Mutat Res Gen Tox En 849 (2020) 503138

Contents lists available at ScienceDirect

Mutat Res Gen Tox En

journal homepage: www.elsevier.com/locate/gentox

Oxidative stress-mediated genotoxicity of malathion in human lymphocytes

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ARTICLE INFO

Keywords:
Comet assay
Formamidopyrimidine DNA glycosylase
Endonuclease III

ABSTRACT

Applying the single-cell gel electrophoresis (comet) assay, we show that the widely used organophosphorus pesticide malathion is cytotoxic, genotoxic, and induces oxidative stress in human lymphocytes.

1. Introduction

Malathion (diethyl, 2-[(dimethoxyphosphinothioyl]butanedioate) is a non-systemic broad-spectrum organophosphate pesticide widely used for the control of insect pests of crops, gardens, household products, ectoparasites on animals, and in public health pest-eradication programmes [1]. In India, malathion production was more than 3000 metric tons in 2017-18 [2]. Malathion is ubiquitous in the global environment due to its high-volume production, indiscriminate use, and stability [3]. The human population can be exposed to malathion from residues in food, drinking water, residential areas where malathion is sprayed, malathion-containing personal use products, and during pesticide application [1,4–7]. Malathion can cause decreased acetylcholinesterase activity, metabolic disturbance, oxidative stress, hepatotoxicity, neurotoxicity, immunotoxicity, cytotoxicity, and genotoxicity in target and non-target species, including humans [9–17]. Malathion exposure has been associated with the risk of cancer, particularly leukemia and lymphoma [18,19]. The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has classified malathion as 'probably carcinogenic to humans' (Group 2A) [8]. Nevertheless, its use in agriculture and in vector-control programmes is permitted in India [2], USA [20] and some European Union countries [21].

Malathion has been tested for genotoxic potential in a broad range of assays, but results have been inconsistent. The pesticide can induce chromosomal aberrations, sister chromatid exchanges (SCEs), micronucleus (MN) formation, DNA damage, and decreased mitotic index in the lymphocytes of pesticide applicators working in cotton fields, vegetable gardens, and vector-eradication programmes [22–24,16]. An increased frequency of MN was observed in human lymphocyte cultures treated with surface water of the Asopos river (Greece) containing 0.01-0.04 μg/L malathion residue [25]. Malathion also induced DNA damage, DNA-protein crosslinks, chromosomal aberrations, SCEs, MN formation, and altered the pattern of hypoxanthine-guanine phosphoribosyl transferase (hprt) mutations in *in vitro* and *in vivo* studies [13,26–33]. In contrast, some studies have indicated that malathion is not genotoxic. No change in proliferation or MN level was observed in the lymphocytes of malathion-exposed workers involved in the Mediterranean fruit fly eradication program in California [27]. Malathion did not induce mutagenicity, chromosomal aberrations, SCEs, or MN formation in bacterial strains, *Drosophila melanogaster*, Chinese hamster ovary cells, V79 cells, or mice [8,34–38]. Although malathion genotoxicity remains controversial, genotoxicity and oxidative stress have been proposed as mechanisms linking malathion exposure to health outcomes [12,13,39]. Malathion may generate reactive oxygen species (ROS) leading to oxidative stress [9,40], but the types of oxidative DNA damage induced by malathion have not been identified.

We have evaluated malathion-induced oxidative-stress-mediated genotoxicity in primary cultures of human lymphocytes. Human lymphocytes are an important system for chemical testing [41]; they are believed to be the primary cells involved in the initiation and progression of hematological malignancies. Malathion-induced oxidative stress was analyzed by measuring generation of ROS, lipid peroxidation (LPO), levels of glutathione (GSH), and activities of the enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione transferase (GST). Genotoxicity was estimated by measuring DNA single-strand breaks (SSBs), double-strand breaks (DSBs), and alkali-labile sites by single-cell gel electrophoresis (SCGE) (alkaline comet assay) [42]. Oxidative DNA damage was characterized by using formamidopyrimidine DNA glycosylase (Fpg) and endonuclease III (Endo-

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https://doi.org/10.1016/j.mrgentox.2020.503138
Received 8 July 2019; Received in revised form 12 January 2020; Accepted 13 January 2020
Available online 17 January 2020
1383-5718/

III) lesion-specific bacterial DNA repair enzymes, which recognize the presence of oxidized purines and pyrimidines [43], in a modified comet assay.

