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Research Article

GENOTOXICITY OF METHYL PARATHION AND ANTIMUTAGENIC ACTIVITY OF *SALVIA OFFICINALIS* L. (SAGE) EXTRACTS IN SWISS ALBINO MICE

JIJA MATHEW* AND JOHN E. THOPPIL

Cell and Molecular Biology Division, Department of Botany, University of Calicut, Pin- 673635,Kerala,India.Email:jijachintu@gmail.com.

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ABSTRACT

Prevention is a more effective strategy than treatment of chronic diseases. Incorporation of phytochemicals with bioactive properties into everyday diet may help to protect cellular systems from genotoxic damage. The present study aimed to evaluate the genotoxicity of the organophosphorus insecticide methyl parathion (MP) and the genoprotective effects of *Salvia officinalis* L. (Sage) in Swiss albino mice using micronucleus and comet assay. Single intraperitoneal doses of MP at concentrations ¼ LD_{50} and ½ LD_{50} elicited statistically significant increase ($P<0.001$) in frequency of micronucleated erythrocytes and DNA damage. MP was proved to be more mutagenic and cytotoxic than the positive control ethyl methanesulphonate. Pretreatment with Sage at concentrations 50, 75 and 100 mg/kg b. w. produced significant decrease in the frequency of MP induced micronuclei ($P<0.001$) and DNA damage ($P<0.001$). This study, therefore, confirmed the mutagenicity of MP and nutraceutical value of Sage to prevent the onset of chronic diseases.

Keywords: Antimutagenicity; genotoxicity; nutraceutical; methyl parathion; *Salvia officinalis*

INTRODUCTION

The advent of the industrial revolution has seen a significant increase in the number of new chemical entities released in the environment [1]. These new chemical entities entering the ecosystem may linger in the environment for countless years and may pose serious threat to the ecosystem and public health. Among these xenobiotics, agrochemicals form a considerable share and among agrochemicals, pesticides constitute the major fraction. Pesticides act selectively against certain organisms without adversely affecting others. Absolute selectivity, however, is difficult to achieve and most pesticides are toxic also to humans. Toxicity, in particular genotoxicity, of pesticides on non-target organisms and their influences on ecosystems are of worldwide concern [2].

Organophosphates are a group of compounds that have historically been used as pesticides. They act by inhibiting acetylcholinesterase hydrolysis of acetylcholine, resulting in acetylcholine accumulation in neuromuscular synapses [3]. More recently, it has been reported that organophosphates produce oxidative stress in different tissues, such as liver, blood and brain [4, 5, 6] through the formation of reactive oxygen species [7]. Methyl parathion (O, O–dimethyl O-4-nitrophenyl phosphorothioate) is an organophosphorous insecticide and classified as extremely toxic by WHO.

According to Vidyasagar et al. [8], methyl parathion has enhanced capacity for alkylation of informational macromolecules due to its extra methyl group. Since DNA is the carrier of inherited information, any change in its structure may potentiate serious biological changes. Although there are a multitude of instances where methyl parathion poses adverse impact on human health, its role in agriculture is undeniable. These crucial but mutually opposing features of methyl parathion demand further investigations aimed at developing some mechanisms that can inhibit or at least minimize the genotoxic effects of methyl parathion during their inevitable exposure.

Dietary interactions that decrease the mutagenic load and abnormal biological responses appear to be one of the plausible approaches for prevention of the genotoxic effects of environmental mutagens. Thus, manipulation of the diet may be a non-invasive approach to minimize the effects of genotoxicants [9]. The refinement of this idea led to the development of the concept of functional food or nutraceuticals. They are not just drugs, which have no nutrient value, but are food supplements which have both nutritional as well as therapeutical value [10].

Most of the members of the Lamiaceae family possess a wide range of biological and pharmacological activities that may protect tissues against genotoxic effects of environmental toxicants and therefore lower the risk of human chronic diseases [11]. *S. officinalis* (Sage), belong to the family Lamiaceae, has been reported to have genoprotective effects [12, 13, 14].

