INTRODUCTION

The phytochemicals are secondary metabolic products produced by plants in response to environmental stresses. Thousands of these phytochemicals have been identified and when consumed in human diet, may affect chronic disease risk. Of well over 2000 preparations known to the modern practitioner of Ayurveda, nearly 1500 are of plant origin [1]. Susruta Samhita refers to 700 drugs including a small number, which were not available in the country in that time. India has been exposed for well over a century to the application of allopathic medicines with their definite merits as well as failings.

The studies carried out on antimutagenic effects of the ayurvedic therapies were critically reviewed, their protocol studied, and the significant results have been pointed out as this effect may account for their therapeutic effect to great extent [2]. The ayurvedic herbs have antimutagenic and antiviral activity occupy an important place in the ayurvedic system of medicine and are used in the treatment of various ailments either alone or from a part of various formulations [3].

The mutagenicity refers to the induction of permanent transmissible changes in the structure of genetic material within cells and organisms [4,5]. The mutations are caused by mutagens such as reactive oxygen species (ROS), ultraviolet radiation, ionizing radiation, pollution, and are also the end products of normal metabolic processes of aerobic organisms [6-8]. Oxidative stress caused by ROS is known to cause tissue injury and can include damage to DNA, proteins, and lipids [9,10]. Oxidative injury to DNA occurs when oxygen radicals react with DNA [6]. If not repaired, the changes in nucleic acid bases and the breaks in the DNA chain that occur after free radical reactions lead to DNA mutation and mutagenic forms of DNA [4,10].

The term genotoxicity is a broader concept than mutagenicity and describes the capacity of the compounds to affect the DNA structure or the cellular apparatus and topoisomerases, which are responsible for the genome fidelity. Genotoxic effects on DNA are not always related to mutations [11,12]. The mutations are created mainly by external factors, including chemical and physical agents, called mutagens. In addition, the mutations can occur spontaneously due to errors in DNA replication, repair, and recombination. In general, mutations can be grouped into negative, neutral, positive, lethal, and sublethal. The mutagenic changes that occur in germline cells can be passed to future generations, whereas somatic mutations may contribute to the pathogenesis of various pathological conditions, including cancer [13-16].

Antimutagenic agents are able to counteract the effects of mutagens. Therefore, knowledge on the mode of action of certain mutagenic compounds provides a basis for an explanation of how antimutagenic compounds work: Identifying the antimutagenic compounds are among the most promising areas of research in recent years [17]. In this study, the mutagenic and antimutagenic potential of Liv52 was evaluated by employing Salmonella mutagenicity test with and without metabolic activation.
MATERIALS AND METHODS

Materials
Liv52 syrup
Liv52 syrup is a patented product of Himalaya drugs and Co. India comprising a standardized mixture of the plants Capparis spinosa (15.6 mg/ml), Cichorium intybus (15.6 mg/ml), Solanum nigrum (6.4 mg/ml), Terminalia arjuna (6.4 mg/ml), Cassia occidentalis (3.2 mg/ml), Achillea millefolium (3.2 mg/ml), and Tamarix gallica (3.2 mg/ml).

Chemicals
Nutrient broth and agar were purchased from HiMedia Laboratories Pvt. Ltd. 2-aminofluorene (2-AF), 2-anthraquinine (2-AN), benzo(a)pyrene (BaP), 4-nitro-O-phenylenediamine (NPD), butylated hydroxyanisole (BHA), D L-tocopherol (Vitamin E), naphthoflavones, phenobarbital sodium, cumene hydroperoxide, 2-aminoacridine, dantron, D-biotin, and L-histidine were purchased from Sigma Chemical Company; NADP and glucose 6-phosphate were from Sisco Research Laboratories, India, and Dextrose, KCl, MgCl₂, and other buffer components were from SD Fine Chemicals, India.

Salmonella tester strains and mutagenicity test
Salmonella typhimurium tester strains TA1537, TA1538, TA100, and TA102 were kindly supplied from Bruce N Ames Laboratory, University of California, USA. An optimum association of standard tester strains for maximum detection of mutagens was used in this study [18].