2. Materials and methods

2.1. Chemicals

Technical grade malathion (CAS 121-75-5; 95 %), dimethyl sulfoxide (DMSO) (67-68-5), Ficoll-Paque (17-5442-02), N-acetylcysteine (NAC) (616-91-1), trypan blue (72-57-1), NADPH (2646-71-1), ethyl methane sulphonate (EMS) (62-50-0; 100 %), 5-5′-dithiobis-2-nitrobenzoic acid (DTNB) (69-78-3; 98 %), 1-chloro-2,4-dinitrobenzene (CDNB) (97-00-7; 99 %), and hydrogen peroxide (H_2O_2) (7722-84-1; 98 %) were purchased from Sigma Chemicals Co., St. Louis, MO. Quercetin (6151-25-3; 99 %), glutathione (GSH) (70-18-8; 99), NaCl (7647-14-5; 99.5 %), KCl (7447-40-7; 99.5 %), glucose (50-99-77; 98 %), NaOH (13-10-73; 97 %), dimethyl formamide (68-12-2; 99.9 %), tris HCl (1185-53-1; 99 %), and bovine serum albumin (BSA) (9048-46-8; 98 %) were purchased from Sisco Research Laboratories, Mumbai, India. Dichlorodihydrofluorescein diacetate (DCFH-DA) (4091-99-0; 98 %) and XTT (X6493) were purchased from Thermo-Fisher Scientific. *N,N,N,N* tetramethylethylenediamine (TEMED) (110-18-9; 99 %), ethylenediaminetetraacetic acid (EDTA) (6381-92-6; 99 %), thiobarbituric acid (TBA) (504-17-16; 98 %), ethidium bromide (EtBr) (1239-45-8; 95 %), Triton X-100 (9002-93-1; 98 %), normal-melting-point agarose (NMPA), low-melting-point agarose (LMPA) (9012-36-6; 98 %), phosphate buffered saline (PBS) (TL1033), RPMI 1640 (AL1624), fetal bovine serum (FBS) (RM 9955), penicillin-streptomycin solution (A002A), Na_2HPO_4 (7558-79-4; 99 %), 2-thio-2-nitrobenzoic acid (69-78-3; 98 %), disodium-EDTA (6381-92-6; 99.5 %), HEPES (75277-39-3; 99 %), and ethanol (MB228) were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai. Formamido pyrimidine glycosylase (Fpg, M0240S), endonuclease III (Endo-III) (M0268S), and Endo-III reaction buffer (B02688) were purchased from New England Biolabs, Ipswich, MA, USA.

2.2. Isolation and culture of human lymphocytes

Human peripheral blood (5 mL) was collected from non-smoking, non-drinker, medication-free, healthy male and female volunteers (n = 26) aged 20–23 y (21 ± 0.75), by venepuncture, in vacutainer tubes with heparin (Cat. No. 367878, Becton-Dickinson, India Pvt. Ltd). The investigation was conducted with the approval of the Central University of Kerala, Institutional Human Ethical Committee (CUK/IHEC/2017-011) and with informed consent.

Lymphocytes were isolated by the Ficoll-Paque density-gradient method. Pooled lymphocyte samples were used. Briefly, whole blood, 5 mL, was diluted with sterile PBS, pH 7.4, 5 mL, layered on Ficoll-Paque, 5 mL, and centrifuged, 400 × *g*, 20 min, at room temperature (20 °C) to obtain the buffy coat containing lymphocytes. The buffy coat was washed twice with sterile PBS and then with RPMI-1640 medium. Lymphocyte viability (Trypan blue exclusion) was about 95 %. Cells (1×10^4) were cultured in RPMI-1640 medium supplemented with 10 % FBS, 1.5 % phytohemagglutinin (PHA, ThermoFisher Scientific), and 1 % penicillin-streptomycin. Lymphocytes were cultured in a humidified incubator with 5 % CO_2 at 37 °C in 15 mL T25 polystyrene flasks. Lymphocytes were disaggregated by pipetting and used for the exposure experiments. Viability was checked before exposure and found to be > 70 %.

2.3. Positive control

Oxidative stress induction was tested by measuring the effect of NAC, 20 μM [44]. To confirm the induction of genotoxicity and the abilities of Endo-III and Fpg to recognize oxidized bases, EMS (0.15 mM) was used as positive control [45,46].