A great variety of tests and test systems based on microbes, plants and animals have been developed in order to assess the genotoxic effects of xenobiotic agents, including pesticides. Arguably, the most reliable genotoxicity evaluation for human health risk is conducted with mammals, whose enzyme systems and more specifically their monooxygenase enzyme complex, are responsible for the biotransformation of xenobiotic chemicals [15]. In the present investigation, we have evaluated the antimutagenic effect of *Salvia officinalis* (Sage) against methyl parathion induced genotoxicity in mammalian test system (Swiss albino mice) through micronucleus assay and comet assay.

MATERIALS AND METHODS

Assay animals

Eight to ten weeks old Swiss albino mice of either sex, weighing 20 - 25 g obtained from Small animal breeding station, College of Veterinary Sciences, Mannuthy, Thrissur were used as the assay animals. The animals were maintained under standard environmental conditions (25 ± 2°C, relative humidity 45 ± 10%, light and dark cycle of 12 h), fed with standard pellet diet and water *ad libitum*, and 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.

The animals were divided into thirteen groups consisting of three animals each for micronucleus assay. Group one served as negative control (0.5ml distilled water), group two as positive control, treated with ½ LD_{50} (235 mg/kg b. w.) dose of ethyl methanesulphonate and group three, four and five were treated with methyl parathion $^1/_8$ LD_{50}, ¼ LD_{50} & ½ LD_{50} (1.16, 2.32 & 4.64 mg/kg b. w.) dose [16]. Groups six, seven, eight and nine were treated with *S. officinalis* extracts (25, 50. 75 and 100 mg/kg, b.w.) and groups ten, eleven, twelve and thirteen were treated with *S. officinalis* extracts (25, 50. 75 and 100 mg/kg, b.w.) followed by methyl parathion ½ LD_{50} (4.65 mg/kg b. w.) dose. In comet assay the assay animals were divided into nine groups consisting of three animals each. The experimental design was same as the micronuclei assay except the treatment with *S. officinalis* extracts alone; which was not incorporated in comet assay. All the treatments were given intraperitoneally as a single dose.

Insecticide tested

Methyl parathion 50% (O, O-Dimethyl O-4-nitro-phenyl-phosphorothioate – Bayer, Germany) emulsifiable concentrate, (CAS Registry No. 298-00-0) was used as the mutagen in this study.

Procurement and authentication of the plant material

S. officinalis plants were collected from Medicinal and Aromatic plant garden, Ooty and taxonomic authentication was done at Calicut University Herbarium (CALI), Department of Botany, University of Calicut, Kerala, India.

Preparation of methanol extract

Leaves and flowers of *S. officinalis* were cut into small pieces and were shade dried. About 10g of the dried, powdered sample was stirred overnight with 100ml of 70% methanol using a magnetic stirrer. The suspension thus obtained was centrifuged at 4472 g at 4^0C for 15 minutes 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 40^0C in a hot air oven. 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 methanolic extract during treatments. For the HPLC analysis the crude extract was dissolved in 10 ml methanol (HPLC Grade, Merck) and filtered through 0.20 mm membrane filter.

Treatment duration

The treatment durations for various assays were selected based on the recommendations of WHO [16]. In the case of micronucleus test, the sampling interval after dosing was 30 hours and for comet assay, it was 24 hours. For combination treatment with methyl parathion, the *S. officinalis* extract was given 2 hours prior to methyl parathion treatment.

Micronucleus test in bone marrow cells

The micronucleus test was conducted according to the procedure of Schmid [17]. The controls and treated animals were killed at the end of the sampling duration by cervical dislocation. The femur bones were dissected out and 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, fixed in methyl alcohol for five minutes and stained by giemsa – May Grunwald staining method [18]. 1000 polychromatic erythrocytes (PCE) and corresponding normochromatic erythrocytes (NCE) were scored for micronuclei. Analysis of slides was done under oil immersion using the image analyzer system attached to LEICA DM 500 research microscope. The criteria used for the identification of micronuclei were their size smaller than one-third of the main nucleus, no attachment with the main nucleus, and same color and intensity as the main nucleus [19]. The percentage of micronucleated polychromatic erythrocytes (MNPCE) was expressed as mean ± standard error of three animals.

