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ANTIGENOTOXIC PROPERTIES OF NJAVARA RICE *ORYZA SATIVA* L. INDICA AN INDIGENOUS MEDICINAL RICE VARIETY OF KERALA

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Abstract: Njavara rice (*Oryza sativa* L. indica) is known to be a highly nutritious and medicinal variety of rice, indigenous to Kerala. Rice bran contains many bioactive ingredients showing antioxidant, antiproliferative, anti-inflammatory properties and found to be effective against hypercholesterolemia. In the present study the genotoxicity and antigenotoxicity of the water extract of Njavara rice was investigated. The mutagenicity and antimutagenicity of the extract were determined using a *Salmonella* mutation assay. Njavara rice extract was non mutagenic in TA100 strains in the absence of metabolic activation. The extract showed strong antimutagenic activity against sodium azide, a potent mutagen with an inhibition percentage of 64.5 to 87%. The inhibitory mechanism of this rice extract might be partly due to either induction of detoxifying enzymes or inhibition of metabolizing enzymes or the presence of compounds like polyphenols. Further studies must be undertaken to identify the compounds behind these properties.

Index Terms **- Njavara rice, antigenotoxicity, Ames test, chemoprevention.**

I. INTRODUCTION

India is well known for its traditional medicinal system – Ayurveda. As India is rich in plant diversity with a vast variety of medicinal plants, it is not surprising that most of the local population relies on the traditional system of medicine for treatment of many ailments. Plant extracts are widely used to cure diseases from ancient times. Ayurveda has formed a reliable treatment system for many ailments and has now attracted the attention of researchers worldwide. Multitude of the studies focusing on the bioactivity of the plant extracts and their phytochemical profiling are going on. Ayurveda not only provides treatment but also offers to rejuvenate the whole body through right kind of nutrition. In addition to the plant extracts, many tubers and rice varieties are also used for curing diseases.

In Kerala Ayurveda is the most widely used traditional medicine system. Njavara is a rice variety indigenous to Kerala, with extra short growth duration. Njavara also known as Shasthika as its maturity period is sixty days (Jose et al., 2010). Njavara is cultivated in different parts of the state of Kerala and exploration and collection of germplasm revealed that this landrace is generally distributed in the paddy fields of midland and lowland zones. The germplasm has been in rare occurrence. This rice is not only used as a cereal food but also finds importance in Ayurveda because of its medicinal properties.

Njavara kanji-the rice boiled in milk is a rejuvenating drink especially for children, aged and underweight (Sreejayan et al., 2005). Njavara rice paste is found effective for swelling in foot, reduces pain of snake bites and is also used as a healthy baby food (Kumari, 2010). Traditionally Njavara is preferred by physicians for the treatment of neuromuscular disorders such as arthritis, rheumatism, circulatory, respiratory, digestive and nervous system ailments. Njavara is also used for osteoporosis, cirrhosis of liver, piles, ulcers and during snake bites to relieve pain and wound healing (Jaba et al., 2012). Studies on Njavara rice have shown it to have strong antioxidant, antiproliferative and anti-inflammatory properties (Rao et al., 2010; Ajitha et al., 2012; V Shalini et al., 2012). It is also reported to be effective against experimentally induced hypercholesterolemia (Chithra et al., 2015). The aim of this work was to analyze mutagenic and antimutagenic properties of the water extracts of Njavara rice in histidine mutant strain of *Salmonella typhimurium* TA 100.

II. MATERIALS AND METHODS

2.1 Rice preparation

Njavara rice was bought from the market and dehulled. The seeds were powdered and extracted with distilled water for 24 hours. The solution was filtered and evaporated under reduced pressure to obtain powdered Navara rice extract. This powdered extract was used for the mutagenicity and antimutagenicity assay.

2.2 Chemicals

Agar, sodium chloride (NaCl), sodium azide (NaN3), ampicillin, citric acid monohydrate, crystal violet, D-biotin, dextrose, disodium hydrogen phosphate, ethanol 70%, hydrochloric acid (HCl), L-histidine, magnesium chloride hexahydrate (MgCl2·6H2O), magnesium sulphate heptahydrate (MgSO4·7H2O), nutrient broth, potassium chloride (KCl), potassium phosphate, dibasic (K2HPO4), sodium ammonium phosphate tetrahydrate (NaNH4HPO4·4H2O) and sodium dihydrogen phosphate monohydrate (NaH2PO4·H2O).

