

INVESTIGATION OF THE ANTIMUTAGENIC ACTIVITY OF THREE *SALVIA* EXTRACTS

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## ABSTRACT

The human population is continuously exposed to a plethora of diverse chemicals with established mutagenic activity. Dietary interactions that decrease the mutagenic load appear to be one of the plausible approaches for preventing mutations. This study aimed to investigate the genoprotective efficacy of methanol extracts of *Salvia farinacea* Benth., *Salvia microphylla* Kunth and *Salvia splendens* Sellow ex J. A. Schult. 'Blue Ribbon' (Lamiaceae) against the methyl parathion-induced toxicity on bone marrow cells and spermatocytes of Swiss albino mice by chromosomal aberration assay and micronucleus test. Swiss albino mice pretreated with methanol extracts of all the three species of *Salvia* exhibited significant inhibition of chromosomal aberrations and micronuclei formation ( $p < 0.001$ ) induced by methyl parathion. The maximum inhibition of chromosomal aberrations in bone marrow cells and spermatocytes was observed with *S. microphylla* extract ( $2.33 \pm 0.19$  and  $3.33 \pm 0.19$ ) followed by *S. farinacea* ( $4.33 \pm 0.39$  and  $3.67 \pm 0.58$ ) and *S. splendens* 'Blue Ribbon' ( $3.67 \pm 0.19$  and  $4.33 \pm 0.39$ ) extracts. Similarly, the highest inhibition of micronuclei was observed in *S. microphylla* ( $0.27 \pm 0.09$ ) pretreated mice followed by *S. farinacea* ( $0.33 \pm 0.09$ ) and *S. splendens* 'Blue Ribbon' ( $0.37 \pm 0.07$ ) pretreated mice. The bioactive phytochemicals; rosmarinic acid, betulinic acid, myricetin, ascorbic acid and asiatic acid present in these *Salvias* might be the reason for the high genoprotective efficacy. The outcome of the present work ascertains the role of these *Salvia* species as potential nutraceuticals against the non-intentional exposure to methyl parathion and suggests a new avenue in the prophylaxis therapy.

**Keywords:** Antimutagenicity, Chromosomal aberration assay, Genotoxicity, Micronucleus test and *Salvia*.

## INTRODUCTION

Human population is exposed to a great deal of environmental harm that may affect the functioning of specific bio-molecules and thereby damage health at various levels. Some of the chemicals, e.g., pesticides, may be genotoxic to the sentinel species and to non-target species, causing deleterious effects in somatic or germ cells. DNA alterations are known to be indicators of early damage in the affected organisms. However, the adoption of preventive measures is probably more difficult regarding chemicals such as pesticides which are strongly related with a number of human activities such as agriculture, aquaculture, or several household tasks. These crucial but mutually opposing features of pesticides demand further investigations aimed at developing some mechanisms that can inhibit or at least minimize the genotoxic effects during their inevitable exposure.

Antimutagenesis is considered as one of the most feasible ways for inhibiting the negative effects of environmental genotoxicants. The genotoxic effects of toxicants can be minimized by modulation of the physiological detoxification. Many naturally occurring compounds with genoprotective activity are known to protect cellular components from genotoxic damage and prevent diseases<sup>1</sup>. Chemoprevention aimed at inhibiting or delaying the onset of mutagenesis is a rapidly growing area of cancer research. Recent studies have revealed the presence of natural bioactive materials in many different plant species worldwide and the antimutagenicity of these materials is currently under active investigation.

The Lamiaceae family is a large group of plant species that contain substantial amounts of phenolic compounds<sup>2</sup>, so it is considered to be a promising source of natural genoprotectants. The genus *Salvia* is the largest genera of Lamiaceae, with nearly 1,000 species<sup>3</sup>. Many species of *Salvia* possess antimutagenic activity<sup>4, 5, 6, 7</sup>. The genoprotective effects of *Salvia* species is because of the presence of phenolic acids and flavonoids. These phyto-compounds have been reported to exhibit a broad range of biological activities including antioxidant, anti-inflammatory, antimutagenic, and antitumour effects<sup>8, 9, 10, 11, 12, 13</sup>. Regarding phenolic acids, the majority of these compounds in *Salvia* species are unique to *Salvia*<sup>14</sup>.

