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Original Article - Pharmacological study

Assessment of mutagenicity of *Boswellia serrata* extract (Boswegex®) using Bacterial Reverse Mutation test method

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ABSTRACT

Background: Extracted from the Indian branching tree *Boswellia serrata*, boswellic acids derived from the plant's resin hold promise in addressing inflammation, arthritis, and hyperlipidemia. **Objective:** In this study, the mutagenicity of *B. serrata* (Boswegex®) is evaluated. **Materials and Methods:** The potential genotoxic and mutagenic effects of *Boswellia serrata's* aqueous ethanolic extract, known as Boswegex®, were explored using a bacterial reverse mutation assay against Salmonella strains TA98, TA100, TA102, TA1535, and TA1537. Different concentrations ranging from 500 to 6000 µg/plate were tested using both Method 1 (Standard plate incorporation assay) and Method 2 (pre-incubation assay), with DMSO (Dimethyl sulfoxide) as the solvent control. The positive control, 2-aminoanthracene, was used alongside a metabolic activator (S-9 fraction). Given the prolonged use of supplements, an assessment for mutagenicity is vital. In this context, the mutagenicity of *B. serrata* (Boswegex®) was evaluated through the *in vitro* Bacterial Reverse Mutation Test, employing both the Standard Plate Incorporation Assay and the pre-incubation assay. **Results:** Notably, none of the mutagenic assays revealed a mutagenicity increase over background levels. **Conclusion:** These findings collectively indicate that *Boswellia serrata* (Boswegex®) does not exhibit mutagenic properties.

KEYWORDS: *Bacterial Reverse Mutation Test, Boswellia serrata (Boswegex®), Pre-incubation assay, Standard plate incorporation assay*

INTRODUCTION

The Ayurveda medical system employs *Boswellia serrata* extract to treat inflammatory illnesses. The gummy extract is also used to treat a number of illnesses, such as arthritis, diarrhoea, dysentery, lung ailments, and ringworm.[1-3] Triterpenoids,[4] boswellic acid, and 11-keto-boswellic acid[5] are the primary biologically active components of *B. serrata.*

B. serrata has been the subject of many research investigations on its effectiveness for a variety of purposes, although there are either insufficient or few studies on its safety. The potential for cytotoxic, genotoxic, or mutagenic action of *B. serrata* extract must also be tested because it is typically intended for long-term usage. Additionally, because various studies have linked mutagenicity and carcinogenicity, mutagenicity tests aid in lowering the risks associated with genetic or carcinogenetic mutations for individuals.[6]

To the best of the authors knowledge, there is no genotoxicity data available for this *B. serrata.* The mutagenic activity and anti-mutagenicity of the dry extract of *B. serrata* (Boswegex®), a widely used medicinal plant in India, by *in vitro* Bacterial Reverse Mutation tests, a standard plate incorporation assay, and a pre-incubation assay based on Organisation for Economic Co-operation and Development (OECD) Guideline No. 471 were investigated.[7,8]

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METHODOLOGY

Preparation of Boswegex® with Regular boswellic acids

An aqueous ethanolic extract of the gum resin of *Boswellia serrata* was used for manufacturing Boswegex®, which contains Regular boswellic acids. Boswegex® with Regular boswellic acids were standardised to contain 65% by HPLC, to maintain quality and batch-to-batch consistency.

Dried gum was ground into a coarse powder and then extracted using aqueous ethanol. The extraction process was repeated two to three times. The mixed extracts were then filtered under vacuum, and the filtrate was then heated in a rotary evaporator to between 50-60 oC to create a viscous extract. The resinous portion of the extract was then removed by washing, and after that, the organic acids were selectively precipitated by treating with alkaline water and then acidification. A semi-dried cake was then produced by decolorizing, filtering, and washing in water with a neutral pH. To make powder, this was further dried at 50–60 °C in an oven with a vacuum. By using HPLC, this product was further standardised to 65% of boswellic acids.

Characterization of boswellic acids (Boswegex®) by HPLC

Extraction and purification processes were standardised for Boswegex®. The analytical method was standardised based on HPLC. Briefly, HPLC analysis was performed using a Phenomenex Luna C18, 250 × 4.6 mm, 5 µl column with a flow rate of 1 ml/min in Shimadzu liquid chromatography equipped with a UV/Vis detector at a wavelength of 210 nm and 254 nm to detect the peaks. The sample was eluted by injecting 20 µl of mobile phase (acetonitrile, water, and acetic acid (90:10: 0.1) with a run time of 35 min.