Figure 1. Photograph of dehulled Njavara rice used for the study

2.3 Mutagenicity assay

The mutagenicity of Njavara rice extract was determined using Ames test (Maron and Ames, 1983) using histidine mutant *S. typhimurium* TA100 in the absence of S9 activation. As per the standard method 10ml of nutrient broth was inoculated with a single isolated colony of TA100 and incubated for 5-8 h at 37 °C. in a shaker incubator at 120 rpm. Sodium azide was used as a positive control as it is capable of producing frame shift mutations in TA 100 strain. Autoclaved distilled water was used as a negative control in the experiment. Five different concentrations of both freshly prepared positive control and rice extract in distilled water were prepared (0.1µg/100µl - 0.5µg/100µl). The positive, negative control and rice extracts were mixed in the top agar and spread on to the corresponding minimal glucose plates. All plates were in triplicates and were incubated for 24 h at 37 °C. The spontaneous revertant colonies which appeared in the minimal agar were counted using a stereomicroscope.
The mutagenicity of the samples was calculated by the following equation:

$$\mathbf{MR} = \frac{\mathbf{SR + IR}}{\mathbf{SR\ +\ Negetive\ Control}}$$

Where, MR= Mutagenicity Ratio, SR= Spontaneous Reversion rate and IR= Induced Reversion.

2.4 Anti-mutagenicity assay

Antimutagenicity of the rice extract was assessed according to the preincubation method devised my Moron and Ames, 1983. A single fresh colony of standard strain of *S. typhimurium* TA 100 was inoculated in nutrient broth and incubated for 5-8 h at 37 °C. Five different concentrations (0.1µg/100µl - 0.5µg/100µl) of positive controls of sodium azide and Njavara rice extract were prepared. Test was done in triplicates for each concentration and were performed in the absence of S9 mix. 100µl of each concentration of positive control was mixed with 100µl of the corresponding concentration of aqueous rice extract were added to the top agar mix prepared according to the Moron and Ames 1983 method. The number to the revertant colonies spontaneous revertant colonies were counted manually using a stereomicroscope, after an incubation of 24 h at 37°C. The antimutagenicity results were expressed as percent inhibition calculated as described by Tachino et al., (1994).

$$Inhibition\% = 100 - [T/M \times 100]$$

where T is the number of revertant colonies in a plate containing mutagen and compounds and M is the number of revertant colonies in a plate containing the mutagen alone.
The antimutagenic effect was assessed according to the cut off values given by Resende et al., 2012 and Negi et al., 2003, where a sample showing
Inhibition lower than 25% - No antimutagenicity
Inhibition value between 25% and 40% - Moderate antimutagenicity
Inhibition values greater than 40% - Strong antimutagenicity
The cell viability was also tested for all concentrations of rice extract, positive mutagen and the combination of rice and mutagen used in the study. A sample was considered bactericidal when a reduction of cells less than 60 percent as that of the negative control were obtained (Lira et al., 2008).

2.5 Statistical analysis

Statistical analysis was done to test the significance of the mutagenicity and antimutagenicity results. The mean and standard error of means was assessed for the no of the revertant colonies obtained in different concentrations of rice, positive and rice + positive combinations. For the antimutagenicity results One-way ANOVA was done in which P-value <0.01 was considered significant.

III. RESULTS AND DISCUSSION

3.1 Results of Mutagenicity assay

Mutagenicity ratios of Njavara rice extract were found of 0.012 or less at all concentrations. Table.1 shows the number of revertants/plate of the positive control, and Njavara rice extracts, in the absence of S9 metabolic activation. Mutagenicity ratio of positive (mutagen-sodium azide) was found in the ratio of 0.04-0.09. The mutagenicity ratio of rice extract and the number of revertant colonies were less than the negative control. As evident from the graph in figure.4 there is no dose response increase in the mutagenicity ratio of rice when compared to positive mutagen sodium azide which showed a dose dependent increase in the no of reverent and the mutagenicity ratio. According to standards set for Ames test by Maron & Ames (1983), an extract can be mutagenic when there is twofold increase in the number of revertant colonies over negative control. Thus, the results clearly indicate that the Njavara rice extract is nonmutagenic without metabolic activation.

Table 1. The number of revertant colonies per plate expressed as mean and standard error in positive control, Njavara rice extract and rice in combination with the mutagen.