Metabolic activation
Rat liver S9 fraction was used where induction of liver enzymes was done using phenobarbitone and beta-naphthoflavone [20,21]. S9 mixture was prepared freshly before the test by adding cofactors (8 mm MgCl₂, 33 mm KCl, 5 mm glucose 6-phosphate, and 4 mm NADP at the pH 7.4) to 50 µl of S9 [19].

Methods
Ames Salmonella/mammalian - microsome mutagenicity test (an in vitro short-term mutagenicity test) was used in this study. S. typhimurium tester strains TA1537, TA1538, TA100, and TA102 were used for mutagenicity testing. The mutagenicity study was conducted using tester strains TA1538 and TA100 against various standard mutagens with and without metabolic activation.

Mutagenicity assay
All the tester strains of S. typhimurium were analyzed for mutagenicity assay with and without S9 mix. The molten soft agar of 2 ml, various concentrations of the test compound (10 to 500 µl) of Liv52, 0.1 ml of overnight bacterial culture, and 0.5 ml of S9 mix (wherever used) were added, gently mixed and poured over minimal glucose agar medium and incubated at 37°C for 48 hrs. After incubation, the plates were counted for his⁻ revertants. The experiment was performed in duplicate using the similar procedure for all the concentrations. The results were determined as mean of six plates per point with standard deviation and viable cell count as approximately 1-2 × 10⁹ cfu/ml. The positive mutagenic controls including 4-Nitro-O-phenylenediamine (-S9) for TA1538 and TA100; aminoacridine (-S9) for TA1537; cumene hydroperoxide (-S9) for TA102, 2-aminofluorene, 2-antramine, and Benzo(a)pyrene (+S9) for TA1538 and TA100, Emodin (+S9) for TA1538, and dantron (+S9) for TA102 were used. All the solutions were prepared freshly by dissolving in dimethyl sulfoxide.

Antimutagenicity assay
For antimutagenicity assay, plate incorporation method and preincubation method were used by incorporating the tester strains TA1538 and TA100. For plate incorporation assay to 2 ml of the molten soft agar various concentrations of test compound (10 to 500 µl), 0.1 ml of overnight bacterial culture, 0.1 ml of known appropriate positive mutagen control, and 0.5 ml of S9 mix (wherever used) were added aseptically and poured over minimal glucose agar medium and incubated at 37°C for 48 hrs. For antimutagen control, BHA and Dl tocopherol were used.

For preincubation method, 0.1 ml of overnight culture, various concentrations of test compounds, optimal concentration of appropriate known mutagen control, and 0.5 ml of S9 mix were added in sterile tubes, mixed thoroughly using vortex mixture and incubated at 37°C for 20-30 minutes. This mixture was added to molten top agar and poured on the surface of minimal glucose agar and continued further as in plate incorporation method. The results were expressed as the mean of six plates from two independent experiments with standard deviation. Antimutagenicity or percentage of inhibition of mutagenicity by Liv52 was calculated using formula as given below.

\[
\text{Antimutagenicity/percentage of inhibition of mutagenicity} = \frac{(a-b)}{(a-c)}\times 100
\]

Where,
- \(a\) = number of his⁻ revertants induced by positive mutagen,
- \(b\) = number of his⁻ revertants induced by the positive mutagen in the presence of test Compound,
- \(c\) = number of his⁻ revertants induced by the test compound alone.

Statistical evaluation
Statistical analysis was performed using Karl Pearson Correlation Coefficient method. Statistical Package for the Social Sciences (SPSS) ver.15.0 was used.

RESULTS AND DISCUSSION
The present investigation depicts the antimutagenic potential of various concentrations of Liv52 in S. typhimurium reverse mutation assay. The test preparation was found to be nontoxic to the tester strains at different doses with or without metabolic activation. The characteristics his⁻ revertants patterns of the standard tester strains to various standard mutagens were shown in Table 1. The viable count was approximately 1-2 × 10⁹ cells/ml in all the tester strains (TA1537, TA1538, TA100, and TA102).

Liv52 failed to induce his⁻ revertants in all the four tester strains either in the presence or absence of metabolic activation by plate incorporation method thereby found to be nonmutagenic in Ames test (Table 2).

The effect of Liv52 on NPD induced mutation frequency by plate incorporation method was depicted in Table 3. It was very clearly defined and proved that Liv52 inhibited NPD induced his⁻ revertants significantly in both the strains that were dose-dependent. In TA1538, the highest inhibitory effect was 48.4% and in TA100 it was 47.2% at the dose of 100 µl of Liv52 per plate.

