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PLANT-MEDIATED SILVER NANOPARTICLES USING *Semecarpus anacardium* LINN AND BIOLOGICAL EVALUATION OF TOXICOLOGY STUDIES IN ANTI-MUTAGENICITY

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ABSTRACT

Semecarpus anacardium a common medicinal plant, has multiple uses in traditional system of medicine and in particular it is used as a Vermifuge for centuries. The plant and its extracts have been evaluated for number of activities like anti-inflammatory, cardio-tonic, sedative and neuron-muscular. The plant extract was evaluated for Antimutagenicity and mutagenicity studies in order to confirm the safety of its usage. Ethanol extracts of *Semecarpus anacardium* showed no mutagenicity up to 5 mg/plate when tested with *Salmonella typhimurium* TA97a, TA98, TA100, TA102 and TA1535 strains with or without metabolic activation. On the other hand ethanol extract of *Semecarpus anacardium* showed a significant protective effect against mutagenicity induced by mutagen in *S. typhimurium* TA98 and TA100 strain with or without metabolic activation. The results of these studies indicate that *Semecarpus anacardium* is non-mutagenic in Ames test, exhibit protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain.

Key Words: Semecarpus anacardium; Antimutagenicity; Mutagenicity; Salmonella typhimurium

INTRODUCTION

Semecarpus anacardium (SA) linn (Family: Anacardiacea) is distributed in sub-Himalayan region, tropical and central parts of India. The nut is commonly known as "marking nut" and in the vernacular as "Ballataka" or "Bhilwa". Detoxified nut of *Semecarpus anacardium* were incorporated in prescription for toxic conditions, obstinate skin diseases, tumours, malignant growth, fevers, haemoptysis, excessive menstruation, vaginal discharge, deficient lactation, constipation, intestinal parasites. The biological effects of *Semecarpus anacardium* documented in both traditional and modern scientific literature. The plant and its extracts, have been extensively investigated for their Antibacterial enhancing effects. Alcoholic extracts of *Semecarpus anacardium* has shown anti-cancer activity. Bioflavonoids have been implicated as responsible for the anticancer activity. The major chemical entities shown to be responsible for the Anticancer action of Semecarpus anacardium is the Bioflavonoids like biflavones A, C, A1 and A2. The other major chemical constituents isolated and characterized from the alcoholic extract are tetrahydrorobustaflavone, semecarpuflavone, jeediflavone and gulluflavone. We have investigated the mutagenic activity and anti-mutagenicity of ethanol extract of *Semecarpus anacardium* a widely used medicinal plant, by Ames test and chromosomal aberration test based on OECD guidelines [1-16].

MATERIALS AND METHODS

Plant materials and chemicals

The ethanol extract of *Semecarpus anacardium* (Batch# BM/06001) was obtained in powder form from Managing director, Dr.Yuvaraj M.Pharma.,Ph.D.,D.Sc.(CSN Drug discovery & regulatory group Ltd, Bangalore-560041, India. Dimethyl sulfoxide (DMSO-CAS No. 67-68-5), nicotinamide adeninedinucleotide phosphate sodium salt

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(NADP-CAS No. 214-664-6), D-glucose-6-phosphate disodium salt (CAS No. 3671-99-6), L-histidine monohydrate (CAS No. 7048-02-4), D-Biotin (CAS No 58-85-5) were purchased from Sigma Chemical Co. The S9 microsome fraction was prepared in house from the livers of rats treated with sodium phenobarbitol (Venitt et al., 1990). Standard mutagens: 2-aminofluorene (CAS No 613-13-8), Mitomycin C (CAS No 56-07-7), 4-nitroquinolene-1-oxide (CAS No 56-57-5), sodium azide (CAS No 26628-22-8) were obtained from Sigma. Oxoid nutrient broth No. 2 (Oxoid) and Difco bacto agar (Difco) were used for the preparation of bacterial growth media.

Ames assay

S. typhimurium strains TA97a, TA98, TA100, TA1535 and TA102 were obtained from Bruce Ames Laboratory, Molecular and Cell Biology, University of California, and checked for their viable counts and genotype characteristics. Plate incorporation method Maron and Ames [17] using histidine-dependent strains of S typhimurium TA97a, TA98, TA100, TA102 and TA1535 in the presence and absence of metabolic activation system (S9liver fraction) was adopted for assessing the mutagenicity. *Semecarpus anacardium* was tested for its mutagenic properties at five different concentrations viz., 5, 2.5, 1.25, 0.625 and 0.312 mg/plate. 100 µl of various concentrations of *Semecarpus anacardium* dissolved in DMSO were added to 2 ml top agar mixed with 100µl of bacterial culture and then poured on to a plate containing minimal glucose agar.