2.4. Stock solution

Malathion stock solution was prepared in 1 % DMSO. Lymphocytes were treated by adding stock solution to fresh supplemented RPMI growth medium.

2.5. Toxicity assays

The IC_{50} of malathion was determined. Lymphocytes grown to 80–100 % confluence were treated with malathion (up to 2 mg/mL). Lymphocytes (1×10^4) were seeded in medium (100 μL) in each well of a 96-well microtiter plate, in triplicate. The plate was incubated for 24 h at 37 °C. The vehicle control was 1 % DMSO, which was not toxic. Control and malathion-treated lymphocytes were incubated for 24 h at 37 °C and then cell viability was evaluated using the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay. In brief, after the 24 h incubation, XTT (1 mg/mL) solution (freshly prepared), 50 μL, was added to each well. The 96-well plate was incubated for 2 h at 37 °C. In viable cells, XTT is reduced to orange formazan product, measured at 450 nm with a plate reader (EnSpire™ Multimode Plate Reader, PerkinElmer, Inc.). Colour is directly proportional to the number of viable cells. The assay was conducted in triplicate and repeated thrice. IC_{50} was determined by the Probit method [47]. IC_{50} for malathion was 244 μg/mL. Concentrations chosen for further studies were 50, 100, and 150 μg/mL.

2.6. ROS generation

ROS generation was analyzed by the DCFH-DA method [48]. Briefly, lymphocytes were cultured in RPMI medium for 24 h at 37 °C. Lymphocytes (2 H 10^5) were seeded in a 96-well plate (white/transparent, flat bottom) for 24 h malathion treatment, in triplicate. After 24 h, cells were incubated with serum-free medium + DCFH-DA (1 μM), 100 μL, for 30 min in the dark at 37 °C, washed twice with PBS, and harvested in PBS. Conversion to fluorescent DCF was measured (excitation, 480 nm; emission, 530 nm) with the plate reader. Values were expressed as pmol DCF formed/min/mg protein.

2.7. Lymphocyte lysate

Lymphocytes (5×10^6 /mL) treated with malathion, or controls, were cultured in RPMI for 24 h and then harvested and centrifuged. Pellets were sonicated on ice in cold buffer (5 mM potassium phosphate (pH 7.4) + 0.9 % NaCl and 0.1 % glucose), 1 mL, and then spun, 10 min, 10,000 × g, at 4 °C. Supernatant was collected and used for biochemical analyses.

2.8. Lipid peroxidation (LPO)

Malondialdehyde (MDA) levels were measured by the thiobarbituric acid-reactive substances (TBARS) method of Ohakawa et al. [49], with minor modifications. The reaction mixture (0.1 M EDTA, 75 μl, and 1 % TBA in 0.5 M NaOH, 250 μl, was added to cell lysate, 1 mL. Samples were kept in a boiling water bath for 15 min, cooled to room temperature, and absorbance measured at 532 nm. TBARS were expressed as nmol MDA/mg protein.

2.9. Superoxide dismutase (SOD)

SOD activity was determined by inhibition of quercetin autooxidation (Kostyuk and Potapovich [50]). The incubation, 1 mL, contained protein (2 − 3 μg, 500 μL cell lysate); sodium phosphate buffer (16 mM, pH 7.8), 250 μL; TEMED, 8 mM, 100 μL; and EDTA, 80 μM, 100 μL. Addition of quercetin, 0.15 % in DMF, 50 μL, started the reaction, and

autoxidation was monitored at 406 nm for 3 min. The decrease in absorbance following addition of cell lysate was monitored. The amount of protein that inhibits quercetin oxidation by 50 % was defined as 1 unit and values were expressed as unit/mg protein.

2.10. Catalase (CAT)

CAT activity was determined according to the method of Aebi [51]: decomposition of H_2O_2 (final concentration, 880 μM). The reaction mixture contained phosphate buffer (pH 7.4), 1.5 mL, H_2O_2 solution, 1.2 mL, and cell lysate, 300 μL. After addition of lysate, decomposition of H_2O_2 was monitored at 240 nm for 3 min and expressed as μM H_2O_2/min/mg protein.