Fig. 1 Highlighted the effects of Liv52 on NPD induced mutation frequency in TA1538 and TA100 by preincubation method. Further, there was a significant enhanced inhibitory effect on preincubation (63.1%) in TA1538 and 61.8% in TA100.

The effect of Liv52 on 2-aminofluorene, 2-anthraquinine, and cigarette smoke condensate-induced mutation frequency in TA1538 by plate incorporation method was well defined in this study (Table 4). From these data, it was obvious that in TA1538, Liv52 reduced his⁻ revertants induced by the above said indirect mutagens, in a dose-dependent manner. At a dose of 100 µl of Liv52 per plate, the percentage inhibitions of his⁻ revertants were the highest of 99.8 for 2-AF, 99.8 for 2-AN, and 100 for CSC. Further, an increase of the test compound in the bioassay did not make a significant difference in the inhibitory effect (Correlation coefficient were > 0.5 and p<0.005).
The effect of Liv52 on 2-aminofluorene, 2-anthranilic, benzo(a)pyrene, and cigarette smoke condensate-induced mutation frequency in TA100 by plate incorporation method was well defined in this study. From these data, it was obvious that in TA100 also Liv52 reduced its revertants induced by the above said indirect mutagens, in a dose-dependent manner (Correlation coefficient were >0.5 and p<0.005). For the dose of test compound, 100 µl per plate the percentage inhibitions of his revertants were the highest of 100 for 2-AF, 100 for 2-AN, 97.7 for B(a)P, and 100 for CSC (Table 5).

Figs. 2 and 3 depict the effect of Liv52 on the above said indirect mutagens induced mutation frequency in TA1538 and TA100, respectively, by preincubation method. It was clear from the figures that there was a significant enhancement in inhibitory action by Liv52 on preincubation (Correlation coefficient was > 0.5 and p<0.005).

### DISCUSSION

Liv52, a polyherbal ayurvedic preparation was found to be nonmutagenic in Ames test. The antimutagenic potential of Liv52 was demonstrated against NPD induced mutagenesis in the absence of metabolic activation in both the tester strains TA1538 and TA100. Liv52 exhibited antimutagenicity against 2-AF, 2-AN, and CSC induced mutagenesis in TA1538 and also against 2-AF, 2-AN, B(a)P, and CSC in TA100 in the presence of metabolic activation. It was evident from the data that the antimutagenic activity of Liv52 was more potent in the presence of metabolic activation than in the absence of S9 mix (Correlation coefficient was >0.5 and p<0.005). Further, it was noticed that the antimutagenic activity of Liv52 against both direct and S9 dependent mutagens was comparatively more in TA100 than in TA1538. These results indicate that Liv52 inhibits its revertants

### Table 1: Characteristic reversion patterns of standard tester strains to diagnostic mutagens

| Diagnostic mutagens | His revertants/plate |
|---------------------|----------------------|
|                      | TA1537 | TA1538 | TA100 | TA102 |
|                      | −S9 | +S9 | −S9 | +S9 | −S9 | +S9 | −S9 | +S9 |
| Spontaneous revertants | 8±1 | 15±2 | 15±1 | 23±2 | 143±1 | 161±9 | 386±2 | 409±3 |
| Aminocaridine         | 858±51 | - | - | - | - | - | - | - |
| Cumene hydroperoxide  | - | - | - | - | - | - | - | - |
| 4-nitro-o-phenylene diamine | - | - | 671±41 | - | - | - | - | - |
| Sodium azide          | - | - | - | - | 725±56 | - | - | - |
| 2-Anthranilic         | - | 331±16 | - | - | 608±42 | 721±50 | - | - |
| 2-amino fluorene      | - | - | - | - | 633±39 | - | 526±38 | - |
| Benzo (a)pyrene       | - | - | - | - | - | - | 621±40 | - |
| 7,12-dimethyl benzanthracene | - | - | - | - | - | - | 783±56 | - |
| Dantron               | - | - | - | - | - | - | - | - |
| Cigarette smoke condensate | - | - | - | - | 201±9 | - | 433±26 | - |

Results were expressed as the mean of six plates from the two independent experiments with standard deviation. Viable cell count was approximately 1-2 x 10^9 cells/ml.