In animals, including man, foreign chemicals are subject to a series of modifying enzymatic and non-enzymatic reactions aimed at detoxifying the chemical and altering it to water-soluble forms suitable for elimination from the body. These enzymatic reactions are also capable of activating certain chemicals to reactive molecules

that can interact with DNA to produce potentially harmful damage<sup>15</sup>. Hence, *in vivo* mammalian assay systems are preferred, because they facilitate the meaningful extrapolation of the data obtained, to human beings. Because of these reasons, the antimutagenic properties of the methanol extracts of *S. microphylla* Kunth, *S. farinacea* Benth. and *S. splendens* Sellow ex J. A. Schult. 'Blue Ribbon' against the genotoxic effects of methyl parathion was examined using the chromosomal aberration assay in bone marrow cells and spermatocytes and micronucleus test in bone marrow cells of Swiss albino mice.

## MATERIALS AND METHODS

## Assay animals

Eight to ten weeks old Swiss albino mice (*Mus musculus* L.) of either sex, weighing 20-25 g, were obtained from Small animal breeding station, College of Veterinary Sciences, Mannuthy, Thrissur. The animals were maintained under standard environmental conditions ( $25 \pm 2^\circ\text{C}$ , relative humidity  $45 \pm 10\%$ , light and dark cycle of 12 hours), fed with standard pellet diet and water *ad libitum*. The experiments were conducted in accordance with the ethical norms and guidelines put forth by the Ministry of Environment and Forest, Government of India (Reg. No: 426/01/C/CPCSEA) and the Institutional animal ethics committee, University of Calicut.

## Treatment duration

Chromosomal aberration analysis on mouse bone marrow cells and spermatocytes was conducted after 24 hours treatment. Analysis of the micronuclei was conducted in bone marrow cells after 30 hours treatment. For combination treatment with methyl parathion, the *Salvia* extracts were given 2 hours prior to methyl parathion treatment.

## Experimental design

Three mice per group were analyzed for each data point in the experiments, viz., treatment with  $\frac{1}{2}$  LD<sub>50</sub> doses of the methyl parathion, *Salvia* extracts (25, 50, 75 and 100 mg/kg, b.w.) and *Salvia* extracts (25, 50, 75 and 100 mg/kg, b.w.) followed by methyl parathion  $\frac{1}{2}$  LD<sub>50</sub> (4.65 mg/kg b. w.) dose. Negative control mice were administered 0.5 ml distilled water and positive control mice were given  $\frac{1}{2}$  LD<sub>50</sub> (235 mg/kg b. w.) dose of ethyl methanesulphonate for each of the three experiments. All the treatments were given intraperitoneally as a single dose.

## Chemicals

Methyl parathion (O, O-dimethyl O-4-nitro-phenyl-phosphorothioate) 50% emulsifiable concentrate, (CAS Registry No. 298-00-0) was used as the mutagen and ethyl methanesulphonate (CAS Registry No. 62-50-0) was used as the positive control in this study.

## Procurement and authentication of the plant material

*S. farinacea* was collected from Ambalavayal, Wayanadu District, Kerala, *S. microphylla* from Kunoor, Ooty District, Tamilnadu and *S. splendens* 'Blue Ribbon' from Anackampoil, Calicut District, Kerala, India. Botanical identity of these samples was confirmed and the voucher specimens (CALI 123703, CALI 123704 and CALI 123708) were deposited at the Herbarium (CALI) of Department of Botany, Calicut University.

## Preparation of methanol extract

Aerial parts of *S. farinacea*, *S. microphylla* and *S. splendens* 'Blue Ribbon' were cut into small pieces and shade dried. About 20 g of the dried, powdered sample was stirred overnight with 200 ml of 70% methanol using a magnetic stirrer. The suspension thus obtained was centrifuged at 15,000 rpm at 4°C for 15 min using REMI C-24 BL high speed refrigerated centrifuge. The supernatant was collected and the methanol and water were removed by keeping the supernatant at 40°C in a water bath. Dried extract was stored in a glass bottle with airtight lid and was kept under refrigeration. Double distilled water was used for re-dissolving the dried crude methanol extract during treatments.