2.11. Glutathione (GSH)

GSH content was measured by the method of Tietze [52]. Lymphocytes (1×10^7/mL) were washed twice with PBS and lysed with ice-cold extraction buffer (0.1 % Triton-X and 0.6 % sulfosalicylic acid in KPE buffer) 1.0 mL. KPE buffer is 0.1 M potassium phosphate buffer +5 mM EDTA, pH 7.5. Precipitated protein was removed by centrifugation (5000 × g, 5 min). The assay was performed by adding the reagents to tubes in the following order: cell lysate, 200 μL; GSH reductase (1 unit/mL), 200 μL; DTNB, 6 mM, 300 μL. Tubes were mixed well and then were incubated at room temperature for 3 min. Then, NADPH, 0.3 mM, 300 μl, prepared in stock buffer solution (125 mM sodium phosphate, 6.3 mM EDTA, pH 7.5) was added. After incubation at room temperature, 5 min, 5-thio-2-nitrobenzoic acid was measured at 412 nm. Total GSH was determined from a standard curve and expressed as μM GSH/mg protein.

2.12. Glutathione transferase (GST)

GST activity was assayed spectrophotometrically according to the method of Habig and Jakoby [53]. The reaction mixture (1 mL) contained 0.25 M potassium phosphate buffer (pH 7.0) +2.5 mM EDTA, 880 μL; GSH, 0.1 M, 10 μL; at 25 °C, CDNB, 25 mM, 10 μL; and lysate, 100 μL. For the blank, PBS, 100 μL, was used. The reaction mixture was incubated at 30 °C; absorbance at 340 nm was recorded every min to 3 min and the GST activity was expressed as μmol GS-DNB formed/min/mg protein.

2.13. Protein

Protein levels were determined by the Bradford [54] assay with BSA standard.

2.14. Comet assay

Lymphocytes were gently mixed with Hanks' balanced salt solution (HBSS, 1 ml) and centrifuged at 200 × g for 3 min at 4 °C. The pellet was resuspended in PBS (pH 7.4, Ca^{2+} and Mg^{2+} free) and the adjusted to 1×10^4 cells/mL. Cell suspension, 50 μL, was mixed with low-melting-point agarose (1 % LMPA; prepared in 0.1 M sodium phosphate buffer, pH 7.2 + 0.9 % NaCl), 50 μL.

2.14.1. Alkaline, Endo-III-, and Fpg-modified comet assay

The alkaline, Endo-III-, and Fpg -modified comet assays were carried out simultaneously in two different experimental sets. The alkaline comet assay was performed with the protocol of Singh et al., slightly modified [42]. Slides were prepared by layering cell suspension, 80 μL, on slides pre-coated with 1 % normal-melting-point agarose (NMPA) (prepared in 0.1 M sodium phosphate buffer, pH 7.2 + 0.9 % NaCl). The cell suspension was uniformly smeared and immediately covered with a cover glass. Then, slides were kept for 10 min at 4 °C in a refrigerator to solidify the agarose. After agarose solidification cover glass was removed and the slides were placed in cold lysis buffer (2.5 M NaCl, 100 mM Na_2-EDTA, 10 mM Tris–HCl, pH 10.0 adjusted with 0.26 M NaOH, 1 % Triton X-100, 10 % DMSO, freshly prepared and added just before use) overnight (> 12 h) at 4 °C in a dark chamber, for lysis. After lysis, the slides were rinsed with chilled distilled water, placed on a specially designed horizontal electrophoresis platform with an alkaline electrophoresis buffer (0.03 M NaOH and 1 mM Na_2-EDTA, pH > 13) for 20 min at 4 °C, followed by electrophoresis in the same buffer and at the same temperature for 30 min; constant 300 mA; 0.70 V/cm.

The endo-III- and Fpg-modified assays were performed following the method of Collins et al. [55] with some modifications. Lysis was performed as above. Immediately after lysis, slides were washed three times with buffer (40 mM HEPES, 100 mM KCl, 0.5 mM EDTA, 0.2 mg/mL BSA, pH 8.0) for 5 min at room temperature. For evaluation of oxidative DNA damage, four slides were prepared separately from each group: (a) Fpg reaction buffer, (b) Fpg, (c) Endo-III reaction buffer, and (d) Endo-III. Fpg and Endo-III slides of the control were exposed to Endo-III or Fpg, diluted 1:1000 or 1:3000 respectively, following the recommendation of the supplier (New England Biolabs). Briefly, slides were incubated with Endo-III (0.5 U, 75 μL) or Fpg (0.13 U) [55]. Control slides were treated with reaction buffer, 75 μL. Endo-III, Fpg and control slides were incubated for 30, 45, or 60 min at 37 °C, respectively. After enzyme treatment, cover glasses were removed and the slides were placed in a horizontal electrophoresis tank with alkaline electrophoresis buffer for 20 min, followed by electrophoresis as described above. All steps were performed in the dark.