Comet assay

The comet assay was performed according to the method of Singh et al. [20] with slight modifications. At the end of the 24 hour treatment period the mice femurs were dissected out and bone marrow was flushed out from each femur into 3 ml of phosphate buffered saline. 50 µl of bone marrow cell suspension was mixed with 200µl of 1% molten low melting point agarose, at 37^0C and 50 µl of the mixture was rapidly spread on frosted slides pre-coated with 1% regular melting agarose and immediately covered with a long cover slip. The slides were placed on a tray and kept for 10 min on a cooling plate to solidify. After solidification the cover slip was gently removed and the slides were kept in lysis buffer (2.5M NaCl, 0.1M EDTA, 0.01M Tris HCl, 1% sodium lauryl sarcosinate and 1% Triton X-100) at 4^0C for 1 hour. Slides were then incubated for 10 minutes in electrophoresis buffer (0.3M NaOH, 1mM EDTA and 0.2% DMSO) for equilibration and electrophoresis was carried at 25V, 300mA for 30 minutes. After electrophoresis, slides were neutralized by keeping in neutralization buffer (0.4 M Tris HCl, pH 7.5) for 10 minutes.

Silver staining

The slides were fixed for 10 minutes in fix solution (15% TCA, 5% ZnSO4 7H2O and 5% Glycerol) and washed twice with distilled water. After drying at room temperature, slides were stained with freshly prepared stain solution [66 ml of solution A (5% Sodium Carbonate) and 34 ml of solution B (0.1% Ammonium Nitrate, 0.1% Silver Nitrate, 0.25% Tungstosilicic acid and 0.15% Formaldehyde)] for 35 minutes in dark. The stained slides were washed twice with distilled water and immersed in stop solution (1% Acetic Acid) for 5 minutes. After drying at room temperature, slides were analyzed and images of comets were taken with LEICA ICC 50 integrated camera attached to LEICA DM 500 research microscope. The captured images of 100 comets from each slide were analyzed by comet analysis software (CASP).

The comets were classified into five different categories based on the method of Garcia et al. [21]. The comets categorized according to the range of tail DNA% is as follows: category 0 (no damage) – <1%; category 1 (low damage) – >1–25%; category 2 (medium damage) – >25–45%; category 3 (high damage) – >45–70% and category 4 (very high damage) – >70%.

Statistical analysis

The data was analyzed for mean values and standard error (mean ± 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

Micronucleus test in bone marrow cells

The results of micronucleus assays on the clastogenic and aneugenic potential of methyl parathion and inhibitory effect of *S. officinalis* extract against the genotoxicity of methyl parathion in bone marrow cells of Swiss albino mice are shown in Tables 1 and 2. Exposure of mice with methyl parathion showed a significant increase in the frequency of micronuclei ($P<0.001$) compared to the negative control group. All test concentrations ($^1/_8$ $LD_{50,}$ ¼ LD_{50} & ½ LD_{50}) of methyl parathion increased the frequencies of micronuclei in mouse bone marrow cells, with the increases most pronounced at the highest concentration (½ LD_{50}) ($P<0.001$; Table 1). The mean value of the PCE/NCE ratio in methyl parathion treated mice was also decreased in all the test concentrations in a dose dependent manner; ascertain the genotoxicity of the pesticide. The percentage of MNPCE induced by methyl parathion ½ LD_{50} dose was not significantly different from that caused by ½ LD_{50} (235 mg/kg b. w.) dose of ethyl methanesulphonate (positive control) ($P>0.05$).

Table: 1. Incidence of micronuclei in bone marrow cells of Swiss albino mice treated with distilled water, EMS and methyl parathion (Values indicate mean ± S. E. of three animals).