## Chromosome aberration assay in bone marrow cells

The controls and treated animals were injected intraperitoneally with 4 mg/kg b.w. of colchicine, as a cytostatic chemical, 2 hours before the end of the exposure period. After 2 hours, the animals were killed by cervical dislocation. The femur bones were dissected out and the bone marrow was flushed out into 3 ml of pre-warmed 0.56% potassium chloride solution. The cell suspension was incubated at 37°C for 25 min and the cells were harvested by centrifuging at 500 rpm for 5 min. The cell button was suspended in two drops of potassium chloride solution and was fixed using freshly prepared chilled Carnoy's fluid (3 parts absolute alcohol : 1 part glacial acetic acid). Three repeated washings were made using Carnoy's fluid and after the third washing, the cells were suspended in few drops of fixative. Later the suspension was dropped onto clean pre-chilled slides. Air was blown over the slides and they were warmed for few seconds<sup>16</sup>. Later the slides were stained using 4% giemsa for 10 min followed by rinsing with double distilled water<sup>17</sup>. Hundred well-spread metaphase plates were examined for chromosomal aberrations from each animal of a treatment group and the values were expressed as mean  $\pm$  standard error. Analysis of slides was done under oil immersion using the image analyzer system attached to LEICA DM 500 research microscope.

## Chromosome aberration assay in spermatocytes (meiosis)

The procedure of Evans et al.<sup>18</sup> was used in the study of chromosome aberration assay in spermatocytes. The testes were dissected out from the mice and transferred to 1.2% trisodium citrate solutions. They were washed and transferred to 3 ml of fresh trisodium citrate solution. The tunica layer was removed and tubules of both testes were clubbed together. The tubules were then teased and the cell suspension obtained was incubated at 37°C for 25 min. The cells were collected by centrifuging the suspension at 500 rpm for 5 min. The cell button was suspended in two drops of trisodium citrate solution and was fixed using fresh chilled Carnoy's fluid. Three repeated washings were made using Carnoy's fluid and after the third washing the cells were suspended in few drops of fresh fixative. The slides were prepared by the same method of chromosome aberration assay in bone marrow cells. Hundred well spread diakinesis and metaphase plates were examined for chromosomal aberrations from each animal of a treatment group and the values were expressed as mean  $\pm$  standard error.

## Micronucleus test in bone marrow cells

The procedure described by Schmid<sup>19</sup> was adopted for carrying out micronucleus test. The bone marrow was flushed out from each femur into one drop of human AB serum. It was then mixed well and smeared on clean dry slides using haemocytometer cover slip. The slides were dried and were fixed in methyl alcohol for five min and were stained by giemsa - May Grunwald staining method<sup>20</sup>. Thousand polychromatic erythrocytes (PCE) and corresponding normochromatic erythrocytes (NCE) were scored for micronuclei. The percentage of micronucleated polychromatic erythrocytes (MNPCE) was expressed as mean  $\pm$  standard error of three animals.

## High performance liquid chromatography (HPLC) analysis of methanol extracts

The HPLC (Shimadzu) separation was performed on a Reverse Phase C-18 (phenomenex) column, with mobile phase acetonitrile:methanol (90:10) in an isocratic mode; flow rate: 1 ml/min; running time 25 min; detection wavelength: 254 nm; injection volume: 20  $\mu$ l. The identification of constituents was carried out by HPLC/MS analysis, by comparison and combination of their retention times, UV-vis and mass spectra of the peaks with those of authentic reference samples.

## Statistical analysis

The data was analyzed for mean values and standard error (mean  $\pm$  SE) for all groups. Statistical comparisons were made using Students *t*-test and  $P < 0.05$  was considered significant. The analyses were performed by using the statistical software SPSS 17.

## RESULTS AND DISCUSSION

Swiss albino mice treated with  $\frac{1}{2}$  LD<sub>50</sub> dose of methyl parathion resulted in statistically significant increase in the frequency of chromosomal aberrations in bone marrow cells (Fig. 1) and spermatocytes (Fig. 2) compared to the negative control group ( $p < 0.001$ ). The mutagenicity of methyl parathion was further confirmed from the results of micronucleus test (Fig. 3 & 4).

