an assay of the antimutagenic activity of these two plant extracts was the focus of this study.

Therefore, the objectives of this research was to use the bacteria reverse mutation test (the Ames test) to determine the spontaneous mutation rate of the *E.coli* WPA uvrA strain, to determine the level of mutagenicity of Acridine mutagen to *E. coli* WP2 uvrA. Also, to assay the antimutagenic activity of Aloe vera gel and garlic aqueous extract.

MATERIALS AND METHODS

The two plant samples used in this study (Garlic bulbs) were purchased at the Oke-odo market in the Ifako-Ijaye area of Lagos state while the Aloe vera plant were obtained from a private garden at Ogba Lagos state. The plants materials were certified in the herbarium of the Department of Botany and Microbiology, University of Ibadan.

The tester (mutant) strain used is the *Escherichia coli* Tryptophan auxotroph strain, *E. coli* WP2 uvrA. It is one of the several strain suggested by Ames *et al.*, (1973) for use in the bacterial reverse mutation assay. It was obtained from the *E. coli* Genetic Stoc Centers, MCD Biology Department, Yale University, New Haven, Connecticut, U.S.A. It has a strain designation of WP2 uvrA and *E. coli* Genetic stock centre designation of CGSC #5379. This strain carries a mutation of an *E. coli* B gene with the following chromosomal markers. Lon-II, Sul A1, trpE 65(OC) and uvrA 155.

Garlic bulbs were peeled and oven dried. The dried bulbs were blended into fine powder using a seed and nut grinder. Fifty grams of powdered garlic sample was dissolved in 250cm³ of hot distilled water and was continuously stirred for about 20 minutes. This was left standing for 24 hours in a corked conical flask. The extract was obtained by sieving the garlic and water mixture (after 24 hours) with a net sieve. Filtration was repeated using a whatman No.4 filter paper. The filtrate obtained was freeze dried at -72⁰C using an Edwards Modulyo (FDII) freezer drier.

During, analysis, a known weight of garlic powder was dissolved in distilled water and the solution was filter sterilized using a 0.2µm Millipore filter. Aloe-Vera gel was obtained by peeling the green epidermal layer of the leaf. The succulent parenchyma tissue obtained were liquidized using Phillip blender at ultra high speed. No water was added, the resultant gel was filter sterilized using 0.2µm filter. The gel was thereafter stored in a sterile bottle. Sterility test of the two plant extracts was carried out before use. All the media used were prepared according to the method of Maron and Ames (1983).

Spontaneous Mutation Rate of *E. coli* WP2uvrA: A drop of broth culture was aseptically spread over the plates of minimal glucose plate with glass spreader. The plates were incubated at 37⁰C and the *E. coli* that was able to growth (has been spontaneously mutated) were counted after 24 hours.

Mutagenic Activity of Acridine Orange: The mutagenic activity of acridine orange was tested at

2 concentrations; 100µg/plate and 50µg/plate. 1mg/ml of the acridine orange was prepared with sterile distilled water and filter sterilized using 0.2µm hydrophobic membrane filter. This was diluted with sterile distilled water to 100µg/ml and 50µg/ml. 0.1ml of the mutagen and 0.1ml of the broth culture of the bacteria were added to 2mls of molten tryptophan-top agar in the test tubes. Each tube was briefly vortex for 4 second before pouring on a minimal glucose agar plates. A control containing sterile distilled water in place of tryptophan in the top agar was set up along. The tryptophan-top agar contained 100ml of top agar and 10ml of 0.5nM of tryptophan dispensed in 2ml quantities in test tubes. The plates were incubated after the top agar had solidified at 37°C for 48 hours. The revertant colonies were counted thereafter.

Antimutagenic Activity of Plant Extract: Antimutagenic activity of the samples were assayed at different concentrations for aloe vera gel, 0.1ml, 0.2ml and 0.5ml of the 100% gel were used while garlic extract 0.1g/ml, 0.25g/ml and 0.5g/ml of the freeze dried sample were used. The method of McCann *et al.*, (1975b) was employed in the assay.