These plates were incubated at 37^0C for 48 h and his+ revertant colonies were manually counted and the results were shown as the mean of the three plates with standard deviation. The influence of metabolic activation was tested by adding 500 µl of S9 mixture. The experiments were analysed in triplicate And was repeated to confirm the result. The criteria employed to interpret the results of Ames test as positive were similar to those used in regulatory guidelines OECD test guideline No. 471(1997). The number of induced mutation should be at least twice the activity observed in negative control and there must be a reproducible dose response curve.

Concurrent positive and negative (DMSO) controls were used in the study. The standard mutagens used as positive controls in each experiments were without metabolic activation, 4-nitroquinoline-1-oxide (5µg/plate) for strain TA97a and TA98, sodium azide (5µg/plate) for strain TA100 and TA1535, mitomycin-C (0.02mg/plate) for TA102. In case of positive controls with metabolic activation, 2-aminoflurene (20µg/plate) for TA97a, TA98, TA100, TA1535 and TA102 were used.

Anti-mutagenicity test: Based on the results of mutagenicity testing, *Semecarpus anacardium* were tested for its antimutagenic properties [18] at five different concentrations viz., 5, 2.5, 1.25, 0.625 and 0.312 mg/plate. Dimethyl sulphoxide (DMSO) was used as solvent control. The S9 mix (500 µg/ml) or phosphate buffer for the presence and absence of metabolic activation, 100 µg/ml of the respective positive control (without metabolic activation sodium azide for TA100 and 4-nitroquinolene-1-oxide for TA98 in case of with metabolic activation 2-aminofluorene for both the strains), 100 µl of the appropriate concentration of the extract, 100 µg/ml of respective bacterial culture, were added to sterile capped tubes and incubated in an incubator for 30 m at 37 ± 1°C.

After incubation, the mixture was added to sterile tubes containing 2 ml of top agar kept at 45 ± 2 °C in a water bath. The tubes containing the mixture and top agar were gently mixed and then overlaid onto the surface of minimal glucose agar plates prepared under aseptic conditions contained in 100 µg/ml 10 mm plate. After solidification, the plates were inverted and incubated at 37 ±1°C for 48-72 h. Plating was done in triplicates. Positive and negative control (DMSO) plates were also prepared in triplicates. The inhibition rate of mutagenicity (%) was calculated with respect to the number of revertant colonies in the control group treated with the corresponding mutagen by the following assay [19].

RESULTS

All the strains of S. typhimurium viz., TA97a, TA98, TA100, TA102 and TA1535, exposed to different concentrations of *Semecarpus anacardium* did not show two-fold or greater increase in the mean number of revertants as compared to the negative control group as given in Table 1. All strains used in the study exhibited marked increase (>10-fold) in the number of revertants when treated with positive control agents.

The results confirmed the sensitivity of the tester strains to mutagens and thus the validity of the assay. The results indicated that the mean number of histidine revertants in the treatment groups were comparable to the mean number of revertants in the negative control group in all the five S. typhimurium tester strains viz., TA97a, TA98, TA100, TA102 and TA1535 both in the absence and presence of metabolic activation. Ethanol extract of *Semecarpus anacardium* upto 5mg/plate in the presence and absence of metabolic activation was found to be non-mutagenic to all the five S. typhimurium tester strains.

On the other hand, ethanol extract of *Semecarpus anacardium* showed a significant dose dependent anti-mutagenic activity, in S. typhimurium TA98 and TA100 strain with or without metabolic activation which is shown in Table 2 and 3. *Semecarpus anacardium* exhibit protection against the mutagenicity induced by 4-nitroquinolene-1- oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain.