Bacterial reverse mutation assay

The Boswegex® was evaluated for a bacterial reverse mutation test against *Salmonella typhimurium-Histidine auxotrophic strains* TA98, TA100, TA102, TA1535, and TA1537 at different concentrations ranging from 500, 1000, 2000, 4000, 5000, and 6000 µg/plate for both Method 1 (Standard plate incorporation assay) and Method 2 (pre-incubation assay) using DMSO (Dimethyl sulfoxide) as solvent control. The positive controls like 2-Aminoanthracene for "with metabolic activator (*i.e.*, S-9 fraction)" and Mitomycin C, 9-Aminoacridine, Sodium Azide, and 2-Nitrofluorene for "without metabolic activator "were also simultaneously tested. All tests were performed with and without the S-9 fraction, *i.e.*, the metabolic activator collected from NaPB-βNF (Sodium phenobarbital-β-Naphthoflavone) induced rat livers. All the experiments were conducted in triplicate (n = 3), the data were analysed statistically, and the results were expressed as mean ± SD. All combinations of positive controls used for the respective tester strains in the assay are listed in Table 1.

Table 1: Positive Controls

Strain	Activation	Positive controls	Concentration per plate (µg)
TA98	+	2-Aminoanthracene	4
TA98	-	2-Nitrofluorene	2
TA100	+	2-Aminoanthracene	4
TA100	-	Sodium azide	1
TA1535	+	2-Aminoanthracene	4
TA1535	-	Sodium azide	1
TA1537	+	2-Aminoanthracene	4
TA1537	-	9-Aminoacridine	50
TA102	+	2-Aminoanthracene	4
TA102	-	Mitomycin C	0.5

Preparation and Standardisation of Stock cultures

Two days prior to the experiment, tester strains were subcultured from the glycerol stock on SCDA (Soya bean Casein Digest Agar) plates, and plates were incubated at 37 °C for 24 to 48 hours. After an incubation, bacterial suspensions were prepared in sterile saline by dissolving 3–4 colonies of test cultures. The total numbers of cells were adjusted to 10^9 CFU/ml by measuring 0.17 OD at 620 nm with a digital colorimeter.

Preparation of Vogel-Bonner and Glucose minimal medium

The medium (Vogel-Bonner Medium E) was prepared under aseptic conditions (Table 2). Add salts in the order indicated to warm water in a 2-liter beaker or flask placed on a magnetic stirring plate. Allow each salt to dissolve completely before adding the next. Adjust the volume to 1-liter. Distribute into two 1-liter glass bottles. Autoclave, loosely capped, for 30 minutes at 121 °C. When the solutions have cooled, tighten the caps.

Table 2: Vogel-Bonner medium E (50X)

Ingredient	Per liter
Warm distilled H_2O (45°C)	670 ml
Magnesium sulfate ($MgSO_4$• 7 H_2O)	10 g
Citric acid monohydrate	100 g
Potassium phosphate, dibasic (anhydrous) (K_2HPO_4)	500 g
Sodium ammonium phosphate ($NaHNH4(PO_4$ • 4 H_2O)	175 g

Add 15 g of agar to 930 ml of distilled water in a 2-liter flask. Autoclave for 20 minutes using slow exhaust. When the solution has cooled slightly, add 20 ml of sterile 50X VB salts and 50 ml of sterile 40% glucose. For mixing, a large magnetic stir bar can be added to the flask before autoclaving. After all the ingredients have been added, the solution should be stirred thoroughly. Pour 30 ml into each petri plate (Table 3).

Table 3: Minimal glucose plates

Ingredient	Per liter
Agar	15 g
Distilled H_2O	930 ml
50X VB salts	20 ml
40% glucose	50 ml

Preparation of Top Agar

The top agar was prepared by dissolving 6 g of Agar and 5 g of NaCl in 1000 ml of distilled water in a water bath. Top agar was autoclaved at 121 °C for 20 minutes at 15 psi of pressure.