### Table 2: Nonmutagenicity of Liv52 syrup in tester strains TA1537, TA1538, TA100 and TA102 with and without S9 mix by plate incorporation method

| Various concentrations of Liv52 syrup | His revertants/plate |
|--------------------------------------|----------------------|
|                                      | TA1537 | TA1538 | TA100 | TA102 |
|                                      | −S9 | +S9 | −S9 | +S9 | −S9 | +S9 | −S9 | +S9 |
| Spontaneous revertants               | 9±1 | 15±2 | 13±2 | 21±2 | 14±10 | 158±12 | 378±29 | 412±36 |
| 10 µl/plate of Liv52 syrup           | 9±1 | 13±1 | 13±1 | 23±2 | 129±10 | 159±13 | 371±29 | 408±29 |
| 25 µl                               | 12±3 | 10±2 | 16±1 | 21±1 | 139±12 | 170±11 | 385±35 | 412±31 |
| 50 µl                               | 13±2 | 12±1 | 21±2 | 29±2 | 153±11 | 149±10 | 391±31 | 422±29 |
| 75 µl                               | 10±2 | 10±2 | 12±1 | 23±1 | 133±9 | 158±12 | 402±39 | 410±51 |
| 100 µl                              | 9±1 | 15±2 | 12±1 | 19±1 | 155±16 | 168±1 | 394±29 | 429±21 |
| 200 µl                              | 8±1 | 14±1 | 23±2 | 24±1 | 166±12 | 148±9 | 408±34 | 437±20 |
| 500 µl                              | 10±1 | 10±1 | 15±1 | 26±2 | 143±11 | 150±12 | 378±18 | 426±16 |

Results were expressed as the mean of 6 plates from two independent experiments with standard deviation. Viable cell count was approximately 1-2 X 10^9 cells/ml.

### Table 3: Antimutagenicity of Liv52 against NPD induced mutagenesis in TA1538 and TA100 without S9 mix by plate incorporation method

| Various concentrations of Liv52 syrup | TA1538 NPD (5 µg/plate) | % inhibition | TA100 NPD (20 µg/plate) | % inhibition |
|--------------------------------------|--------------------------|--------------|--------------------------|--------------|
| Spontaneous revertants               | 14±2                     | -            | 138±12                   | -            |
| 10 µl/plate of Liv52 syrup           | 60±9±52                  | 14.3         | 491±43                   | 12.4         |
| 25 µl                                | 450±36                   | 35.4         | 426±32                   | 28.5         |
| 50 µl                                | 364±31                   | 48.1         | 375±29                   | 41.2         |
| 75 µl                                | 321±29                   | 54.4         | 352±26                   | 46.9         |
| 100 µl                               | 263±10                   | 63.1         | 292±21                   | 61.8         |
| 200 µl                               | 271±19                   | 61.9         | 290±20                   | 60.3         |
| 500 µl                               | 268±23                   | 62.4         | 294±23                   | 61.3         |

Results were expressed as the mean of 6 plates from two independent experiments with standard deviation. Viable cell count was approximately 1-2 X 10^9 cells/ml. (Correlation coefficient were > 0.5 and p value were < 0.005).
### Table 4: Antimutagenicity of Liv52 syrup against 2-AF 2-AN, CSC induced mutagenesis in TA1538 with S9 mix by plate incorporation method

| Various concentrations of Liv52 syrup | Histidine revertants/plate | 2-AF (5 µg/plate) | % inhibition | 2-AN (1 µg/plate) | % inhibition | CSC (100 µg/plate) | % inhibition |
|--------------------------------------|----------------------------|--------------------|--------------|--------------------|--------------|-------------------|--------------|
| Spontaneous revertants               |                           | 23±2              | -            | 25±2              | -            | 22±1             | -            |
| 10 µl/plate of Liv52 syrup           |                           | 612±51            | 51.1         | 636±43            | 51.6         | 206±17           | -            |
| 25 µl                                |                           | 311±23            | 76.4         | 321±26            | 72.8         | 127±9            | 42.9         |
| 50 µl                                |                           | 162±11            | 99.3         | 191±16            | 95.6         | 39±4             | 90.8         |
| 75 µl                                |                           | 22±2              | 100.2        | 28±2              | 99.5         | 24±3             | 98.9         |
| 100 µl                               |                           | 23±1              | 100          | 24±2              | 100.2        | 20±3             | 104          |
| Vitamin E (10 µl/plate)             |                           | 110±9             | 85.2         | 103±9             | 87.2         | 51±6             | 84.2         |
| BHA (100 µg/plate)                  |                           | 201±18            | 69.8         | 206±18            | 70.4         | 86±9             | 65.2         |

Results were expressed as the mean of 6 plates from two independent experiments with standard deviation. Viable cell count was approximately 1.2 X 10^6 cells/ml.

