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In-vitro Anti-mutagenic Activity of *Asparagus racemosus*: An Ayurvedic Medicinal Plant

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ABSTRACT

Asparagus racemosus is a plant traditionaly used in epilepsy, as a brain tonic, cardiac disorders, hypertension, habitual abortions, weakness of the uterus, excessive bleeding during menstruation. The current study evaluated the antimutagenic pottential of methanolic (RME) and aqueous methanolic extract (RAE) extracted from *A. racemosus*. Ames assay was used to acess the antimutagenic potential of RME and RAE (2.5×10^3,1.0×10^3, 0.5×10^3, 0.25×10^3, 0.10×10^3 and 0.01×10^3 μg 0.1 mL plate^{-1}) that was added with mutagenic activation of TA98 and TA100 strain of *Salmonella typhimurium*. *A. racemosus* extract RME and RAE have been found to have effective in the inhibition of mutation induced by NPD and sodium azide. Among the two extracts, RAE showed maximum inhibition of 49.2% followed by RME having inhibition of 40.63% in Co-incubation mode. Current study indicated that *A. racemosus* can be used as a new source of anti-mutagenic.

Key words: Plant extracts, *Asparagus racemosus, Salmonella typhimurium*, antimutagenic

INTRODUCTION

Asparagus racemosus is a plant belonging to Family Liliacea is one such important medicinal plant promoting general well-being by increasing cellular vitality and resistance (Goyal *et al*., 2003). *A. racemosus* is widely distributed across the globe with distribution ranges from tropical Africa, Java, Australia, Srilanka, Southern parts of China and India, but it is mainly cultivated in India (Kirtikar and Basu, 1984). Among the traditional uses of *A. racemosus*, Shatavari is indicated in epilepsy, (Gomase and Sherkhane, 2010), as a brain tonic, helps in regulating cardiac disorders and hypertension (Venkatesan *et al*., 2005), male genital dysfunctions, oligospermia, spermatogenic irregularities and other male disorders such as painful micturition (Sahu *et al*., 2002; Dartsch, 2008). It is extensively used in ayurvedic formulations for digestive discomfort, indigestion, amebiasis and piles (Sharma *et al*., 2012). In females, it has been prescribed by the doctors in habitual abortions, weakness of the uterus, excessive bleeding during menstruation (Nevrekar *et al*., 2002). The crude extract has been shown to have an antioxidant effect against oxidative damage in terms of protection from lipid peroxidation, protein oxidation, depletion of protein thiols and the levels of the antioxidant enzyme, superoxide dismutase and catalase were improved (Kamat *et al*., 2000; Kamat and Devasagayam, 1995, 1996; Bhatnagar *et al*., 2005; Karmakar *et al*., 2012). *A. racemosus* extracts have been demonstrated to

possess hepatoprotective activity, augmented by restoration of glutathione levels, inhibiting the production of free radicals, acting as a scavenger and reducing the free radical generation via inhibition of hepatic CYP2E1 activity (Ergul *et al.*, 2010; Palanisamy and Manian, 2012). The study of anti-mutagenic properties of the natural plant products are useful in phytotherapy because these compounds serve as a beneficial alternative to traditional therapeutics that possibly will accomplish clinical goals with reduced adverse events

MATERIALS AND METHODS

Reagents and strains: *S. typhimurium* strains TA98, TA100 were kindly provided by Professor Bruce Ames (University of California at Berkeley, USA. Sodium azide for TA100, 4-nitro-ophenylenediamine (NPD) for TA98, Dimethyl sulfoxide (DMSO) were procured from M/S Sigma Chemicals Co. (St Louis, MO, USA). Solvent methanol was procured from SRL, India.

***A. racemosus* extract preparation:** The roots of *A. racemosus* were collected in the month of September, 2012 from Palampur (HP), India. The roots of *A. racemosus* were cleaned to remove the majority of dust and soil. The roots were chopped into small fragments to increase the surface area for the drying purposes. The small fragments were dried under shade, and the dried roots were ground in the powder form. The powdered material (3.1 kg) was subjected to successive extractions. The maceration was carried out using solvent as methanol (3×3L), 80% methanol (3×3L) and the menstrum collected was concentrated and dried to powder form using rota vapour to render 115 g of an extract of methanol extract (RME) and 135 g of aqueous methanolic extract (RAE).