Preparation of S-9 (Rat liver)

The male rat was dosed exogenously by inducing NaPB/βNF (Sodium phenobarbital/β-Naphthoflavone) for 24 h. After a period of induction, the rat was sacrificed, and the liver was homogenised under aseptic conditions. The ingredients mentioned above were added in reverse order. The solution was prepared fresh and kept in an ice bath in chilled conditions. Any leftover S9 or S9 mix was discarded (Table 4).

Table 4: Preparation of S-9 Mix

Ingredients	Standard S-9 Mix (Per 50ml)
Rat liver S9	2.0 ml
$MgC1_2$-KC1 salts	1.0 ml
1 M glucose-6-phosphate	0.25 ml
0.1 M NADP	2.0 ml
0.2 M phosphate buffer, pH 7.4	25.0 ml
Sterile distilled water	19.75 ml

Detection of bacterial mutagenic reversion of Boswegex®

Determining the solubility of Boswegex®

The solubility of Boswegex® was checked by dissolving it in a DMSO (Dimethyl sulfoxide) solvent at 5–60 mg/ml. At a concentration of 60 mg/ml, the test substance Boswegex® gave a clear solution without precipitation, and hence this concentration was used for further studies. Based on the solubility of Boswegex®, DMSO (Dimethyl sulfoxide) was selected as the solvent control.

Determining the Precipitation of Boswegex®

Boswegex® was uniformly dissolved in DMSO (Dimethyl sulfoxide) to get concentrations of 5, 10, 20, 40, and 60 mg/ml of the solvent. One hundred microliters of each of these dilutions (corresponding to 500, 1000, 2000, 4000, and 5000 µg) were mixed with 2 ml of molten soft agar containing 0.5 mM histidine or biotin and overlaid on Vogel Bonner agar plates in triplicates. The plates were observed after 2 hours for clarity of agar plates. At this concentration, Boswegex® was observed without precipitation, and hence this concentration was used for further studies (Table 5).

Table 5: 0.5 mM histidine/biotin solution

Ingredient	Per 250 ml
D-Biotin(Molwt 244.31)	30.5 mg
L-Histidine.HC1 (Molwt 209.63)	26 mg

Determining the preliminary toxicity of Boswegex®

One hundred micro liters of each respective concentration (500, 1000, 2000, 4000, 5000 µg/plate) were mixed with 2 ml of soft agar containing 0.5 mM histidine and biotin, 500 µl of S-9 mix/0.2 M Sodium phosphate buffer (for the test in the presence or absence of metabolic activation), and 0.1 ml of overnight TA 100 culture. These were mixed and overlaid onto VB agar plates in triplicate. Similarly, solvent control was also performed. After the solidification, agar plates were incubated at 37 °C for 48–72 hours. The experiment was conducted at various concentrations of Boswegex®, and mean numbers of revertant colonies were obtained. The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent test strain. The results were compared with those of the solvent control for both methods in the presence and absence of metabolic activators. The revertant colonies observed were also within the limits of the established number of spontaneous revertants. The number of revertant colonies in the positive controls was increased by 5.4 to 30.6 and 5.6 to 26.1-fold in methods 1 and 2 under identical conditions. The mean number of revertant colonies at all the tested concentrations was recorded and compared to the solvent control both in the presence and absence of metabolic activation. Similarly, the intensity of the bacterial background (4+) was also comparable to that of the solvent control plates. OECD guidelines 471 "Bacterial Reverse Mutation Test" were used for this study[9]. For better understanding, we have given it in terms of coding from 1+ to 4+, where 4+ indicates that the background lawn has shown good growth without any impact on the growth.

Detection of mutagenicity by Standard Plate incorporation assay (Method 1)

Agar was mixed with a suspension of bacterial cells and the test ingredients described in Table 1 before being promptly plated onto VB agar plates. Revertant colonies were counted after an appropriate period of incubation (48–72 hours) at 37 ± 1 °C and compared to the number of spontaneous revertants in the solvent control plates.

Detection of mutagenicity by Pre-incubation assay (Method 2)

In sterile test tubes, the test constituents of 100 µl test compound, 0.5 ml of S-9 / 0.2 M sodium phosphate buffer, and 100 µl tester strains were added and incubated at (37 ± 1) °C for 30-45 min in the incubator. After pre-incubation, 2 ml of soft agar was added to it and poured onto minimal glucose agar plates (VB plates). After an incubation of 48–72 hours, revertant colonies were counted and compared with the number of spontaneous revertants in the solvent control plates.