### Table 5: Antimutagenicity of Liv52 syrup against 2-AF, 2-AN, CSC, and B (a) P induced mutagenesis in TA100 with S9 mix by plate incorporation method

| Various concentrations of Liv52 syrup | Histidine revertants/plate | 2-AF (10 µg/plate) | % inhibition | 2-AN (1 µg/plate) | % inhibition | B (a) P 2.5 µg/plate | % inhibition | CSC (100 µg/plate) | % inhibition |
|--------------------------------------|----------------------------|--------------------|--------------|--------------------|--------------|----------------------|--------------|-------------------|--------------|
| Spontaneous revertants               |                           | 161±13             | -            | 159±14             | -            | 163±16              | 539±51       | 165±12           | -            |
| 10 µl/plate of Liv52 syrup           |                           | 525±13             | 803±72       | 362±29             | 68.5         | 327±36              | 432±31*      | 295±20           | 51.3         |
| 25 µl                                |                           | 299±36             | 62.1         | 209±22             | 92.2         | 24±18               | 86.4         | 226±29           | 72.2         |
| 50 µl                                |                           | 192±21             | 91.5         | 160±20             | 99.8         | 169±12              | 98.4         | 175±19           | 96.3         |
| 75 µl                                |                           | 170±13             | 97.5         | 150±11             | 100.2        | 161±14              | 100.5        | 160±23           | 101.9        |
| 100 µl                               |                           | 158±16             | 100.8        | 153±18             | 100.9        | 163±18              | 100          | 162±20           | 100.1        |
| Vitamin E (20 µl/plate)             |                           | 161±14             | 100          | 173±21             | 97.8         | 165±11              | 99.5         | 176±12           | 95.9         |
| BHA (100 µg/plate)                  |                           | 263±23             | 72           | 292±26             | 79.3         | 236±28              | 80±6         | 232±28           | 74.9         |

Results were expressed as the mean of 6 plates from two independent experiments with standard deviation. Viable cell count was approximately 1.2 X 10^6 cells/ml.

This ayurvedic product may have better antioxidant activity endowed with the ability to intercept the free radicals generated by cellular metabolism or exogenous sources, such as those resulting from the action of cyclophosphamide, thereby preventing their damage to lipids, amino acids, proteins, polyunsaturated fatty acid double bonds, and DNA bases [25]. The aqueous extracts of PM and BF can act directly on compounds that induce mutations in DNA, chemically or enzymatically inactivating them, may inhibit the metabolic activation of promutagenic agents, or may scavenge reactive molecules, as explained [23]. Thus, the considerable presence of antimutagen in Liv52 certainly contributed to their effective antimutagenic activity.

By this investigation, we clearly observed that the Liv52, which is routinely used in ayurvedic practice, have considerable antimutagenic activity, show no cytotoxic activity and may contribute to reducing the
chromosomal damage. Further, Liv52 having antimutagenic potential in it may play an important role in neutralization of various dietary mutagens and may act as a prophylactic agent against human ill-health attributable to mutation. Thus, the consumption of this ayurvedic product can bring added benefits and protection to individuals and also improving their quality of life and health. Further study in these aspects using some other in vitro and in vivo will test be warranted before finalizing the product as more successful.

CONCLUSION

Liv52 was found to be nonmutagenic in Salmonella assay, whereas manifested the antimutagenic potential both with and without metabolic activation. The enhanced antimutagenic activity of Liv52 on preincubation indicates that the antimutagenic factor(s) may be desmutagenic in nature. The exact mechanism by which Liv52 exerts antimutagenic potential is not known. The possibility of having diverse antimutagenic factors in Liv52 which act by different mechanisms are strongly indicated.

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