2.14.1.1. Staining. After electrophoresis, slides were gently washed three times with 0.4 M Tris–HCl buffer (pH 7.5) at 4 °C to neutralize excess alkali, dehydrated with ethanol for 10 min, and air-dried overnight. All slides were then stained with EtBr (20 μg/mL), 75 μL per slide for 10 min in dark. After staining, slides were dipped once in chilled distilled water to remove excess stain and coverslips were placed over the slides.

2.14.1.2. Analysis. Slides were examined with a fluorescence microscope (Leica DMI3000 B, Carl Zeiss/ Leica, Germany) equipped with an appropriate filter, at 40 × magnification. The images of 100 randomly selected non-overlapping cells from each group, in triplicates, were captured with a CCD camera (Leica DFC425 C). DNA damage was analyzed using CASP 1.2.3 software (CASPlab). Tail intensity (% DNA in tail) was used as a parameter of genotoxicity.

2.15. Data evaluation and statistical analyses

2.15.1. Biochemical assays

All the analyses were made in replicates and values are represented as mean ± SEM. Significance of differences between control and treated groups was obtained by one-way analysis of variance (ANOVA) followed by Tukey HSD post hoc test.

2.15.2. Comet assay

To estimate the effects of the treatments, one-way analysis of variance (ANOVA) with Tukey's test was performed. All analysis was done with SPSS (version 16.0) (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Toxicity

The results of the malathion toxicity experiments are shown in Fig. 1. NAC was not protective ($p > 0.05$).

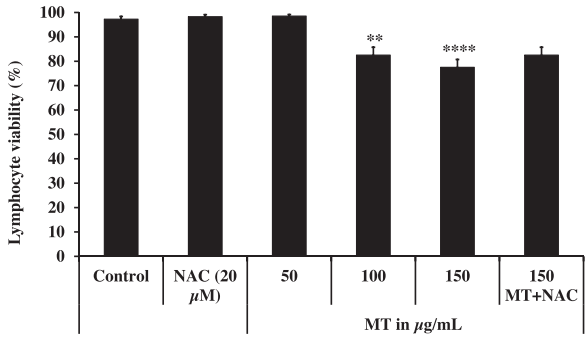

Fig. 1. Lymphocyte viability (%) results. Values without * are not significantly different from control; significant *vs* control at **p < 0.01; **** p < 0.0001 levels.

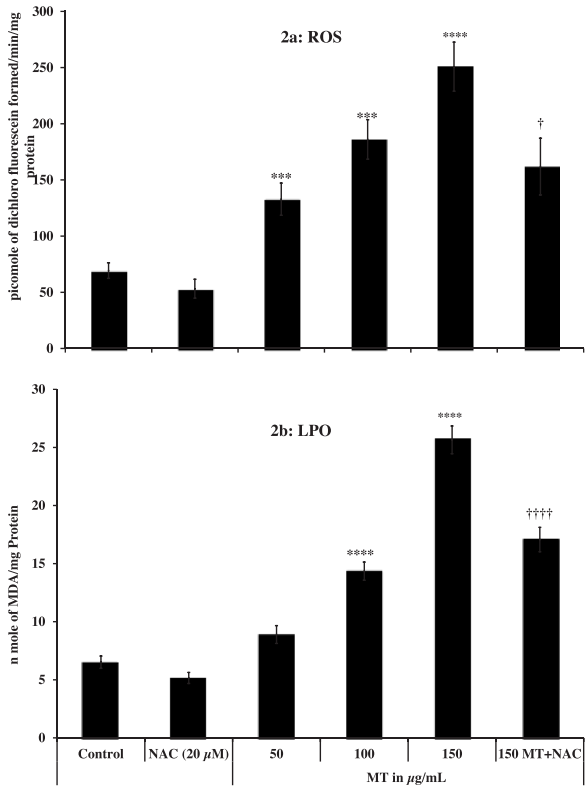

Fig. 2. Reactive oxygen species (ROS) generation (a) and lipid peroxidation (LPO) (b) results. Values without * are not significantly different from control or (†) NAC; significant *vs* control at *** p < 0.001; **** p < 0.0001 and significant *vs* malathion, 100 μg/mL at †p < 0.05; †††† p < 0.0001 levels.