Acceptability of the test

The Experiments were considered acceptable because of the following criteria:

1) Regular background growth in the solvent control was observed.
2) The spontaneous reversion rate in the solvent control was within the range.
3) The positive control substances produced a significant increase in mutant colony frequencies when compared to the respective solvent control plates.

Evaluation and interpretation of results

When the number of revertants in the treatment groups is twice as high as the solvent control, as shown at a minimum of three concentration levels, the test is considered positive for the tester strains TA 98 and TA 100. The number of revertants should be three times that of the solvent control for tester strains TA1535, TA1537, and TA102, and this should be obvious at least three concentration levels.

Statistical analysis

Data were analysed for obtaining mean and standard deviation. Data were also analysed for differences among solvent control and positive control groups using ANOVA. Statistical analysis was carried out using software Graph pad prism (version 5.01). Differences between the solvent control and treatment groups was tested by Dunnett's "t" test at a 5% level ($p \leq 0.05$) of significance. Data were represented as Mean ± SD revertant colonies.

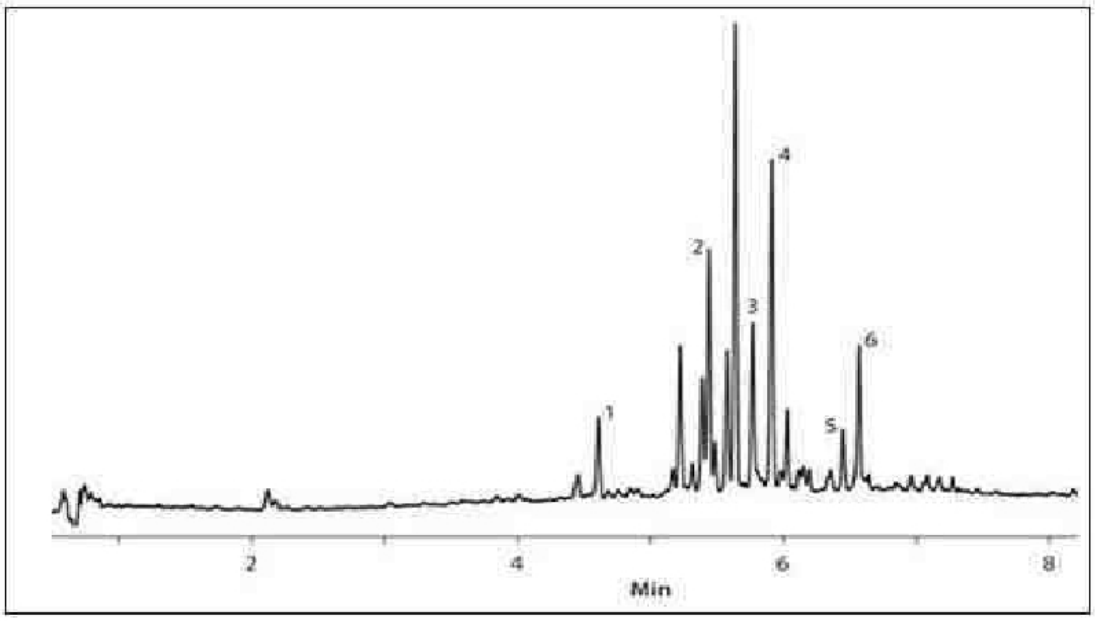
RESULTS

Characterization of boswellic acids (Boswegex®) by HPLC

The characterization of boswellic acids was done using HPLC. The peaks 1 to 6 represented 11-keto-

β-boswellic acid (KBA), 3-O-acetyl-11-keto-β-boswellic acid, α- boswellic acid, β- boswellic acid, 3-O-acetyl-α- boswellic acid, and 3-O-acetyl-β-boswellic acid, respectively (Figure 1).

Figure 1: A representative HPTLC image shows the elution profile of the major boswellic acids in Boswegex at 210nm



Effect of Boswegex® on the Bacterial Reverse Mutation Method

The results were compared with those of the solvent control for both methods in the presence and absence of metabolic activators.

The revertant colonies observed were also within the limits of the established number of spontaneous revertants (Table 6 and Table 7).