Treatments	Dose (mg/kg) b.w.	Duration (hrs)	MNPCE/ 1000 PCE	% of MNPCE	No. of NCE/ 1000 PCE	PCE/NCE ratio
-ve control (Distilled water)	0.5 ml / animal	30	0.67 ± 0. 67	0.07 ± 0.07	1090 ± 12.14	0.92 ± 0.01
+ve control (EMS)	235 mg/kg b.w.	30	12.33 ± 1.77	1.23 ± 0.18	2316 ± 14.45	0.43 ± 0.02
Methyl Parathion	1.16 mg/kg b.w.	30	9.67 ± 0.33	0.97 ± 0.03	1509 ± 14.45	0.66 ± 0.02
Methyl Parathion	2.32 mg/kg b.w.	30	10.33 ± 0.88	1.03 ± 0.09	1701 ± 11.56	0.59 ± 0.006
Methyl Parathion	4.65 mg/kg b.w.	30	15.67 ± 0.88	1.57 ± 0.09	2403 ± 19.08	0.42 ± 0.003

The toxicity of four doses (25, 50, 75 and 100 mg/kg b. w.) of the methanolic extracts of *S. officinalis* was analyzed and the results obtained were compared with that of solvent control group. The *S. officinalis* extract at the doses mentioned; had no significant effect on inducing micronuclei in bone marrow cells of Swiss albino mice (P>0.02). The PCE/NCE ratio was also increased with increase in concentrations of the extract (Table 2).

Screening of the antigenotoxic effect of the *S. officinalis* was done by pretreatment with four doses (25, 50, 75 and 100 mg/kg b. w.) of the extract followed by methyl parathion (½ LD_{50}) treatment. All the four doses of the *S. officinalis* extract proved effective in reducing the mutagenic effect of methyl parathion to a statistically significant degree with the antimutagenic effect most obvious at the highest concentrations 50, 75 and 100 mg/kg b. w. (P<0.001; Table 2). The lowest concentration 25 mg/kg b. w. also caused significant reduction in the frequency of micronuclei (P<0.003).

Table: 2. Incidence of micronuclei in bone marrow cells of Swiss albino mice treated with methanolic extracts of *S. officinalis* and pretreatment with *S. officinalis* extracts followed by methyl parathion (Values indicate mean ± S. E. of three animals).

Treatments	Dose (mg/kg) b.w.	Duration (hrs)	MNPCE/ 1000 PCE	% of MNPCE	No. of NCE/ 1000 PCE	PCE/NCE ratio
S. officinalis	25	30	0.67 ± 0. 67	0.07 ± 0.07	1019 ± 17.34	0.98 ± 0.04
	50	30	1 ± 0.58	0.10 ± 0.06	821 ± 14.45	1.22 ± 0.02
	75	30	1.33 ± 0.33	0.13 ± 0.03	629 ± 17.34	1.59 ± 0.04
	100	30	1.67 ± 0. 67	0.17 ± 0.07	466 ± 2.31	2.15 ± 0.001
S. officinalis + MP	25 + 4.65	30	7.33 ± 0.88	0.73 ± 0.09	1574 ± 1.16	0.64 ± 0.001
	50 + 4.65	30	2.33 ± 0.88	0.23 ± 0.09	760 ± 17.34	1.32 ± 0.04
	75 + 4.65	30	2.33 ± 0.88	0.23 ± 0.09	758 ± 5.20	1.32 ± 0.009
	100 + 4.65	30	2.67 ± 0.88	0.27 ± 0.09	860 ± 17.34	1.16 ± 0.04

Comet assay in bone marrow cells

The comet assay is a rapid, simple and sensitive technique for measuring DNA strand breaks in individual cells. Tail DNA %, tail length, tail moment and olive tail moment are the popular parameters in the comet assay. In the present study comet assay was used to detect the genotoxicity of methyl parathion and the efficacy of *S. officinalis* extracts in reducing the methyl parathion induced DNA damage.