Serial no	Concentration ug/ml	Positive	Rice	Rice + positive
1	0.1	584 ± 13.3	93 ± 5.2	195 ± 10.5
2	0.2	949 ± 12.7	74 ± 1.8	148 ± 5.0
3	0.3	1161± 19.4	73 ±7.6	151 ± 6.9
4	0.4	1378 ± 14.6	61 ± 5.2	186 ± 5.1
5	0.5	1507 ± 21.3	59 ± 3.5	208 ± 6.7

All values are expressed as mean and standard error of means of three measurements.
Positive control- Sodium azide.
Negative control- Distilled water
Mean and SEM of the no of revertants colonies in negative control- 140 ± 1.8
Spontaneous reversion rate – 128

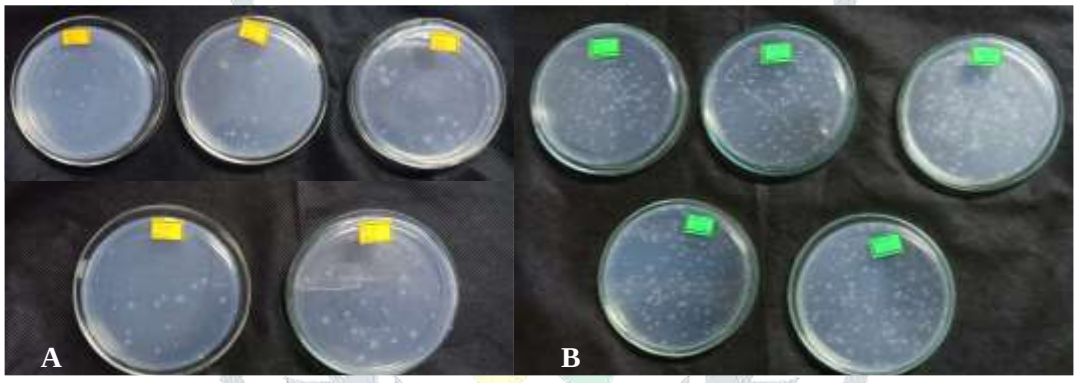

Figure 2. Plates with the revertant colonies of Salmonella typhimurium TA100 A. revertants in different concentrations of Njavara rice extract B. revertant colonies in different concentrations of rice and mutagen association

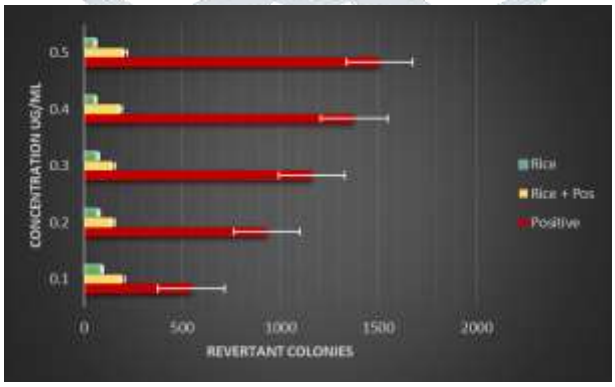

Figure 3. Mean number of revertants per plate and the corresponding standard error in different concentration of Rice extract, mutagen alone and mutagen-extract mixture

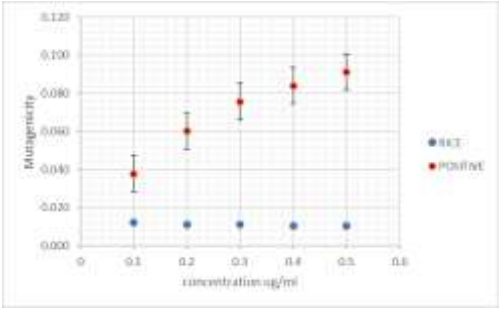

Figure 4. Mutagenicity ratio of rice compared to mutagen at different concentrations along with standard error of mean.

