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Research Article

Genotoxicity studies of dry extract of *Boswellia serrata*

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Abstract

Purpose: *Boswellia serrata, a common medicinal plant, has multiple uses in traditional medicine and, in particular, for the treatment of inflammatory diseases. The plant and its extracts have been evaluated for a number of activities, namely, anti-inflammatory, analgesic, anti-arthritic and antipyretic. In this study, the plant was subjected to genotoxicity studies in order to ascertain an aspect of the safety of the drug.*

Results: *Dry extracts of B. serrata 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. In addition, the extract showed significant protective effect against mutagenicity induced by mutagen in S. typhimurium TA98 and TA100 strains with or without metabolic activation. Similarly, in vitro chromosomal aberration assay did not reveal any significant alterations up to 5 mg/culture as compared to the negative control both in the presence and absence of metabolic activation (S9 mix).*

Conclusion: *The results of these studies indicate that B. serrata is non-mutagenic in Ames test, and is protective against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strains. It was also non-clastogenic in the in vitro chromosomal aberration study.*

Key words: *Boswellia serrata; Chromosomal aberration; Mutagenicity; Salmonella typhimurium; Antimutagenicity.*

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INTRODUCTION

Boswellia serrata extract is used for the treatment of inflammatory diseases in Ayurvedic system of medicine. The extract is gummy and also used for a variety of medical conditions including arthritis, diarrhea, dysentery, pulmonary diseases and ring worm[1-3]. The major bioactive constituents of *B. serrata* are triterpenoids[4], boswellic acid, 11-keto-β-boswellic acid[5].

To the best of our knowledge, there are no genotoxicity data available for this plant, and, therefore, we have investigated the mutagenic activity, anti-mutagenicity and chromosomal aberration of the dry extract of *B. serrata*, a widely used medicinal plant in India, by Ames test and chromosomal aberration test based on Organisation for Economic Co-operation and Development (OECD) guidelines.

MATERIALS AND METHODS

Plant materials and chemicals

The dry extract of *B. serrata*(Batch# NR/QCD/SPC/010) was obtained in powder form from Natural Remedies Pvt Ltd, Bangalore- 560041, India. Dimethyl sulfoxide (DMSO-CAS No. 67-68-5), nicotinamide adenine dinucleotide phosphate sodium (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), and D-Biotin (CAS No 58-85-5) were purchased from Sigma Chemical Co., while minimal essential medium (MEM, CAT no. 41090-036) was procured from Gibco. S9 microsome fraction was prepared in-house from the liver of rats treated with sodium phenobarbitol[6].

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), Benzo(α)pyrene (CAS No 200-028-5) were also 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[7] 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. *B. serrata* 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 *B. serrata* 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 two 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[8]. 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, *B. serrata* was tested for its anti-mutagenic properties at five different concentrations viz.,

Table 1: Mutagenic activity of dry extract of *B. serrata*

Dose Level (mg/plate)	Revertant colonies / plate (mean 9n=3) ± S. D.)									
	TA97a		TA98		TA1535		TA100		TA102	
	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
NC(DMSO)	184 ± 5	187 ± 4	20 ± 2	20 ±3	13 ± 2	14 ± 2	181 ± 2	184 ± 6	294 ± 6	304 ± 6
5	178 ± 3	184 ± 6	20 ± 2	20 ± 1	12 ± 2	10 ± 1	186 ± 4	183 ± 7	299 ± 9	298 ± 2
2.5	178 ±2	187 ± 3	19 ± 2	20 ± 2	13 ± 3	12 ± 1	186 ± 5	182 ± 5	298 ± 2	302 ± 3
1.25	178 ± 7	188 ± 2	20 ± 2	21 ± 2	14 ± 2	12 ± 1	184 ± 6	180 ± 3	298 ± 3	304 ± 5
0.625	179 ± 3	188 ± 3	19 ± 2	21 ± 2	11 ± 1	13 ± 2	184 ± 6	183 ± 3	296 ± 5	298 ± 2
0.312	178 ±5	186 ± 5	20 ± 1	20 ± 1	14 ± 2	12 ± 2	186 ± 4	185 ± 5	299 ± 8	302 ± 4
PC SA	NA	NA	NA	NA	1152 ± 27	NA	2059 ± 15	NA	NA	NA
PC 4NQNO	1345 ± 25	NA	1428 ± 23	NA	NA	NA	NA	NA	NA	NA
PC MMC	NA	NA	NA	NA	NA	NA	NA	NA	3046 ± 39	NA
PC 2AF	NA	2152 ± 25	NA	1338 ± 16	NA	681 ± 8	NA	2165 ± 17	NA	3129 ± 27