Table 6: Spontaneous natural revertants observed without metabolic activation

Test Strains	Without S-9 activation			
	R1	R2	R3	Mean ± SD
TA98	19	21	27	22± 4.1
TA100	50	57	65	57± 7.5
TA102	202	225	231	219± 15.3
TA1535	7	8	9	8± 1.0
TA1537	7	5	8	7± 1.5

Data was expressed as mean ± SD, Where *p* value is ≤ 0.05

Table 7: Spontaneous natural revertants observed with metabolic activation

Test Strains	With S-9 activation			
	R1	R2	R3	Mean ± SD
TA98	28	25	23	25± 2.5
TA100	54	62	65	60± 5.6
TA102	220	214	227	220± 6.5
TA1535	4	7	8	6 ± 2.0
TA1537	8	5	6	6 ± 1.5

Data was expressed as mean ± SD, Where *p* value is ≤ 0.05

Preliminary Toxicity of Boswegex® on TA 100 without metabolic activation and with metabolic activation were expressed in Table 8 and Table 9.

For tester strains TA 98 and TA 100, the test is considered positive when the number of revertants in the treatment groups shows twice that of the solvent control, and this should be evident at a minimum of three concentration levels.

Table 8: Preliminary Toxicity of Boswegex® on TA 100 without metabolic activation

Test conc./ plate	Without S-9 activation				Bacterial lawn intensity
	R1	R2	R3	Mean ± SD	
DMSO (Dimethyl sulfoxide)	50	57	65	57 ± 7.5	4 +.
500 µg	52	57	54	54 ± 2.5	4 +.
1000 µg	60	57	59	58 ± 1.5	4 +.
2000 µg	88	90	78	85 ± 6.4	4 +.
4000 µg	55	70	62	62 ± 7.5	4 +.
6000 µg	56	55	50	53 ± 3.2	4 +.

Data was expressed as mean ± SD, Where *p* value is ≤ 0.05

Table 9: Preliminary Toxicity of Boswegex® on TA 100 with metabolic activation

Test conc./ plate	Without S-9 activation				Bacterial lawn intensity
	R1	R2	R3	Mean ± SD	
DMSO (Dimethyl sulfoxide)	52	61	59	57 ± 4.7	4 +.
500 µg	57	52	54	54 ± 2.5	4 +.
1000 µg	51	48	56	51 ± 4.0	4 +.
2000 µg	59	53	58	56 ± 3.2	4 +.
4000 µg	55	60	62	59 ± 3.6	4 +.
6000 µg	51	48	59	52 ± 5.6	4 +.

Data was expressed as mean ± SD, Where *p* value is ≤ 0.05

For tester strains TA1535, TA1537, and TA102, the number of revertants should be three times that of the solvent control, and this should be evident at a minimum of three concentration levels (Table 10-15).

Effect of Boswegex® by statistical analysis

The statistical analysis of the combined data of the two procedures showed no statistically significant increase in the number of revertants in any of the five concentrations of Boswegex® examined as compared to the respective controls in any of the five Salmonella strains.

DISCUSSION

The toxicity and Genotoxicity information of many herbal formulations is still insufficient, despite the strong interest in traditional herbal medicine being utilized to treat a variety of ailments. The majority of people think that products derived from natural sources are safe and shouldn't be risky. To ensure safety, an evaluation of the Genotoxicity of the *Boswellia serrata* extract is necessary.

Many plants contain mutagenic components, such as tannins, anthraquinones, and furocoumarins.[10] Because of this, screening medicinal plants to determine their mutagenicity is essential. It should be mandatory to conduct additional tests before consuming plants that have the potential to cause mutagenicity.

The Genotoxicity of a known mutagen is also increased by certain plant extracts. Accordingly, these extracts appear to be co-mutagenic and hence possibly carcinogenic, showing that they could be dangerous. When an extract is found to be

antimutagenic in relation to a recognized mutagen, this does not always mean that it is additionally antimutagenic in relation to another mutagen.