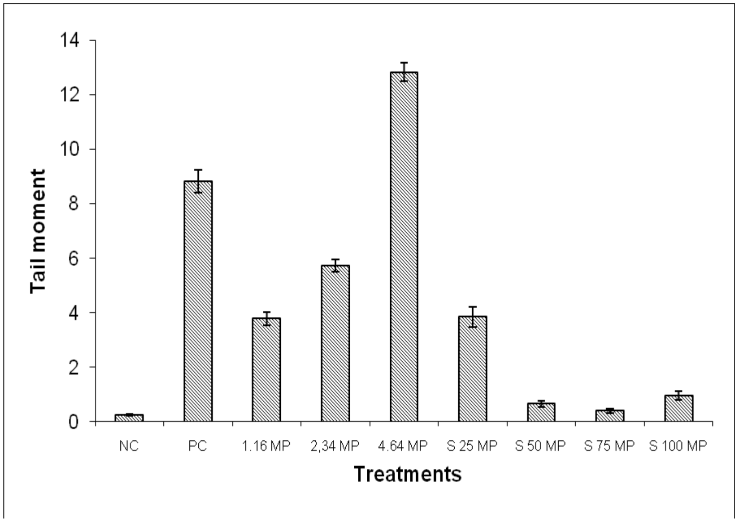

Fig. 1: Graph showing the tail moment in bone marrow cells of mice exposed to double distilled water (NC), EMS (PC), methyl parathion (MP) (1.16 – 4.64 mg/kg b.w.) and combination of *S. officinalis* extracts (S 25 – S 100 mg/kg b.w.) and methyl parathion (4.64 mg/kg b.w.)

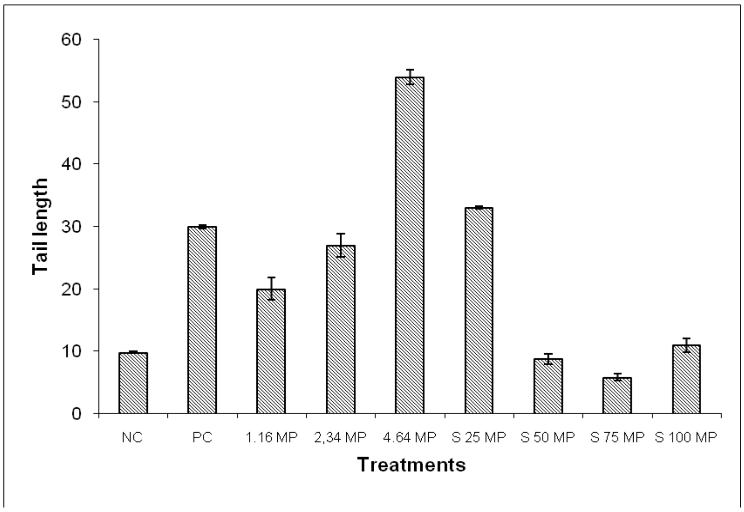

Fig. 2 :Graph showing the tail length in bone marrow cells of mice exposed to double distilled water (NC), EMS (PC), methyl parathion (MP) (1.16 – 4.64 mg/kg b.w.) and combination of *S. officinalis* extracts (S 25 – S 100 mg/kg b.w.) and methyl parathion (4.64 mg/kg b.w.)

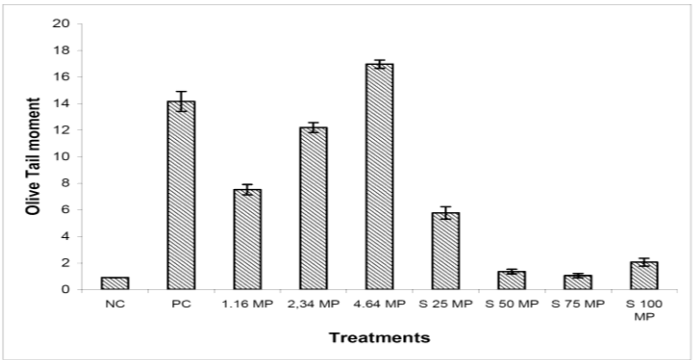

Fig. 3 Graph showing the olive tail moment in bone marrow cells of mice exposed to double distilled water (NC), EMS (PC), methyl parathion (MP) (1.16 – 4.64 mg/kg b.w.) and combination of *S. officinalis* extracts (S 25 – S 100 mg/kg b.w.) and methyl parathion (4.64 mg/kg b.w.)