MUTAGENICITY TEST

The *Salmonella* histidine point mutation assay proposed by Maron and Ames (1983) was followed using *S. typhimurium* strains TA98 (frameshift mutation test) and TA100 (base pair substitution test). The fresh cultures of tester strains TA98 and TA100 of *S. typhimurium* having density of 1-2×10^9 CFU mL^{-1} were used to investigate the antimutagenic activity of different extracts of *A. racemosus*. The minimal agar plates were prepared one day before experiment. Top agar was autoclaved and stored at 4°C, before the initiation of the experiment. It was melted and kept at 45°C. Co-incubation and pre-incubation two modes of experimentation were followed. The concentrations of the test sample used for investigating the antimutagenicity were 2.5×10^3, 1.0×10^3, 0.5×10^3, 0.25×10^3, 0.10×10^3 and 0.01×10^3 µg 0.1 mL $plate^{-1}$. All these concentrations of test samples were dissolved in DMSO under sterile condition. The negative control was run with different concentration of extracts, to verify its toxicity. The concentrations were considered non-toxic if the number and size of the revertant colonies in the negative control were equivalent to that of spontaneous revertant colonies. The fraction will be non-toxic if the intensity of background lawn is equivalent to the control having bacteria culture only. For the determination of toxicity of test sample, 0.1 mL of sample with 0.1 mL of freshly grown culture was added to the top agar which was maintained at 45°C. The mixture was then spread over the minimal agar plates which were then incubated at 37°C for 48 h. The anti-mutagenic effect of the different concentration of the extracts was determined against the known mutagen which is the characteristic of each strain depending on the reversion event. The mutagenicity of mutagen was also checked on the tester strains, in order to ensure the responsiveness of tester strain and efficacy of promutagen. The confirmation of the effect of mutagen on tester strain was determined by taking 0.1 mL of freshly grown culture along with 0.1 mL of the mutagen specific for the tester strain were added to the

soft agar, then poured on to the minimal glucose agar plates after thorough mixing. Then plates were incubated at 37°C for 48 h and effect of mutagen was accessed by counting the number of revertant colonies. In order to determine the antimutagenic potential of the different concentrations of the extracts co-incubation mode of experiment was followed. In co-incubation experimentation, 0.1 mL of bacterial culture, 0.1 mL of NPD or 0.1 mL of sodium azide and 0.1 mL of non-toxic concentrations of the extracts were added to 2 mL of top agar containing 0.5 mm histidine/biotin. The anti-mutagenicity potential of extract was also determined in pre-incubation experiment mode. In the pre-incubation mode of experimentation, equal volumes of the mutagens and the extracts were mixed in sterile capped tubes and allowed to stand for 30 min at 37°C under continuous shaking and 0.2 mL of this was added to 2 mL of soft agar with 0.1 mL of fresh *Salmonella* culture. The dose inhibiting 50% of mutagenicity (IbD50) was inferred from the dose-response curve and used as an indication of antimutagenicity potency (De *et al*., 1992). All the test samples were assayed using triplicate plates per run and each experiment was conducted twice in order to make estimation of variation. The activity of each extract was expressed as the percentage decrease of reverse mutation:

$$\text{Activity (\%)} = \frac{a - b}{a - c} \times 100$$

$$\text{Percent of control} = \frac{b}{a} \times 100$$

Where:
a = No. of histidine revertants induced by mutagen (NPD, sodium azide)
b = No. of histidine revertants induced by mutagen in the presence of extract
c = No. of revertants in the negative control

RESULTS AND DISCUSSION

The mutagenic and anti-mutagenic effects of *A. racemous* extracts RME and RAE induced by NPD in TA98; Sodium azide in TA100 is presented in Table 1 and 2, respectively. The extract of *A. racemosus* at a concentration of 0.1 mL did not show any effect on spontaneous revertants of both TA98 and TA100 *S. typhimurium* tester strain. In this study, anti-mutagenic activities of RME and RAE were evaluated by determining reductions in the number of His revertant mutations induced by selecting positive mutagens in *S. typhimurium* strains TA98, TA100.

Only the positive control showed significantly higher number of revertants than the treatment groups. The result of negative control experiment in which RME and RAE extracts were added but no mutagen have shown an insignificant number of histidine revertants in both the strains. RME and RAE reduced the frameshift mutation induced by NPD in TA98 and sodium azide induced mutation in TA100 strains of *S. typhimurium*. RME have shown a 40.63% inhibition in the formation of revertants in TA98 strain at co-incubation mode and 45.7% inhibition at pre-incubation mode. The extract has shown 40.1 and 42.89% inhibition at co-incubation and pre-incubation mode in TA100 tester strain.