Additionally, when given in addition to this second or another mutagen, it's easy to make the same extract co-mutagenic. As a result, an extract may exhibit both antimutagenicity and mutagenicity.[11-12]

Table 10: Average No. of revertants obtained by Method-1 and 2 without Metabolic Activation

Average No. of revertants (*n=3*) /plate ± SD										
Test Conc. /plate	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
DMSO (0.1 ml)	**M- 1**	**M-2**	**M- 1**	**M-2**	**M- 1**	**M-2**	**M- 1**	**M-2**	**M- 1**	**M-2**
	27.0 ± 1.0	23.0 ± 2.0	56.0 ± 1.7	20.0 ± 0.0	224.0 ± 3.6	203.0 ± 3.6	8.0 ± 1.0	8.0 ± 1.0	7.0 ± 1.0	9.0 ± 2.0
500 µg	23.0 ± 1.0	22.0 ± 1.0	35.0 ± 2.6	21.0 ± 1.0	228.0 ± 5.6	248.0 ± 2.6	7.0 ± 1.0	7.0 ± 1.0	7.0 ± 1.0	7.0 ± 2.0
1000 µg	24.0 ± 2.0	23.0 ± 3.6	39.0 ± 2.6	25.0 ± 2.6	246.0 ± 4.0	255.0 ± 7.9	7.0 ± 1.0	7.0 ± 2.6	8.0 ± 2.0	9.0 ± 1.7
2000 µg	27.0 ± 1.0	19.0 ± 2.6	31.0 ± 1.7	29.0 ± 2.6	231.0 ± 7.2	289.0 ± 3.6	7.0 ± 0.0	8.0 ± 1.7	8.0 ± 1.0	8.0 ± 0.0
4000 µg	29.0 ± 2.0	28.0 ± 2.0	36.0 ± 3.0	25.0 ± 5.0	236.0 ± 3.6	255.0 ± 3.0	7.0 ± 1.7	7.0 ± 0.0	8.0 ± 1.0	8.0 ± 1.7
6000 µg	28.0 ± 2.0	29.0 ± 2.0	30.0 ± 0.0	23.0 ± 0.0	212.0 ± 5.3	234.0 ± 2.6	6.0 ± 1.0	7.0 ± 1.0	7.0 ± 0.0	9.0 ± 1.0
Positive Control	413 ± 2.6	496 ± 5.2	430 ± 12.5	456 ± 5.6	1246 ± 2.6	1140 ± 5.0	185 ± 4.6	209 ± 7.9	214 ± 4.0	187 ± 6.6

Data was expressed as mean ± SEM, Where *p* value is ≤ 0.05

Table 11: Method 1 (Direct plate incorporation method) without Metabolic Activation Individual Plate Revertant Colony Counts

Concentration (/plate)	Plate TA 98	Plate TA 100	Plate TA 102	Plate TA 1535	Plate TA 1537
Solvent Control DMSO (0.1 ml)	26	58	227	8	7
	28	55	225	7	8
	27	55	220	9	6
a) 500 µg	23	32	229	8	7
	22	36	233	6	6
	24	37	222	7	8
b) 1000 µg	24	38	250	7	8
	26	37	246	6	6
	22	42	242	8	10
c) 2000 µg	27	30	229	7	8
	26	30	239	7	7
	28	33	225	7	9
d) 4000 µg	31	36	240	6	7
	27	33	235	6	9
	29	39	233	9	8
e) 6000 µg	28	30	210	5	7
	26	30	218	7	7
	30	30	208	6	7
Positive control	410	420	1245	180	210
	415	426	1249	189	218
	414	444	1244	186	214

Table 12: Method 2 (Direct plate incorporation method) without Metabolic Activation Individual Plate Revertant Colony Counts

Concentration (/plate)	Plate TA 98	Plate TA 100	Plate TA 102	Plate TA 1535	Plate TA 1537
Solvent Control DMSO (0.1 ml)	21	20	200	8	7
	23	20	202	9	9
	25	20	207	7	11
a) 500 µg	23	22	249	7	7
	22	20	245	6	9
	21	21	250	8	5
b) 1000 µg	19	24	258	6	7
	24	23	261	10	10
	26	28	246	5	10
c) 2000 µg	20	26	290	9	8
	16	30	292	9	8
	21	31	285	6	8
d) 4000 µg	28	20	255	7	9
	26	25	252	7	6
	30	30	258	7	9
e) 6000 µg	31	23	232	7	8
	27	23	233	8	9
	29	23	237	6	10
Positive control	490	450	1145	200	180
	499	457	1140	212	188
	499	461	1135	215	193