## Chromosome aberration assay in bone marrow cells and spermatocytes

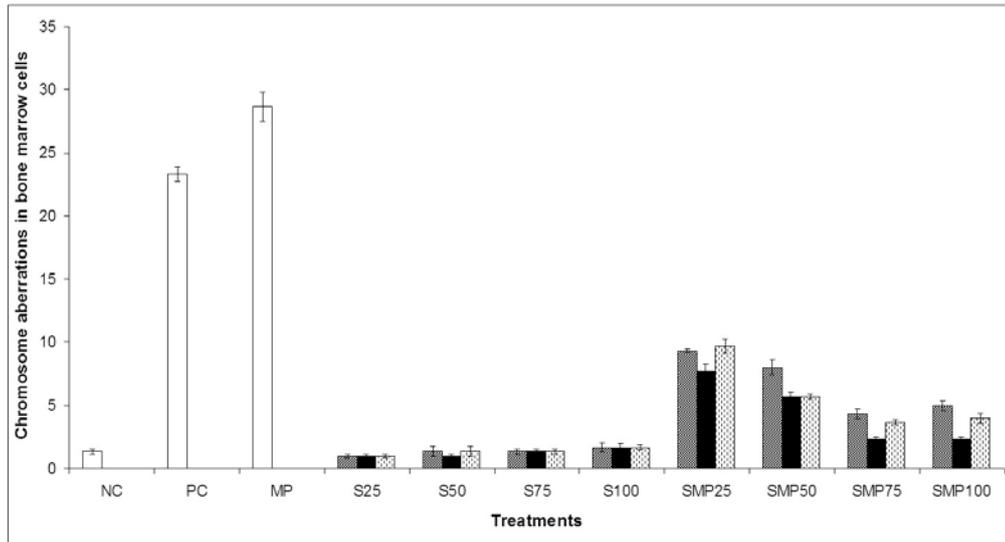
Mutagenic chemicals interact with DNA causing changes in its structure. This may result in the loss, addition, or replacement of bases, thus altering their sequence in the DNA and affecting the fidelity of the genetic message. *In vivo* tests that measure chromosomal aberrations in metaphase cells can detect a wide spectrum of changes in chromosomal integrity. Analysis of metaphase chromosomes in cells from the bone marrow of mammals is a well-established technique for studying chromosome damage *in vivo*. The mammalian bone marrow chromosomal aberration assay can detect clastogenic or aneugenic effects of a test agent. Chromosomal aberrations occur because of lesions in the DNA that lead to discontinuities in the DNA double helix. The primary lesions, which include single and double strand breaks, base damage, DNA-DNA and DNA-protein cross links, alkylations at base or phosphate groups, intercalations, thymine dimers, apurinic and apyrimidinic sites, are recognized by DNA-repair processes. Therefore, the lesions may be corrected or transformed, to reconstitute the original base sequence or produce chromosomal aberrations and gene mutations<sup>21</sup>.

The genotoxicity of four doses (25, 50, 75 and 100 mg/kg b.w.) of the methanol extracts of *S. farinacea*, *S. microphylla* and *S. splendens* 'Blue Ribbon' was analyzed and the results obtained were compared with that of solvent control group. All three extracts at the doses mentioned; had no significant effect on inducing chromosome aberrations in Swiss albino mice ( $P > 0.05$ ).