RAE has shown 49.2 and 16.3% inhibition during co-incubation and pre-incubation mode in TA98 tester strain, moreover 45.4 and 27.2% inhibition was observed during the co-incubation and pre-incubation mode respectively in TA100 tester strain. Among the two extracts, RAE showed

Table 1: Effect of RME on the mutagenicity of NPD in TA98 and sodium azide in TA100 tester strains of *S. typhimurium*

		TA98		TA100	
Treatment	Dose (µg 0.1 mL^{-1})	Mean±SE	Inhibition (%)	Mean±SE	Inhibition (%)
Spontaneous		24.66±1.85		75.33±6.33	
positive control					
NPD		961.00±5.68			
Sodium azide				1194±39.52	
negative control	2.5×10^3	10.66±2.72		84.33±5.60	
	1.0×10^3	8.66±0.33		64±2.51	
	0.50×10^3	9.33±0.88		81.33±1.20	
	0.25×10^3	10.33±1.45		87±0.57	
	0.10×10^3	10±0.57		85.33±2.02	
Co-incubation	2.5×10^3	575±31.97	40.63	748.66±39.70	40.1
	1.0×10^3	632.66±12.67	34.45	726.66±15.93	41.35
	0.50×10^3	633.66±3.84	34.36	730.66±36.79	41.63
	0.25×10^3	853±4.58	11.35	877.33±8.19	28.62
	0.10×10^3	855.33±6.69	11.12	885±17.57	27.86
Pre-incubation	2.5×10^3	525.66±11.79	45.7	718±58.04	42.89
	1.0×10^3	598±75.97	38.11	789±2.08	35.84
	0.50×10^3	593±41.50	38.66	775.33±59.58	37.56
	0.25×10^3	601.33±32.82	37.84	951±20.50	21.95
	0.10×10^3	649±37.11	32.8	923±58.00	24.43
One-way ANOVA					
Positive control and co-incubation		F (5,12) = 114.93	HSD = 88.18	F (5,12) = 37.33	HSD = 175.31
\\positive control and pre-incubation		F (5,12) = 14.27	HSD = 246.42	F (5,12) = 14.68	HSD = 271.33
Two-way ANOVA					
Co-incubation and pre-incubation					
Treatment		F (1,20) = 29.90		F (1,20) = 2.477	
Dose		F (4,20) = 12.71		F (4,20) = 11.04	
Treatment×Dose		F (4,20) = 4.76		F (4,20) =0.58	

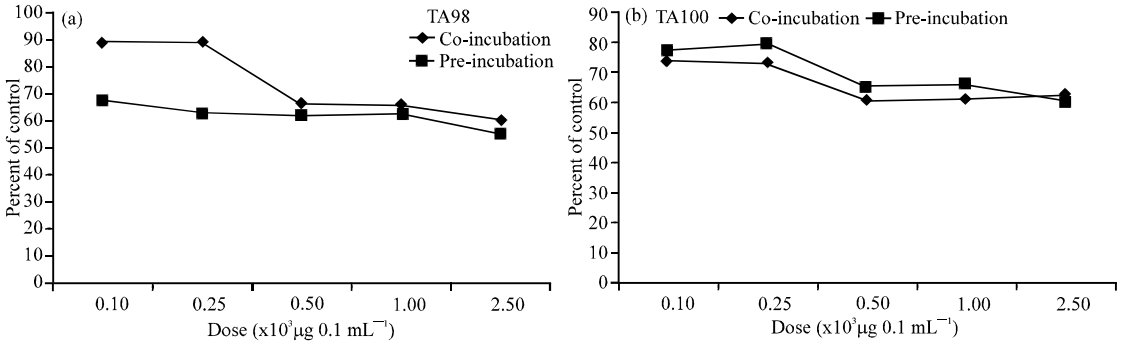

Fig. 1(a-b): Effect of RME on the mutagenicity of NPD in TA98 (a) Sodium azide in TA100, (b) tester strains of *S. typhimurium*

maximum inhibition of 49.2% (Table 1, Fig. 1). On the contrary, RME showed inhibition of 40.63% in Co-incubation mode (Table 2, Fig. 2). The results demonstrated RME and RAE extracts inhibited the mutagenicity induced by NPD in TA98 and Sodium azide in TA100 tester strains of *S. typhimurium*. There are numerous pathways through which natural products show their antimutagenic effects (Bhattacharya, 2011).

Table 2: Effect of RAE on the mutagenicity of NPD in TA98 and sodium azide in TA100 tester strains of *S. typhimurium*

