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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(2): 598-603

ISSN No: 0975-7384

Antimutagenicity of ethanol extract of *Derris brevipes*

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Abstract

Derris brevipes, a common medicinal plant, has multiple uses in traditional system of medicine and in particular it is used as a memory-enhancing agent 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 *Derris brevipes* 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 *Derris brevipes* 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 *Derris brevipes* 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: *Derris brevipes*; Antimutagenicity; Mutagenicity*; Salmonella typhimurium.*

Introduction

Derris brevipes also referred to as *Herpstis monniera*, *Bacopa monniera*, water hyssop, and "Brahmi" has been in use since time immemorial as nerve tonic for improvement of memory. *Derris brevipes* is a perennial creeping plant found throughout India in wet, damp and marshy areas[1-2]. An infusion of the plant has been used in Indian folklore as a nerve tonic [3]. Traditionally, it was used as a brain tonic to enhance memory development, learning and concentration[4] and to provide relief for the patients suffering from anxiety or epileptic disorder [5].

The biological effects of *B. monnieri* are documented in both traditional and modern scientific literature. The plant and its extracts, and the isolated bacosides have been extensively investigated

for their memory and cognition enhancing effects. Alcoholic extracts of *B. monnieri* has shown cognition facilitating effect in normal rats [6] and also inhibited the amnesic effects of scopolamine, electroshock and immobilization stress [7]. Vohora et al.[8] have shown the potential protective effect of *B. monnieri* in Phenytoin-induced cognitive deficit in mice by both acquisition and retention of memory without affecting its anticonvulsant activity. The butanolic extract of *B. monnieri* has shown memory- enhancing effects in rats by increasing recognition index in differential exploration of familiar and new objects [9]. Recently, Kishore and Singh [10]reported that anterograde administration of alcoholic extract of *B. monnieri* in mice facilitated anterograde memory and attenuated anterograde experimental amnesia induced by scopolamine and sodium nitrite.

The major chemical entities shown to be responsible for the memory-facilitating action of *B. monnieri* is the steroidal saponins bacoside A and B [11]. The other major chemical constituents isolated and characterized from the alcoholic extract are dammarane type triterpenoid saponins with jujubogenin and pseudojujubogenin as the aglycones, including bacosides A_1–A_3 [12-14], bacopasaponins A–G [15-17]. Since there is no evidence of antimutagenicity data available, we have investigated the mutagenic activity and anti-mutagenicity of ethanol extract of *Derris brevipes* a widely used medicinal plant, by Ames test and chromosomal aberration test based on OECD guidelines.

Materials and methods

Plant materials and chemicals

The ethanol extract of *Derris brevipes* was obtained in powder form from CRC group,Chromosoft Pvt Ltd, Bangalore- 560041, India. Dimethyl sulfoxide (DMSO-CAS No. 67-68-5), nicotinamide adeninedinucleotide phosphate sodium salt (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 [25].

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, 1984 using histidine-dependent strains of *S. typhimurium* TA97a, TA98, TA100, TA102 and TA1535 in the presence and absence of metabolic activation system (S9 liver fraction) was adopted for assessing the mutagenicity. *Derris brevipes* 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 *Derris brevipes* 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 were 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[26]. 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, *Derris brevipes* were tested for its anti-mutagenic 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 µl) or phosphate buffer for the presence and absence of metabolic activation, 100 µl 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 µl 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 100×10 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

Table 1: Mutagenic activity of ethanol extract of *Derris brevipes*

Conc. (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±155	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= Dimethyl Sulfoxide, PC = Positive control, 4NQNO = 4-Nitroquinolene- N-oxide, SA = Sodium azide, MMC = Mitomycin C, 2AF = 2-Aminofluorene, NA = Not Applicable, n = No. of replicates

All the strains of *S. typhimurium* viz., TA97a, TA98, TA100, TA102 and TA1535, exposed to different concentrations of *Derris brevipes*, 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 *Derris brevipes* 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 *Derris brevipes* 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 *Derris brevipes* exhibit protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain.

Table 2: Inhibition of mutagenicity by ethanol extract of *Derris brevipes* in *S. typhimurium* (TA98 assay system)

Dose Conc. (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 *Derris brevipes* in *S. typhimurium* (TA100 assay system)

Dose Conc. (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. *Derris brevipes*, also referred to damp and marshy areas [1-2]. An infusion of the plant has been used in Indian folklore as a nerve tonic [3]. Traditionally, it was

used as a brain tonic to enhance memory development, learning and concentration [4] and to provide relief of patients with anxiety or epileptic disorder [5]. These characteristics make them a good therapeutic prospect of study.

Our purpose was to investigate the possible mutagenic, anti-mutagenic properties of *Derris brevipes* extracts with Ames assay. The result obtained *Derris brevipes* 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 *Derris brevipes* 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 *Derris brevipes* a promising candidates for further studies. However, invivo genotoxicity studies are in progress.

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