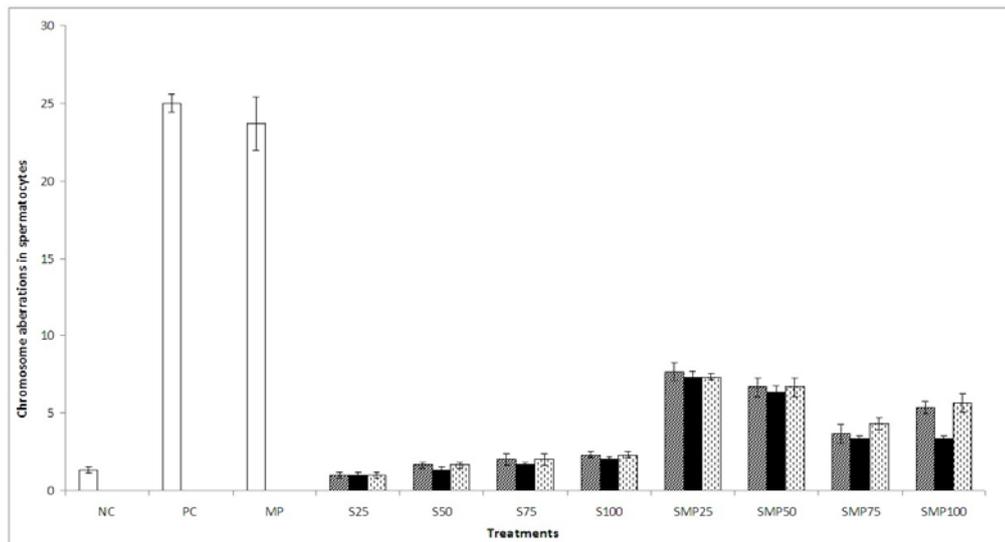
Pretreatment with *S. microphylla* and *S. splendens* 'Blue Ribbon' exhibited excellent inhibitory activity against the methyl parathion-induced chromosomal aberrations (Fig. 1). Results showed that antimutagenic potentiality of *S. farinacea* was lesser compared to other two species (Fig. 1). However its effect on the inhibition of chromosome aberrations was statistically significant when compared with the positive control and methyl parathion alone-

treated group ( $p < 0.001$ ). The maximum genoprotective effect was seen in *S. microphylla* pretreated group and the lowest frequency of chromosomal aberrations was observed with the highest concentrations 75 and 100 mg/kg b.w. *S. farinacea* and *S. splendens* 'Blue Ribbon' extracts at 75 mg/kg b.w. exhibited maximum inhibitory effect and the slight increase in the frequency of chromosomal aberrations at the highest dose 100 mg/kg b.w. may be due to the slight cytotoxic nature of the extracts at higher concentrations.

In spermatocytes of Swiss albino mice, reduction in the percentage of chromosomal aberrations to such a low level, which is close to that of the solvent control was shown by pretreatment with extracts of *S. microphylla* (Fig. 2). *S. splendens* 'Blue Ribbon' and *S. farinacea* also showed significant reduction in the percentage of chromosomal aberrations compared to that of the methyl parathion treated group ( $p < 0.001$ ) (Fig. 2). This assay results showed that antimutagenic potentiality of *S. splendens* 'Blue Ribbon' was lesser compared to the other two species (Fig. 2).



**Fig. 1:** Graph showing chromosome aberrations (excluding chromatid gap) in bone marrow cells of Swiss albino mice exposed to double distilled water (NC), EMS (PC), methyl parathion (MP) (4.65 mg/kg b.w.) and combination of *Salvia* extracts (S 25-S 100 mg/kg b.w.) and methyl parathion (4.65 mg/kg b.w.) *S. farinacea*, *S. microphylla* and *S. splendens* 'Blue Ribbon'



**Fig. 2:** Graph showing chromosome aberrations in spermatocytes of Swiss albino mice exposed to double distilled water (NC), EMS (PC), methyl parathion (MP) (4.65 mg/kg b.w.) and combination of *Salvia* extracts (S 25-S 100 mg/kg b.w.) and methyl parathion (4.65 mg/kg b.w.) *S. farinacea*, *S. microphylla* and *S. splendens* 'Blue Ribbon'

#### Micronucleus test in bone marrow cells

As micronuclei derive from chromosomal fragments and whole chromosomes lagging behind in anaphase and left outside the daughter nuclei in telophase<sup>22, 23</sup>, the micronucleus assay can be considered as a powerful tool to study both clastogenic and aneugenic effects of any toxicants.

Some micronuclei may be originated from fragments derived from broken anaphase bridges<sup>24, 25</sup> formed due to chromosome

rearrangements such as dicentric chromatids, intermingled ring chromosomes or union of sister chromatids. Micronuclei can be easily recognized in cells without the main nucleus, namely erythrocytes<sup>21</sup>. The PCE/NCE ratio was also calculated during the present investigation. The drop in PCE/NCE ratio highlights the retardation in the rate of cell division due to the cytotoxic nature of methyl parathion. According to Adler<sup>26</sup>, an increase in NCEs signals a cytotoxic effect and an increase in PCEs signals a stimulation of proliferative activity due to an early phase of cell depletion.