Table 13: Average No. of revertants obtained by Method-1 and 2 with Metabolic Activation

Average No. of revertants (*n=3*) /plate ± SD										
Test Conc. /plate	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
DMSO (0.1 ml)	**M- 1**	**M-2**	**M- 1**	**M-2**	**M- 1**	**M-2**	**M- 1**	**M-2**	**M- 1**	**M-2**
	33.0 ± 4.4	24.0 ± 3.6	53.0 ± 3.0	26.0 ± 3.6	230.0 ± 8.7	204.0 ± 2.6	8.0 ± 2.0	8.0 ± 0.0	8.0 ± 0.0	10.0 ± 2.0
500 µg	25.0 ± 4.0	22.0 ± 2.0	37.0 ± 6.1	24.0 ± 3.6	213.0 ± 4.4	217.0 ± 1.7	6.0 ± 1.7	6.0 ± 1.0	6.0 ± 1.0	7.0 ± 1.0
1000 µg	24.0 ± 2.0	22.0 ± 2.0	38.0 ± 7.0	24.0 ± 3.6	223.0 ± 3.5	209.0 ± 1.7	7.0 ± 1.0	6.0 ± 1.0	8.0 ± 1.7	9.0 ± 1.0
2000 µg	22.0 ± 2.6	21.0 ± 1.7	34.0 ± 5.6	21.0 ± 1.0	203.0 ± 2.6	235.0 ± 1.7	8.0 ± 1.7	7.0 ± 0.0	8.0 ± 2.6	8.0 ± 0.0
4000 µg	24.0 ± 4.0	29.0 ± 1.0	38.0 ± 3.0	24.0 ± 4.0	189.0 ± 5.3	255.0 ± 1.7	7.0 ± 0.0	7.0 ± 2.0	8.0 ± 2.6	8.0 ± 1.7
6000 µg	22.0 ± 0.0	26.0 ± 2.0	31.0 ± 0.0	23.0 ± 4.4	216.0 ± 5.2	232.0 ± 1.0	5.0 ± 1.0	5.0 ± 1.0	6.0 ± 0.0	9.0 ± 0.0
Positive Control	557 ± 3.5	453 ± 3.0	639.3 ± 3.1	453 ± 3.0	1247 ± 8.2	1140 ± 5.0	197 ± 8.5	208 ± 4.0	202 ± 7.2	187 ± 6.2

Data was expressed as mean ± SEM, Where *p* value is ≤ 0.05

The most widely used tester strains in the pharmaceutical industry were chosen for this investigation due to their high sensitivity, ability to detect a wide range of known bacterial mutagens, and frequent application.[13]

Plant extracts with strong antioxidant properties generally demonstrated good antimutagenic effects. The experiment was conducted at various concentrations of Boswegex® and mean numbers of revertant colonies were obtained.

Table 14: Method 1 (Direct plate incorporation method) with Metabolic Activation Individual Plate Revertant Colony Counts

Concentration (/plate)	Plate TA 98	Plate TA 100	Plate TA 102	Plate TA 1535	Plate TA 1537
Solvent Control DMSO (0.1 ml)	31	53	235	6	8
	30	50	235	8	8
	38	56	220	10	8
a) 500 µg	29	30	211	5	7
	21	41	218	8	6
	25	40	210	5	5
b) 1000 µg	26	31	221	8	6
	24	38	227	7	9
	22	45	221	6	9
c) 2000 µg	20	33	200	9	5
	21	29	204	9	10
	25	40	205	6	9
d) 4000 µg	20	38	195	7	10
	24	35	187	7	5
	28	41	185	7	9
e) 6000 µg	22	31	210	6	6
	22	31	219	5	6
	22	31	219	4	6
Positive control	561	636	1240	196	210
	555	642	1256	206	196
	555	640	1245	189	200

Table 15: Method 2 (Direct plate incorporation method) with Metabolic Activation Individual Plate Revertant Colony Counts