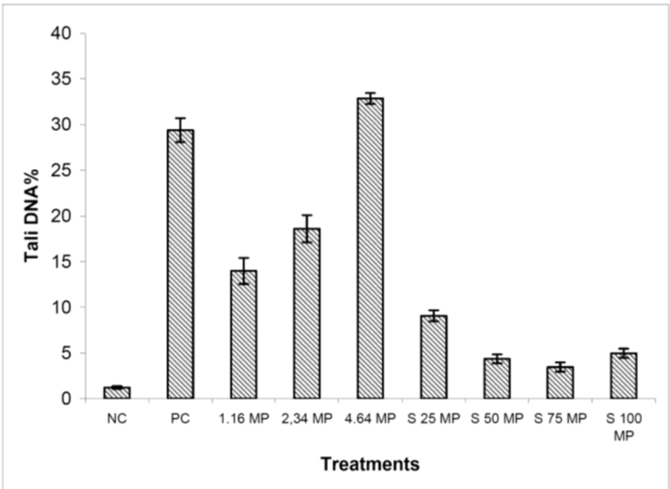

Fig. 4 Graph showing the tail DNA% in bone marrow cells of mice exposed to double distilled water (NC), EMS (PC), methyl parathion (MP) (1.16 – 4.64 mg/kg b.w.) and combination of *S. officinalis* extracts (S 25 – S 100 mg/kg b.w.) and methyl parathion (4.64 mg/kg b.w.)

The DNA damage measured as Tail DNA %, tail length, tail moment and olive tail moment in the bone marrow cells of the methyl parathion treatment groups (Figs 1-4) indicated that the Swiss albino mice exposed to different concentrations of methyl parathion exhibited significantly higher DNA damage ($p < 0.001$) than the negative control group with the damage most prominent at the highest concentration (½ LD_{50}) (Figs 1-4). The bone marrow cells treated with methyl parathion exhibited more DNA damage than that caused by ½ LD_{50} (235 mg/kg b. w.) dose of ethyl methanesulphonate (positive control) (Figs 1-4). The comets of the methyl parathion (½ LD_{50}) treated group belong to the category 3 with high damage where as the ethyl methanesulphonate treated group belong to the category 2 with medium damage.

The efficacy of *S. officinalis* extracts in reducing the methyl parathion induced DNA damage was assessed by pretreatment with four doses (25, 50, 75 and 100 mg/kg b. w.) of the extract followed by treatment with ½ LD_{50} doses of methyl parathion. *S. officinalis* extract at 50, 75 and 100 mg/kg b. w. doses provided bone marrow cells with high protection against methyl parathion induced DNA damage ($P<0.001$; Figs 1-4).

DISCUSSION

Micronucleus test in bone marrow cells

The micronucleus test can detect mutagenic substances in mammals by revealing compounds that cause chromosome breaks or interfere with the mitotic spindle. In the micronucleus test, clastogenic / spindle poison effects can be measured indirectly by counting small nuclei in interphase cells formed from acentric chromosome fragments or whole chromosomes. Micronuclei originate from chromosomal material that has lagged in anaphase. In the course of mitosis, this material is distributed to only one of the daughter cells. It may be included in the main nucleus or form one or more separate small nuclei, *i.e.*, micronuclei[16]. Chromosome breakage and the dysfunction of the mitotic apparatus are two basic phenomena leading to the development of micronuclei in mitotic cells [22]. The micronuclei mainly consist of acentric fragments as demonstrated by DNA content measurements [23]. They may also consist of entire chromosomes and may result from non-disjunction due to malfunction of the spindle apparatus. These larger micronuclei are formed by spindle poisons [24]. Besides these fundamental mechanisms, some micronuclei may have their origin in fragments derived from broken anaphase bridges [25, 26] 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 [16]. The frequency of micronuclei can be evaluated most readily in young erythrocytes, shortly after the main nucleus is expelled.