Results of micronucleus assay showed parallelism to that observed during chromosome aberration assay in bone marrow cells and spermatocytes. Treatment with four doses (25, 50, 75 and 100

mg/kg b.w.) of the methanol extracts of *S. farinacea*, *S. microphylla* and *S. splendens* 'Blue Ribbon' had no significant effect on inducing micronuclei in Swiss albino mice ( $P > 0.05$ ).

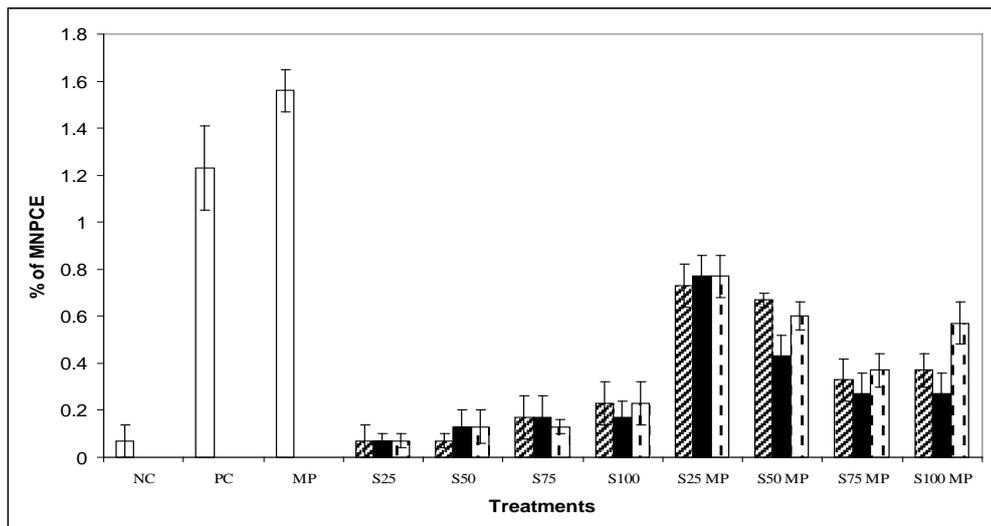


Fig. 3: Graph showing percentage of micro nucleated polychromatic erythrocytes (MNPCE) in bone marrow cells of Swiss albino mice exposed to double distilled water (NC), EMS (PC), methyl parathion (MP) (4.65 mg/kg b.w.) and combination of *Salvia* extracts (S 25-S 100 mg/kg b.w.) and methyl parathion (4.65 mg/kg b.w.) *S. farinacea*, *S. microphylla* and *S. splendens* 'Blue Ribbon'

Pretreatment of mice with methanol extracts of all the three species of *Salvia* showed statistically significant reduction in the frequency of micronuclei compared with that of methyl parathion alone-treated group ( $p < 0.001$ ). The lowest frequency of micronuclei formation was observed in the treatment with *S. microphylla* at 75 and 100 mg/kg b.w. (Fig. 3). *S. microphylla* was very potent even at the lowest dose. The PCE/NCE ratio was

brought back to the level of the solvent control group during the combination treatment of *S. farinacea* and *S. splendens* 'Blue Ribbon' (Fig. 3). The highest value for PCE/NCE ratio was showed by *S. microphylla*. The slight increase in the frequency of micronuclei at the highest dose 100 mg/kg b.w. of *S. farinacea* and *S. splendens* 'Blue Ribbon' extracts may be due to the slight cytotoxic nature of the extracts at higher concentrations.

