

**Research Article****TOXICOLOGY STUDIES OF ALCOHOL EXTRACT OF *DERRIS BREVIPES VAR BREVIPES***Yuvaraj G<sup>\*1</sup>, Sreedevi<sup>3</sup>, JSK<sup>3</sup>, Amruth<sup>3</sup>, Raghu PS<sup>3</sup> and Shankar S<sup>2</sup><sup>1</sup>Advanced Drug Discovery and Toxicology Division, CRC Group, Indore, India<sup>2</sup>Department of Medicinal & Natural Products Chemistry, JSS University, Ooty, India<sup>3</sup>Drug Testing Labs, Department of Pharmaceutical Analysis, JSS University, Ooty, India**\*Corresponding author e-mail:** [mail@crcgroupin.com](mailto:mail@crcgroupin.com), [yuvas2010@gmail.com](mailto:yuvas2010@gmail.com)**ABSTRACT**

*Derris brevipes var 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 a number of activities like anti-inflammatory, cardiotoxic, sedative and neuron-muscular. The plant extract was evaluated for the antimutagenicity and mutagenicity studies in order to confirm the safety of its usage. Ethanol extracts of *Derris brevipes var 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 var brevipes*, showed a significant protective effect against the 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 var brevipes*, is non-mutagenic in the Ames test, exhibit protection against the mutagenicity induced by 4-nitroquinoline-1-oxide, sodium azide and 2-aminofluorene in TA98 and TA100 strain.

**Keywords:** *Derris brevipes var brevipes*, Antimutagenicity, Mutagenicity and *Salmonella typhimurium***INTRODUCTION**

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

The biological effects of *Derris brevipes var brevipes*, 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* shown cognition facilitating effect in normal rats<sup>6</sup> and also inhibited the amnesic effects of scopolamine, electroshock and immobilization stress<sup>7</sup>. The potential protective effect of *Derris brevipes var brevipes* induced cognitive deficit has shown in mice by both acquisition and retention of memory without affecting its anticonvulsant activity<sup>8</sup>. The butanolic extract of *Derris brevipes var brevipes* shown memory enhancing effects in rats by increasing the recognition index in the differential exploration of familiar and new objects<sup>9</sup>. Recently the anterograde administration of alcoholic extract of *Derris brevipes var brevipes* in mice facilitated anterograde memory

and attenuated anterograde experimental amnesia induced by scopolamine and sodium nitrite<sup>10</sup>.

The major chemical entities shown to be responsible for the memory facilitating action of *Derris brevipes var brevipes* is the steroidal saponins bacoside A and B<sup>11</sup>. 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<sub>1</sub>-A<sub>3</sub><sup>12,14</sup>, bacopasaponins A-G<sup>15-17</sup>. Since there is no evidence of antimutagenicity data available, we have investigated the mutagenic activity and anti-mutagenicity of ethanol extract of *Derris brevipes var brevipes*, a widely used medicinal plant, by Ames test and a chromosomal aberration test based on the OECD guidelines.

## MATERIALS AND METHODS

### Plant materials and chemicals

The ethanol extract of *Derris brevipes var brevipes* (Batch# BM/06001) was obtained in powder form from Natural Remedies Pvt. Ltd., Bangalore, 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 microsomes fraction was prepared in house from the livers of rats treated with sodium phenobarbitol<sup>18</sup>.

### 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 Chemical Co., 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<sup>19</sup> 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 var brevipes* 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 var brevipes* dissolved in DMSO were added to 2 ml top agar mixed with 100 µl of bacterial culture and then poured on to a plate containing minimal glucose agar. These plates were incubated at 37°C for 48 h and 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 analyzed in triplicate and were 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<sup>20</sup>. The number of induced mutations 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 experiment was 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-aminofluorene (20µg/plate) for TA97a, TA98, TA100, TA1535 and TA102 were used.

### Anti-mutagenicity test

Based on the results of mutagenicity testing *Derris brevipes var brevipes* were tested for its anti-mutagenic properties<sup>21</sup> at five different concentrations viz., 5, 2.5, 1.25, 0.625 and 0.312 mg/plate. Dimethyl sulphoxide (DMSO) was used as a 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 min at 37 ± 1°C. After incubation, the mixture 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. The 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<sup>22</sup>.

## RESULTS

All the strains of *S. typhimurium* viz., TA97a, TA98, TA100, TA102 and TA1535, exposed to different concentrations of *Derris brevipes var brevipes* did not show a twofold 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 a marked increase (>10-fold) in the number of revertants when treated with positive control agents. The results confirmed the sensitivity of the test 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 the presence of metabolic activation. An ethanol extract of *Derris brevipes var brevipes* up to 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, an ethanol extract of *B. monnieri* 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 var brevipes* exhibit protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminofluorene in TA98 and TA100 strain.

**Table 1: Mutagenic activity of Ethanol extracts of *Derris brevipes var brevipes***

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 ± 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= 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 *Derris brevipes var brevipes* 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 *Derris brevipes var brevipes* 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. *Derris brevipes var brevipes* also referred to as, time immemorial as a nerve tonic for improvement of memory. *Derris brevipes var brevipes* 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 used as a brain tonic to enhance memory development, learning and concentration and to provide relief to patients with anxiety or the epileptic disorder. These characteristics make them a good therapeutic prospect of study.

Our purpose was to investigate the possible mutagenic, anti-mutagenic properties of *Derris*

*brevipes var brevipes* extracts with the Ames assay. The result obtained is *Derris brevipes var brevipes* extracts is non-mutagenic up to 5mg/plate both in the presence and the 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<sup>23-25</sup>. Results of anti-mutagenic activities showed that the ethanol extract of *Derris brevipes var brevipes* was 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<sup>26, 27</sup>. These features make an ethanol extract of *Derris brevipes var brevipes* a promising candidate for further studies. However, *in vivo* genotoxicity studies are in progress.

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