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 = 2-aminofluorene, NA = Not Applicable, n = no. of replicates*

5, 2.5, 1.25, 0.625 and 0.312 mg/plate[9]. 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℃. After incubation, the mixture was added to sterile tubes containing 2 ml of top agar kept at 45 ± 2 ℃ 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℃ for 48–72 h. Plating was done in duplicates. Positive and negative control (DMSO) plates were also prepared in duplicate. 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[10].

Chromosomal aberration assay

Chinese hamster ovary cell line obtained from National Centre for Cell Science, Pune was used for *in vitro* chromosomal aberration study. Monolayer cultures of 80% confluency

were cultured at a cell density of 2.3×10^5 cells per culture and after 24 h they were exposed to the test substance with or without sodium phenobarbitol-induced wister rats S9. As no precipitation and reduced mitotic index was recorded for *B. serrata* at 5 mg/culture, dose levels of 5, 2.5 and 1.25 mg/culture were selected and exposed to cell cultures in duplicate. The criteria employed to interpret the results of chromosomal aberration test as positive were similar to those used in regulatory guidelines[11]. Concurrent positive controls Mitomycin-C without S9 and benzo(A)pyrene with S9 and negative control (DMSO) were used for the study. Cell cultures were incubated at 37°C, harvested at 18h after exposure and stained with 5% Giemsa. A total of about 200 metaphases were observed for structural chromosome aberrations, including both chromosomes and chromatids (i.e., break, deletion, fragments and exchanges). Gaps were recorded but not included in the aberration frequency.

RESULTS

All the strains of *S. typhimurium* viz., TA97a, TA98, TA100, TA102 and TA1535, exposed to different concentrations of *B. serrata*, 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. Dry extract of *B. serrata* up to 5mg/plate both in the presence and absence of metabolic activation was found to be non-mutagenic to all the five *S. typhimurium* test strains. On the other hand, *B. serrata* extract showed a significant dose dependent anti-mutagenic activity, in *S. typhimurium* TA98 and TA100 strain with or without metabolic activation (see Tables 2 and 3). *B. serrata* exhibit protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain

Similarly, i*n vitro* chromosomal aberration assay did not reveal any significant alterations up to a dose of 5mg/culture as shown in Table 4, compared to the negative control both in the presence and absence of metabolic activation (S9 mix) but the positive controls induced aberration. *B. serrata* upto 5mg/ml was non-clastogenic to Chinese Hamster Ovary cell lines both in the presence and absence of metabolic activation.

DISCUSSION

Phytochemicals derived from plants or microbes serve as valuable sources for isolating and characterizing lead molecules with specific functions. This approach assists in identifying compounds that show specific bioactivity. *B. serrata* having triterpenoids[12] as a main constituents is a plant whose extract has been used by traditional healers in southern India to treat specific ailments in reducing pain and inflammation of joints of the body. It is also used for treatment of crohn disease and the gum resin is used to treat patients with bronchial asthma and its also shows cytostatic and apoptosis-inducing activity towards human cancer cell line in vitro[13].

The results obtained show that *B. serrata* extract is non-mutagenic up to dose of 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 yielded positive results for mutagenicity[14].