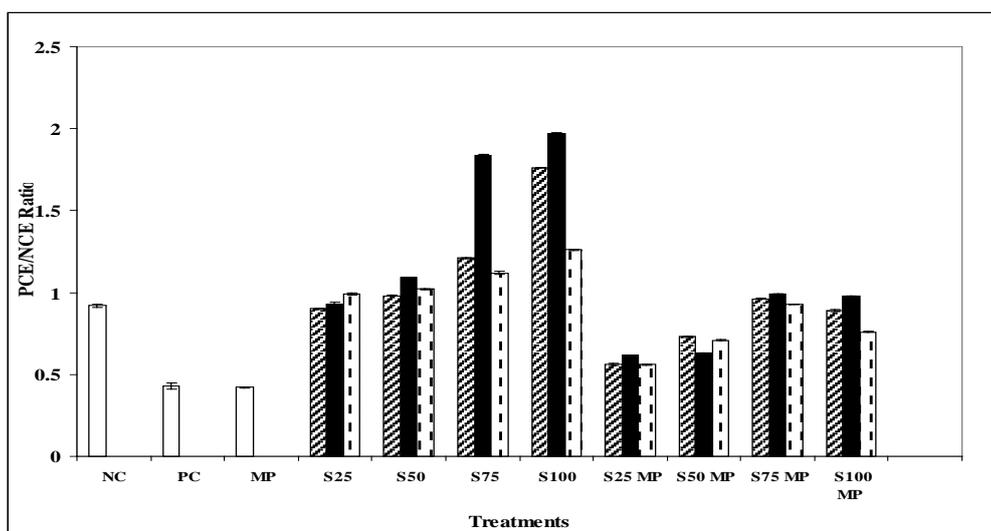


Fig. 4 Graph showing PCE/NCE ratio in bone marrow cells of Swiss albino mice exposed to double distilled water (NC), EMS (PC), methyl parathion (MP) (4.65 mg/kg b.w.) and combination of *Salvia* extracts (S 25 - S 100 mg/kg b.w.) and methyl parathion (4.65 mg/kg b.w.) *S. farinacea*, *S. microphylla* and *S. splendens* 'Blue Ribbon'

Analysis of methanol extracts of *Salvia* spp. by high performance liquid chromatography (HPLC).

HPLC analysis of *S. farinacea* extract showed the presence of several compounds out of which phenols were detected as the most

dominant group followed by fatty acids and terpenes. HPLC analysis enabled to quantify these phytochemicals. Major phytochemical components of the methanol extract were found to be ascorbic acid (phenol) followed by linolenic acid (fatty acid), asiatic acid (triterpene), betulinic acid (triterpene) and carnosol (diterpene) (Table 4).

**Table 4: Chemical components identified by HPLC analysis in the methanol extract of *S. farinacea*, *S. microphylla* and *S. splendens* 'Blue Ribbon'**

S. No.	Compounds	RT	<i>S. farinacea</i>	<i>S. microphylla</i>	<i>S. splendens</i> 'Blue Ribbon'
			Area%	Area%	Area%
1	Betulinic acid	2.76	2.65	28.69	3.72
3	Carnosol	4.60	2.17	1.35	
4	Valerinic acid	5.01			6.06
6	Cinnamic acid	9.30		2.95	11.86
7	Myricetin	11.70	1.6	29.25	
8	Ascorbic acid	12.40	27.23		
9	Vernolic acid	16.10			13.83
10	Rosmarinic acid	16.30		26.54	
11	Linolenic acid	16.31	25.1		
12	Asiatic acid	20.60	21.7		38.18

Phytochemical assessment of the methanol extract of *S. microphylla* showed that it contains several compounds, out of which phenols governed the major portion of the extract, followed by terpenes. Myricetin and rosmarinic acid form major components among phenols and betulinic acid seem to be the major triterpene (Table 4).

Methanol extract of *S. splendens* 'Blue Ribbon' after chemical analysis through HPLC discloses the presence of several terpenes and phenols. Among the phenols, asiatic acid was the major component. Similarly presence of vernolic acid (fatty acid), cinnamic acid (phenol) and valerinic acid (sesquiterpene) also revealed through HPLC analysis (Table 4).

#### Antimutagenicity of *Salvia* extracts

*Salvia* extracts eliciting genoprotective efficacy contain a plethora of compounds including polyphenols and terpenoids. These phytochemicals may act as antioxidants, immune-stimulants and cell proliferation stimulators. Some of the phytochemicals may act in isolation as well as in combination with other constituents. Synergistic effects may be present, and some of the toxic effects generated by active constituents may be countered by other constituents present.