Upon administration of methyl parathion there was significant rise in % MNPCE, and it was dose dependent, indicating methyl parathion induced chromosomal damage in mouse bone marrow cells. Administration of *S. officinalis* extract alone does not produce any significant variation in % MNPCE indicating that, it is devoid of any genotoxicity. Pretreatment with *S. officinalis* decreased the methyl parathion induced formation of micronuclei in PCE due to the inhibition of methyl parathion induced chromosomal damage. 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 [27], an increase in NCEs signals a cytotoxic effect. *S. officinalis* significantly inhibited the same, by decreasing the formation of NCE. The extract at 50, 75 and 100 mg/kg b. w. could bring PCE/NCE ratio above the normal level indicating the high stimulatory effect of the extract.

The results of the present study revealed that methyl parathion is an effective inducer of micronuclei which in turn indicates its high degree of clastogenic / spindle poison nature. Moreover, the cytotoxic nature of methyl parathion is also proved by its inhibitory action on cell division. The high frequency of micronuclei observed by the methyl parathion treatments may be due to high production of reactive oxygen species (ROS) by the pesticide resulting to cell apoptosis. ROS and oxidative stress have been demonstrated to be triggers of apoptosis[28]. Oxidative stress is caused by an imbalance between the production of ROS and an organism's ability to detoxify them or repair the resulting damage. The free radicals formed in this process can damage macromolecule like DNA. Oxidative stress may also be due to the depletion of cellular glutathione (GSH) content below the critical level which prevents the conjugation of xenobiotics like methyl parathion to GSH and thus enables them to freely combine covalently with cell proteins [29].

S. officinalis extracts even at the lowest concentrations used in the current study were potent enough to inhibit the induction of micronuclei caused by methyl parathion. Because of the high antioxidant activity, the *S. officinalis* extract or some of its components act as desmutagen (factors which inactivate mutagens or prevent their interaction with DNA) and suppress the metabolic activation of the methyl parathion.

Comet assay

The Comet assay has been widely accepted as a simple and sensitive tool for assessing DNA damage and repair in individual cells. Its ability to evaluate DNA damage in non-proliferating cells makes it a useful tool to work on any eukaryotic cell [1]. When performed under alkaline conditions, comet assay detects double-stranded breaks, single-stranded breaks, alkali-labile sites, incomplete excision repair (single-stranded breaks), DNA-DNA interactions, and DNA-protein interactions [30, 31]. It has also been used to study oxidative damage [32]. Discrimination of necrosis and apoptosis has also been carried out with the help of this assay [33].

Silver staining

The silver staining method for comet assay is inexpensive and allows preservation of the comet slides for long periods and can be analyzed with a conventional light microscope. The silver staining process is also very useful to keep agarose gels for archival preservation of the samples. Hence the present study follows the silver staining method.

Measurements of DNA damage using comet assay

The first phase of the assay in the present investigation was meant to authenticate the genotoxic potentiality of methyl parathion. Swiss albino mice treated with ½ LD_{50} dose of methyl parathion showed significant increase in tail DNA %, tail length, tail moment and olive tail moment compared to the negative control group.

During the second phase of this assay, antimutagenic potentialities of methanolic extracts of *S. officinalis* were assessed. Pretreatment of mice with methanolic extracts of *S. officinalis* showed a statistically significant reduction in the tail DNA %, tail length, tail moment and olive tail moment values. The slight increase in the tail DNA %, tail length, tail moment and olive tail moment values at 100 mg/kg b.w. may be because of the slight cytotoxic nature of the extract at higher concentrations. Ramos et al. [13] reported that *S. officinalis* and the isolated compounds demonstrated chemo preventive activity by protecting cells against oxidative DNA damage and stimulating DNA repair. Studies of Lima et al. [34] proved that the methanolic extract of *S. officinalis* with a higher content of phenolic compounds than the water extract, conferred better protection against tert-butyl hydroperoxide induced toxicity in HepG2 cells. The results of the present study along with these evidences proved the high genoprotective efficiency of the species.

Mutagenicity metabolism of methyl parathion

Methyl parathion after entering the body of an organism is metabolized, either by glutathione dependent detoxification or by oxidation to form toxic methyl paraxon. It may also react with cholinesterase to cause subsequent toxicity. Detoxification is achieved by degradation reactions that involve either demethylation or dearylation. The resulting desmethyl compounds and dimethyl phosphoric acids are essentially nontoxic [35]. These detoxification reactions are due to the glutathione-dependent alkyl and aryl transferases. The toxic methyl paraoxon may be inactivated within the living organisms by the very same transferases, during which a superoxide radical, which can initiate a chain of reactions is formed [36].