Concentration (/plate)	Plate TA 98	Plate TA 100	Plate TA 102	Plate TA 1535	Plate TA 1537
Solvent Control DMSO (0.1 ml)	25	25	207	8	12
	20	23	203	8	8
	27	30	202	8	10
a) 500 µg	22	25	218	7	8
	20	20	215	6	6
	24	27	218	5	7
b) 1000 µg	22	27	210	7	9
	20	20	207	6	8
	24	25	210	5	10
c) 2000 µg	20	20	237	7	8
	20	22	234	7	8
	23	21	234	7	8
d) 4000 µg	29	24	256	9	9
	28	20	256	7	9
	30	28	253	5	6
e) 6000 µg	26	21	231	5	9
	28	20	232	4	9
	24	28	233	6	9
Positive control	450	450	1145	204	180
	456	456	1140	208	189
	453	453	1135	212	192

The results were compared with those of the solvent control, for both the methods, in the presence and absence of metabolic activator. The revertant colonies observed were also within the limits of established number of spontaneous revertants.

The study indicated that the test substance Boswegex® was not found to be mutagenic in this bacterial reverse mutation test at the mentioned concentrations under the conditions employed.

CONCLUSION

Studies on the mutagenicity and antimutagenicity of traditional medicinal plants are crucial for risk assessment and the development of novel anti-cancer drugs. Therefore, the development of new drugs with anti-mutagenic effects plays an essential role in modern life. All five of our test strains revealed the same results from this study. Therefore, it can be said that Boswegex® did not exhibit mutagenicity in this bacterial reverse mutation test at the tested quantities under the used conditions. These characteristics make Boswegex® a promising subject for more research.

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REFERENCES

1. Krieglstein C F, Anthoni C, Rijcken E J, Laukotter M, Spiegel H U, Boden S E. Acetyl-11-keto-betaboswellic acid, a constituent of a herbal medicine from Boswellia serrata resin, attenuates experimental ileitis. *International Journal of Colorectal Disease*. 2001; 16: 88.
2. Gerhardt H, Seifert F, Buvari P, Vogelsang H, Repges R. Therapy of active Crohn's disease with Boswellia serrata extract H 15. *Zeitschrift fur Gastroenterologie*. 2001; 39: 11–7.
3. Kimmatkar N, Thawani V, Hingorani L, Khiyani R. Efficacy and tolerability of Boswellia serrata extract in treatment of osteoarthritis of knee—a randomized double blind placebo controlled trial. *Phytomedicine*. 2003; 10: 3–7.
4. Babita M, Taneja S C, Sethi V K. Two triterpenoids from Boswellia serrata gum resin. *Phytochemical*, 1995; 39: 453-455.
5. Shailesh A S, Ishwarsinh S R, Bhanubhai N S, Dharmesh A P, Vijay K P. Estimation of Boswellic acids from market formulations of Boswellia serrata extract and 11-keto-βboswellic acid in human plasma by high performance thin layer chromatography. *Journal of Chromatography B*, 2007; 848: 232-238.
6. Hoffmann G R. Mutagenicity testing in environmental toxicology. *Environmental science & technology* 1982; 16 (10): 560A-574A.
7. Dorothy M M, Bruce N A. Revised methods for Salmonella mutagenicity test. *Mutation Research*. 1983; 113: 173-215.
8. Callander R D, Mackay J M, Clay P, Elcombe C R, Elliott B M. Evaluation of Phenobarbital/ β-naphthoflavone as an alternative S-9 induction regime to Aroclor 1254 in the rat for use in in vitro Genotoxicity assays. *Mutagenesis*. 1995; 10: 517-522.
9. Wilcox P, Naidoo A, Wedd D J, Gatehouse D G. Comparison of Salmonella typhimurium TA 102 with Escherichia coli WP2 Tester strains. *Mutagenesis*. 1990; 5: 285-291.
10. Abdillahi H S, Verschaeve L, Finnie J F, Staden J V. Mutagenicity, antimutagenicity and cytotoxicity evaluation of South African Podocarpus species. Journal of Ethnopharmacology, 2012; 139: 728-738.
11. Von Borstel R C, Higgins J A. Janus carcinogens and mutagens. Mutation Research, 1998; 402: 321-329.
12. Zeiger E. Illusions of safety: antimutagens can be mutagens, and anticarcinogens can be carcinogens. Mutation Research, 2003; 543: 191–194.
13. Purves D, Harvey C, Tweats D, Lumley C E. Genotoxicity testing: current practices and strategies used by the pharmaceutical industry. Mutagenesis, 1995; 10: 297–312.