The reputed antimutagenic potentiality of *Salvia* extracts is due to the bioactive components present in it. Vujosevic and Blagojevic and Knezevic-Vukcevic et al.<sup>5, 27</sup> reported the antimutagenic properties of terpenoids of *Salvia*. According to them the protective effect of sage monoterpenoids was through enhanced recombination repair and excision repair. Terpenoid rich essential oils of *Salvia* spp. also modulates mutagenesis by enhanced recombination and inhibition of SOS induction, which is probably caused by inhibition of protein synthesis. The diterpene carnosol acid with ortho-dihydroxyl groups on aromatic ring C inhibited the oxidation through donating H- atoms to scavenge free radicals<sup>8</sup>. Similarly another major diterpene carnosol exhibited potent antioxidative and free radicals scavenging activity<sup>28</sup>. The high antioxidative, anticarcinogenic and antitumour potentialities of the triterpenes betulinic acid<sup>29, 30</sup> and asiatic acid<sup>31, 32</sup> contribute to the genoprotective efficacy of the *Salvia* extracts.

The next category of *Salvia* compounds adding to its genoprotective nature includes phenolics and flavanoids. Studies of Ismail et al.<sup>33</sup> and Maryam et al.<sup>34</sup> stated the direct correlation between total antioxidative activity and flavanoid and phenolic contents. Phapale and Thakur<sup>35</sup> observed fine correlation between total phenolic content and antimutagenic effect. Flavanoids generally have more hydroxyl groups. Besides, ortho-substitution with electron donating alkyl or methoxy group of flavanoids and phenolics increases the stability of the free radical and hence the antioxidant potential. Similar antioxidant activity has been reported for polyphenolics from various sources<sup>36, 37</sup>. Ruch et al.<sup>38</sup> proved that phenolic compounds are very good electron donors, which may accelerate the conversion of hydrogen peroxide to water. Lima et al.<sup>39</sup> explained that phenolic compounds have direct effects on genotoxicants and which would include the antiradical scavenging activity, hydrogen-donating activity and the ability to chelate metal ions. Rice-Evans et al.<sup>40</sup> also proved the ability of phenolic compounds to chelate metal ions.

In the present investigation, rosmarinic acid was found to be one of the major phenolic compounds present in the methanol extract of *S. microphylla*. Furtado et al.<sup>41</sup> stated that the mechanisms underlying the protective effect of rosmarinic acid might be its putative antioxidant activity. Iuvone et al.<sup>42</sup> stated that rosmarinic acid inhibits reactive oxygen species formation, lipid peroxidation, DNA fragmentation, caspase-3 activation and tau protein hyperphosphorylation. Similarly, ascorbic acid, another major phenol has potent antioxidative and free radicals scavenging activity<sup>43</sup>. Aherne and O'Brien<sup>44</sup> reported that myricetin, one of the major flavanoid compounds helps to reduce DNA damage through its direct antioxidant activity and by enhancing DNA repair. The phenolic compound cinnamic acid present in *S. splendens* 'Blue Ribbon' was also reported to have high antioxidant and radical scavenging activity<sup>45</sup>.

The analysis of phytochemical constitution of the extracts revealed that the methanol extracts of the three *Salvia* spp. used in the present investigation contain the aforesaid compounds, which may exert the multifaceted action on the free radicals generated by methyl parathion. In *S. microphylla* the presence of rosmarinic acid, betulinic acid, myricetin and carnosol may cause high genoprotective activity. Asiatic acid, carnosol, betulinic acid, ascorbic acid, linolenic acid and diferulic acid were the major components of *S. farinacea* extract. Some of the components such as rosmarinic acid, myricetin and carnosol are absent in *S. splendens* 'Blue Ribbon' extract but effective components like asiatic acid, cinnamic acid and betulinic acid are present. This may be the reason for the significant antimutagenic potency of the species comparable to *S. farinacea* and *S. microphylla*.

Thus the outcome of the present work ascertains the role of *S. microphylla*, *S. farinacea* and *S. splendens* 'Blue Ribbon' as potential genoprotectants against the non-intentional exposure to methyl parathion.

#### CONCLUSION

Methanol extracts of *Salvia farinacea*, *Salvia microphylla* and *Salvia splendens* 'Blue Ribbon' are strong inhibitors of methyl parathion-induced genotoxicity in Swiss albino mice. HPLC analysis revealed that genoprotective potential of these *Salvia* spp. may be due to the abundance of compounds like rosmarinic acid, carnosol, asiatic acid, myricetin, cinnamic acid, betulinic acid and ascorbic acid.

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