The ability of organophosphates to react with DNA by transalkylation may reasonably explain their mutagenecity [37]. Wild [38] focused attention on the electrophilic activity as the fundamental cause of the toxicity of these compounds and considered DNA alkylation as one of the reasons for the production of chromosomal aberrations. PO-C is the common structural group of all organophosphates, where phosphorous and carbons are electrophilic sites which offer insight into the understanding of the reactions of organophosphates with nucleophiles. A nucleophile can preferentially attack either phosphorous or carbon atom with subsequent cleavage of P=O or C=O bonds and undergo phosphorylation or alkylation, as the case may be. Such reactions of nucleophilic substitution constitute the primary chemical lesions, resulting ultimately in cytotoxic or genotoxic effects [39]. Benke and Murphy [40] found that liver microsome oxidase in the liver of rat oxidizes methyl parathion, containing phosphorothioate (P=S), to oxon (P=O) and subsequently to methyl paroxons. Vijayraghavan and Nagarajan [41] consider these oxons, which are highly toxic compounds, to account for the profound cytotoxic effect of methyl parathion.

Antimutagenicity of *Salvia officinalis* extracts

The reputed antimutagenic potentiality of *S. officinalis* extracts is due to the bioactive components present in it. Antimutagenic properties of terpenoid fractions of *S. officinalis* tested in mammalian system *in vivo* by Vujosevic and Blagojevic [14] showed that post-treatment with sage suppressed the effects of Mitomycin C significantly. Knezevic-Vukcevic et al. [12] reported that the protective effect of sage monoterpenoids was through enhanced recombinational repair and excision repair. Terpenoid rich essential oil of *Salvia* also modulates mutagenesis by enhanced recombination and inhibition of SOS induction, which is probably caused by inhibition of protein synthesis. The diterpene carnosic acid with ortho-dihydroxyl groups on aromatic ring C inhibited the oxidation through donating H atoms to scavenge free radicals [42].

The next category of *S. officinalis* compounds adding to its genoprotective nature includes phenolics and flavanoids. Studies of Ismail et al. [43] stated the direct correlation between total antioxidative activity and flavanoid content. Flavanoids generally have more hydroxyl groups. Besides, orthosubstitution with electron donating alkyl or methoxy group, 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 [44, 45]. Ruch et al. [46] proved that phenolic compounds are very good electron donors, which may accelerate the conversion of hydrogen peroxide to water. Lima et al. [34] 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. [47] also proved the ability of phenolic compounds to chelate metal ions. *S. officinalis* was well known for its phenolic structure-based antioxidative potency [48].

Thus the outcome of the present work ascertains the role of *S. officinalis* as potential nutraceuticals against the non-intentional exposure to methyl parathion and suggests a new avenue in the prophylaxis therapy.

CONCLUSION

Methyl parathion was proved as a potent mutagen in both the mutagenicity assays and was more effective than ethyl methanesulphonate in inducing DNA damage. The toxic nature of methyl parathion was also evident from the lowering of PCE/NCE ratio in methyl parathion treated mice. Experiments conducted to check the non mutagenic nature of *S. officinalis* (Sage) extracts alone at the said concentrations showed positive results. Combination treatment of *S. officinalis* extracts followed by methyl parathion revealed the protective effects of the extracts. Analysis of the results of the present study together with the previous reports proved that *S. officinalis* extracts or some of its components act as desmutagen through antioxidant activity and suppression of metabolic activation of the methyl parathion. Terpenoids and phenolic compounds of *S. officinalis* have antimutagenic activity. The manifestation of antimutagenic nature of the *S. officinalis* extracts, observed in the present study may be due to these compounds, which, when given as pretreatment, scavenged the methyl parathion induced superoxide and other reactive oxygen metabolites at the time of their formation itself. However, further investigations for the individual constituent's action and mechanisms of action are necessary.

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