The results of anti-mutagenic activities showed that the extract was highly effective in reducing the mutagenicity caused by the mutagen 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene (Table 2 and 3). The mutagen 2 Aminoflurone works by causing framshift mutation by forming adducts on the C8 position of guanine in DNA in

Table2: Inhibition of mutagenicity by dry extract of *B. serrata* in *S. typhimurium* TA98 assay system

Dose (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 ± 2	–	22± 2	–
0.312	1020 ± 4	33	564 ± 4	32
0.625	819 ± 4	46	487 ± 4	42
1.25	665 ± 5	56	408 ± 2	52
2.5	87 ± 3	95	61 ± 2	95
5	21± 3	100	22 ± 4	100
PC	1501 ± 4	–	824 ± 4	–

Key: *NC= negative control, PC= positive control*

Table3: Inhibition of mutagenicity by dry extractof *B. serrata* in *S. typhimurium* TA100 assay system

Dose (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 ± 20	–	158 ± 13	–
0.312	635 ± 17	62.66	492 ± 41	75.12
0.625	555 ± 30	69.21	380 ± 25	83.46
1.25	239 ± 25	95	300 ± 31	89.42
2.5	221± 16	96.57	250 ± 28	94.47
5	186 ± 11	100	148 ± 25	100
PC	1400 ± 28	–	1500 ± 69	-

Key: *NC= negative control, PC= positive control*

Table 4: Results of chromosomal abberation study

Concentrations →		VC[a]		1.25[b]		2.5[b]		5[b]		PC[c]	
Metabolic activation		–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
No. of metaphases counted		200	200	200	200	200	200	200	200	200	200
Type of Aberrations Observed	Ring	1	0	0	0	0	0	0	0	1	2
	Chromosomal Gap	0	0	0	0	0	0	0	0	0	0
	Chromosomal Break	0	0	0	0	0	1	0	0	5	4
	Chromatid Gap	0	0	0	0	0	0	0	0	0	0
	Chromatid break	0	1	0	0	0	0	0	0	4	1
	Fragment	0	0	0	0	0	0	1	0	5	3
	Ploidy	0	0	0	0	0	0	0	0	1	4
Total N° of Aberrations		1	1	0	0	0	1	1	0	16	14
Total N° of Metaphases with Aberrations		1	1	0	0	0	1	1	0	16	14
Aberrations/Metaphase (Cell)		1	1	0	0	0	1	1	0	1	1
% Metaphase with Aberrations		0.5	0.5	0	0	0	0.5	0.5	0	8	7

[a] Vehicle control; [b] dry extract of B. serrata (mg/ml); [c] positive controls; mitomycin-C (–S9) Benzo(α)pyrene (+S9)

presence of microsomal activation. In contrast sodium Azide requires no activation by hepatic microsomal enzymes to damage DNA and induce mutagenicity. This suggests that this extract may inhibit microsomal enzymes activation or that they may directly protect DNA strands from the electrophilic metabolites of the mutagen. Many triterpenoids have antioxidants depending on the redox potential, either accept or donate electrons, which may alternatively reduce them protective against mutagen [15]. Our results from these experiments on antimutagenicity suggests that triterpenoids might contain antioxidants which protects from the mutagens.

In chromosomal abberation assay, no significant alterations were revealed up to 5mg/culture as shown in Table 4 when compared to the negative control, both in the presence and absence of metabolic activation (S9 mix). Since we got no mutagneicity, clastogenicity and antimutagenicty we thought that our extract could be working with similar mechanisms mentioned below. Ant carcinogenic/ antimutagenic activity derived from medicinal plants may be due to a variety of mechanisms such as inhibition of gentoxic effects, inhibition of cell proliferation, signal transduction modulation, antioxidant activity and scavenging of free radicals, induction of detoxication enzymes, induction of cytoskeletal proteins which play a key role in mitosis [16]Our findings are similar to the data previously reported on the genotoxicity in respect of other plant extracts[17-19].

CONCLUSION

The results of these studies indicate that *B. serrata* is non-mutagenic in Ames test, exhibits protection against the mutagenicity induced by 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene in TA98 and TA100 strain, and is non-clastogenic in *in vitro* chromosomal aberration study. These features make the dry extract of *B. serrata*a a promising candidate for further studies.

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