		TA98		TA100	
Treatment	Dose (µg 0.1 mL^{-1})	Mean±SE	Inhibition (%)	Mean±SE	Inhibition (%)
Spontaneous		18.33±2.02		74±4.04	
positive control					
NPD		1273±55.18			
Sodium azide				1090.66±94.06	
Negative control	2.5×10^3	10±1.52		66.66±4.17	
	1.0×10^3	11±1.00		66.66±2.72	
	0.50×10^3	10.66±2.60		64.33±6.17	
	0.25×10^3	14.33±1.85		60±4.93	
	0.10×10^3	13±0.57		58.66±2.18	
Co-incubation	2.5×10^3	651.66±22.22	49.2	626±55.24	45.4
	1.0×10^3	668.66±12.83	47.9	659±11.78	42.1
	0.50×10^3	828.33±72.11	35.2	698.66±47.92	38.2
	0.25×10^3	836.33±23.02	34.6	724±9.01	35.6
	0.10×10^3	962.33±25.36	24.6	840.66±11.83	24.2
Pre-incubation	2.5×10^3	1067.33±51.36	16.3	811.66±9.83	27.2
	1.0×10^3	1136±18.03	10.8	866.33±15.85	21.9
	0.50×10^3	1174±11.13	7.8	848±39.20	23.6
	0.25×10^3	1211±57.53	4.9	911.33±26.29	17.4
	0.10×10^3	1215±25.15	4.5	894±20.51	19
One-way ANOVA					
Positive control and co-incubation		F (5,12) = 31.19	HSD = 245.90	F (5,12) = 12.20	HSD = 295.54
Positive control and pre-incubation		F (5,12) = 6.19	HSD = 245.87	F (5,12) = 4.87	HSD = 266.60
Two-way ANOVA					
Co-incubation and pre-incubation					
Treatment		F (1,20) = 283.38			F(1,20) = 14.70
Dose		F (4,20) = 19.34			F(4,20) = 12.55
Treatment×dose		F (4,20) = 0.74			F (4,20) = 5.71

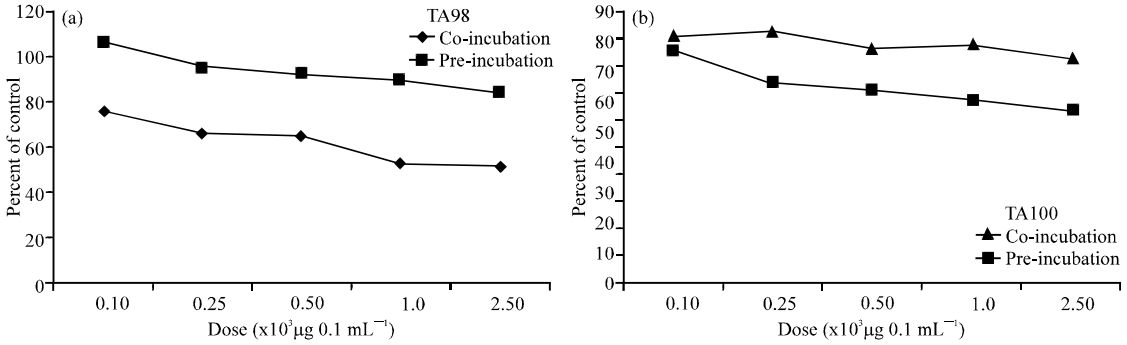

Fig. 2(a-b): Effect of RAE on the mutagenicity of NPD in TA98 (a) Sodium azide in TA100, (b) tester strains of *S. typhimurium*

Natural products have been projected to be potential therapies that reduce the genotoxicity related to the exposure certain therapeutic drugs, free radical damage and environmental contaminants. Vitamins and natural compounds are the major sources of anti-mutagenic compounds which are isolated from plants (Ames, 1983, 1984; Bandyopadhyay *et al.*, 2013).

The primary use of these compounds is to reduce the risk of mutation and cancer.

The protection mechanism of these natural products is complex and multifactorial (Newmark, 1996). The consumption of natural products helps in the enhancement of the enzymes involved in the metabolism of carcinogens. The effect of heat treated homogenized *Asparagus* on the mutagenicity using *Salmonella typhimurium* TA100 strain was studied and found there was considerable increase in activity upon heat treatment (Yamaguchi, 1992).

In another study, acetone, n-hexane, dicholoromethane, 2-propanol extracts of *Asparagus* were examined for the mutagenicity in TA98 strain of *Salmonella typhimurium* induced by 2-amino-3-methylimidazo [4,5-f] quinolone and 2-amino-3,4-dimethylimidazo [4,5-f] quinoxaline and was observed that n-hexane extract have maximum protective activity (Edenharder *et al*., 1995). In a study *A. offcinalis* juice has been shown to inhibit the mutagenic effects of cyclophosphamide in mice and rats (Asita *et al*., 2008). Anti-mutagenic potential of the RME and RAE extract of *A. racemosus* agrees with the literature in which the extracts possess free radical scavenging property (Kamat *et al*., 2000) in addition due to their ability to decrease cytochrome P450 activity and affecting the process of enzymatic activation (Palanisamy and Manian, 2012). In the present study, we considered anti-mutagenic activity relevant property of RME and RAE extract isolated from *A. racemosus*.

CONCLUSION

RME and RAE extracts obtained from *A. racemosus*, possess anti-mutagenic activity, with RAE showed maximum inhibition of 49.2%. The anti-mutagenic activity is a resultant of the free radical scavenging property, decreased cytochrome P450 activity and prove to be beneficial in treating some symptoms related to antiradical activity like hepatoprotection, depression and other related neurodegenerative diseases.

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