3.2 Antimutagenicity results

When the Njavara rice extract was mixed with equal concentrations of sodium azide, at all five concentrations (0.01-0.05 g/plate), high values of percentage inhibition was obtained. The lowest inhibition of 64.5% was obtained at 0.01g/plate concentration. For all five concentrations used in the present study Njavara rice extract showed strong antimutagenic property which ranged from 64.5% - 87%. The antimutagenicity results showed a considerable decrease in the number of revertants/plate (figure 2 & 3), and an increase in the percent inhibition (% I) of the mutagenic activity of sodium azide at higher concentrations. The results are indicated in figure.5. The One-way ANOVA test also confirmed a significant reduction in the mutagenicity of Sodium azide upon addition of Njavara rice extract as there was considerable reduction in the number of the revertant colonies as depicted by p-value of 0.004. The cell viability tests confirmed that the concentrations of Njavara rice extract above 0.01g/plate are bactericidal as the no of the viable colonies were less than 60% negative control (Lira et al., 2008). The cell viability test for mutagen and association of mutagen with Njavara rice extract have passed the cell viability test as none of the concentrations showed less than 60% as that of normal control. Thus, although the antimutagenic effects of Njavara rice extracts are higher at concentrations above 0.02g/plate these concentrations have bactericidal properties. It shows an inhibition percentage of 64.5 % at 0.01 concentration so, Njavara rice extract can be considered to have a strong antimutagenic effect on sodium azide and thus can be used as a potential chemo preventive agent against frame shift mutations.

Table 2. The mutagenicity ratio of rice and sodium azine and antimutagenic activity of Njavara rice extracts on Sodium azide expressed in % inhibition

Serial no	Mutagenicity (Positive)	Mutagenicity (Rice)	% Inhibition
1	0.04	0.01	64.5
2	0.06	0.01	84.2
3	0.08	0.01	87.0
4	0.08	0.01	86.5
5	0.09	0.01	86.2

Oneway ANOVA showed an F-crit value of 5.317
With a P value < 0.01

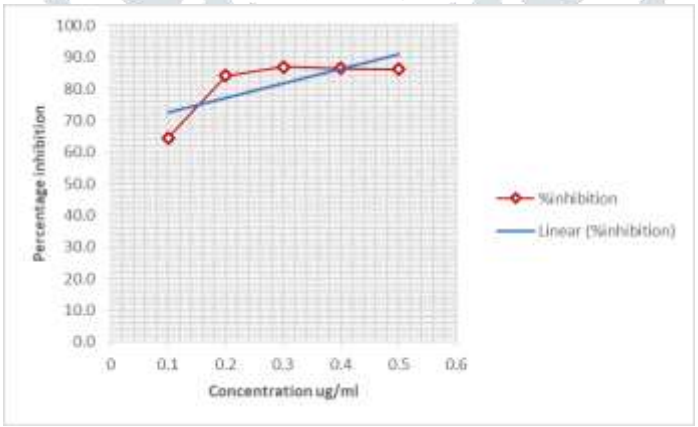

Figure 5. The linear increase in the percentage inhibition of Njavara rice on sodium azide with increasing concentration

Researchers have proved in the past few years that various rice varieties all over the world have antimutagenic properties. Purple rice from Thailand, Hom Nil rice and black glutinous rice have shown strong antimutagenic activities (Sadabpod, Kansadalampai, & Linna, 2010; Punyittayagul et al., 2010). Some works have also assessed the effect of processing and milling fraction on the antimutagenic properties of rice and found that processing activities don't affect its antimutagenic properties (Chun, You, & Cho, 1999 ; Kim, Chun, Ha, & Moon, 1995). In the present study the Njavara rice an endemic rice variety of Kerala, was tested for its mutagenic activity. The results indicated strong antimutagenic activity and bactericidal activity at higher concentrations. This rice variety is widely being used in ayurvedic treatment and therapies. Testing the antimutagenicity is required for the safe use of any formulation as a medicine. In this study antimutagenicity was tested without S9 mix, as Njavara rice pastes are used externally for treating a wide variety of skin ailments.

There have been several reports in the literature, that medicinal plants or fruit juices rich in polyphenols have strong antimutagenic and anticancerous properties (Arriaga-Alba et al., 2008). A study has shown that the crude methanolic extract from Njavara rice bran

contains significantly high polyphenolic compounds (Rao et al., 2010). The presence of these compounds could be the potential reason for its strong antimutagenic activity. Further studies are needed to find the specific compounds responsible for the antimutagenicity. To conclude Navara Rice is safe to use in the Ayurveda medicines as it is non mutagenic. As it shows strong antimutagenic effects against potential mutagen causing frame shift mutations it can be further studied to isolate chemo-preventive compounds which can help reduce the genotoxic effect of mutagens.

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