Counting of Revertant Colonies: After 48 hours of incubation, the plates used in each of the assays were scored and the revertant colonies of *E. coli* WP2 uvrA were counted using an automated colony counter. Plates with too many colonies were enumerated using the method of McCann *et al.*, (1975a). Spontaneous mutation rate was determined by

$$\frac{\text{Number of colonies on plate}}{\text{Number of cell plated}} = \frac{65}{3 \times 10^3}$$

RESULTS

The spontaneously reverting colonies of *E. coli* WP2 uvrA were determined in the Ames assay to have a spontaneous mutation rate of approximately 2.2×10^{-7} . This was obtained from the ratio of number of spontaneously reverting colonies to the

number of cell initially plated out. Mutagenic activity of the acridine orange was higher at 100µg/ml treated plates with 282×10^{12} and lower at 50µg/ml treated plates with 154×10^{12} . This chemical is therefore a strong mutagenic and potentially carcinogenic substance.

The highest concentration of Aloe-vera gel used (0.5ml) showed 43.62% reduction on the effect of the mutagen on the bacteria reversion, as shown in the table 1. The two other concentration 0.2ml and 0.5ml of aloe vera showed 37.22% and 33.69% reduction in revertant of the bacteria colonies.

The garlic aqueous extract showed remarkable antimutagenic activity with a positive dose-response relationship. 0.1/ml of garlic extract showed 72.16% reduction in the mutagenic effect of acridine on the bacterium while with 0.25g/ml of garlic extract the highest antimutagenic activity of the plant extract was obtained. It showed 81.02% reduction of the effect of acridine orange on the mutant strain of *E. coli*.

Table 1.
Effects of Treatment of Mutant Strain *E. coli* WP2 uvrA With Different Extracts and Acridine Orange

Concentrates	No. of Colonies per plant x 10 ¹²	Percentage
Acridine 100µg	282	333.8% increase
50µg	154	136.9% increase
Aloe vera gel 0.1ml	187	33.69% reduction
0.2ml	177	37.23 reduction
0.5ml	159	43.62 reduction
Garlic 0.1g/ml	78.5	72.16 reduction
0.25g/ml	71	74.82 reduction
0.5g/ml	53.5	81.02 reduction

DISCUSSIONS

Spontaneous mutation of *Escherichia coli* WP2 uvrA was determined to have reverted spontaneous rate of approximately 2.2×10^{-7} . This shows that mutation of bacteria occurs naturally even when they are not exposed to a mutagen. However, the frequency is very low. The mutagenic activity of acridine orange dye on tryptophan auxotroph *E. coli* WP2 uvrA strain was

evident in this study. The dye increased the mutation rate of the bacterial strain to 333.8% at 100µg/ml concentration of the dye.

The antimutagenic activity of the plant extract used showed garlic extract to be strongly antimutagenic than aloe vera. The high degree of antimutagenicity of garlic extract is probably due to the wealth of organosulphur compounds it contains. Phytochemical constituents of garlic include ajoene, Allian allin, allylcystein, diallylsulphide and over 100 other biologically useful secondary metabolites have been reported to be inherent in the valuable spice (Dorant, *et al.*, 1993 and Belman, 1983).

The antimutagenicity implies that garlic may have potential anticarcinogenic effect on cancer induced by mutations of the critical genes of the cell cycle. The mechanisms by which garlic may reduce the risk of cancer may not solely be via reduction of mutation; but may involve the antioxidant activity, immune systems enhancing properties and high levels of tannins present in garlic which help reduce incidence of tumors (Chung *et. al*, 1998). Hence, the consumption of this plant spice may actually be giving protection to human body against mutation of cells and cancer induced processed food substances we consumed daily. Also, Aloe vera possesses some activity against mutagens which possibly can be enhanced at higher concentration

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