Table 1. Mutagenic activity of Ethanol extract of *Semecarpus anacardium*

Concentrations (mg/plate)	Revertant Colonies / Plate (Mean (n=3) ± S.D.)									
	TA97a		TA98		TA1535		TA100		TA102	
	-S9	+S9 (10%)	-S9	+S9 (10%)	-S9	+S9 (10%)	-S9	+S9 (10%)	-S9	+S9 (10%)
NC (DMSO)	181 ± 9	191 ± 6	40 ± 10	42 ± 5	14 ± 3	11 ± 3	177 ± 13	183 ± 8	298 ± 10	306 ± 7
0.313	187 ± 5	186 ± 5	43 ± 9	47 ± 4	12 ± 4	13 ± 3	181 ± 9	181 ± 2	300 ± 9	296 ± 8
0.625	182 ± 9	180 ± 10	43 ± 5	39 ± 3	13 ± 3	12 ± 3	181 ± 8	189 ± 2	302 ± 9	305 ± 4
1.25	184 ± 11	182 ± 10	40 ± 4	47 ± 5	16 ± 2	12 ± 1	182 ± 9	186 ± 3	294 ± 6	300 ± 9
2.5	176 ± 14	189 ± 3	41 ± 4	45 ± 6	15 ± 6	12 ± 2	180 ± 7	176 ± 6	295 ± 6	299 ± 6
5	192 ± 7	189 ± 2	42 ± 8	41 ± 4	12 ± 4	11 ± 3	179 ± 8	178 ± 4	286 ± 16	286 ± 7
PC SA	NA	NA	NA	NA	1265±55	NA	2341±133	NA	NA	NA
PC 4NQNO	1913±110	NA	813±110	NA	NA	NA	NA	NA	NA	NA
PC MMC	NA	NA	NA	NA	NA	NA	NA	NA	3537±276	NA
PC 2AF	NA	2287±148	NA	1668±62	NA	674±56	NA	2737 ±57	NA	3194±70

Key: mg = milligram, S.D. = Standard deviation, NC = Negative control, DMSO= DimethylSulfoxide, PC = Positive control, 4NQNO = 4-Nitroquinolene N Oxide, SA = Sodium azide, MMC = Mitomycin C, 2AF = 2Aminofluorene, NA = Not Applicable, n = No.of replicates.

Table 2. Inhibition of mutagenicity by ethanol extract of *Semecarpus anacardium* in *S.typhimurium* TA98 assay system

Dose Concentration (mg/plate)	His+ Revertant Colonies / Plate (Mean ± S.D.)			
	Presence of S9 Mix	% Inhibition of mutagenesis	Absence of S9 Mix	% Inhibition of mutagenesis
NC (DMSO)	21 ± 1	-	23 ± 3	-
0.312	1018 ± 2	33	563 ± 4	63
0.625	818 ± 2	46	484 ± 3	69
1.25	665 ± 5	57	406 ± 4	74
2.5	87 ± 3	96	73 ± 5	97
5	21 ± 3	100	22 ± 4	100
PC	1501 ± 3	-	824 ± 4	-

Key NC= Negative control, PC= positive control

Table 3. Inhibition of mutagenicity by ethanol extract of *Semecarpus anacardium* in *S.typhimurium* TA100 assay system

Dose Concentration (mg/plate)	His+ Revertant Colonies / Plate (Mean ± S.D.)			
	Presence of S9 Mix	% Inhibition of mutagenesis	Absence of S9 Mix	% Inhibition of mutagenesis
NC (DMSO)	179 ± 3	-	172 ± 3	-
0.312	634 ± 5	63	495 ± 3	76
0.625	551 ± 5	70	382 ± 4	84
1.25	240 ± 3	95	301 ± 7	90
2.5	224± 4	96	257 ± 5	94
5	183 ± 3	100	169 ± 1	100
PC	1421± 1	-	1503 ± 4	-

Key NC= Negative control, PC= positive control

DISCUSSION

Traditional use of plants in alternative medicine frequently provides the basis to select which plant extract it is worth studying. *Semecarpus anacardium* is a perennial creeping plant found throughout India in wet, damp and marshy areas. An infusion of the plant has been used in Indian folklore as a nerve tonic. Traditionally, it was used as a brain tonic to enhance memory development, learning and concentration and to provide relief of patients with anxiety or epileptic disorder. These characteristics make them a good therapeutic prospect of study. Our purpose was to investigate the possible mutagenic, anti-mutagenic properties of *Semecarpus anacardium* extracts with Ames assay. The result obtained is *Semecarpus anacardium* extracts is non-mutagenic upto 5mg/plate both in the presence and absence of S9 (Table 1).

The absence of mutagenicity is not characteristic of all natural products in use since other medicinal plants assayed with the Ames test, with or without the S9, have resulted positive for mutagenicity [20-22]. Results of anti-mutagenic activities showed that the ethanol extract of *Semecarpus anacardium* were highly effective in reducing the mutagenicity caused by the mutagen 4- nitroquinolene-1-oxide, sodium azide and 2-aminoflourene (Table 2 and 3),

our results confirm the data previously reported on the antimutagenicity studies done by using plant extracts [23,24].

These features make ethanol extract of *Semecarpus anacardium* a promising candidates for further studies. However, invivo genotoxicity studies are in progress.

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