3.2. Oxidative stress

Malathion caused ROS generation and increased LPO, even at the lowest concentration tested (Fig. 2). In contrast to the toxicity results, NAC was protective.

3.3. Antioxidants

SOD and CAT, enzyme activities were elevated at the two lower concentrations of malathion but dropped again at the highest concentration, and NAC was ineffective (Fig. 3a and b). GST activity and GSH content increased and decreased, respectively, with malathion concentration (Fig. 3c and d). NAC had no effect.

3.4. DNA damage

Data are shown in Table 1. In the untreated control groups, Fpg and Endo-III treatments had no effect on DNA damage. EMS (positive control) caused DNA damage under all analysis conditions. Malathion, especially at the higher concentrations (100 and 150 μg/mL), caused DNA damage, and the damage was significantly increased by Endo-III treatment. NAC had no effect on untreated cells. For cells treated with the highest concentration of malathion and measured with the Endo-III modified assay (only), NAC reduced damage.

4. Discussion

We found that malathion is cytotoxic to human lymphocytes (Fig. 1), possibly due to generation of ROS and LPO. Pesticides, particularly organophosphorus pesticides (OPs), may cause oxidative stress by generating ROS [9,40], causing damage to the cell membrane, protein, lipid, and DNA, resulting in cytotoxicity [56]. 4-Hydroxynonenal (4HNE), a lipid peroxidation product, is cytotoxic [57]. Pesticide-induced oxidative stress-mediated cytotoxicity was seen in rat adrenal pheochromocytoma (PC12) cells [58] and rat lymphocytes [59].

At 50 μg/mL malathion, lymphocytes were viable, despite generation of ROS. Activation of pro-survival signalling pathways may occur in response to the pesticide and this may be a factor in increased cell proliferation in cancer [60]. NAC may scavenge oxidative free radicals generated by the malathion, protecting cells from oxidative stress.

ROS generation was seen in all malathion-treated groups (Fig. 2a), indicating low antioxidant capacity, excess production, or insufficient removal of ROS [61]. Malathion treatment increased TBARS (Fig. 2b), suggesting increased oxidative stress [62,63]. NAC reduced ROS production and LPO elevation induced by malathion, implicating ROS production in malathion toxicity.

SOD and CAT activities showed a biphasic response (Fig. 3a and b). Increased activities at lower concentrations may be an adaptive response to stress [64]. Reduced activities at the highest concentration of malathion could be due to production of ROS beyond the cell's detoxication capacity, leading to accumulation of H_2O_2, which may promote LPO, alter gene expression, and mediate cytotoxicity [61].

Elevated GST enzyme activity in malathion-treated cells (Fig. 3c) may be related to activation of adaptive mechanisms to counteract oxidative stress [65]. Lower concentrations of malathion cause mild GSH depletion and high doses can lead to massive depletion (Fig. 3d), indicating that the antioxidant property of GSH may mitigate malathion-induced ROS generation [66]. Slightly increased GSH in the cells treated with malathion + NAC (Fig. 3d) demonstrates some protective action of this GSH precursor.

Significantly increased % tail DNA was observed in malathion-treated groups, indicating malathion genotoxicity. Our findings are consistent with studies showing elevated DNA damage in lymphocytes of pesticide manufacturing factory workers exposed to pesticides [67]. The % tail DNA measured in the presence *vs* absence of Fpg and Endo-III enzymes may suggest that oxidative stress contributes to malathion-induced DNA damage (Table 1). Other studies have demonstrated malathion-induced DNA damage and oxidative stress in rat lymphocytes [30,39].

The DNA damage observed in lymphocytes (Table 1) following malathion exposure could be a consequence of free-radical attack on DNA due to oxidative stress. For EMS (positive control) treatment, increased % tail DNA resulted from Fpg III treatment, indicating that EMS may act, at least in part, by generating oxidative reactive species [46].

The significant reduction of % tail DNA observed in the combination of 150 μg/mL malathion and 20 μM NAC in the Endo-III modified comet assay indicates that malathion induced oxidative stress may be responsible for oxidative DNA damage, with NAC attenuating this effect by counteracting the stress.

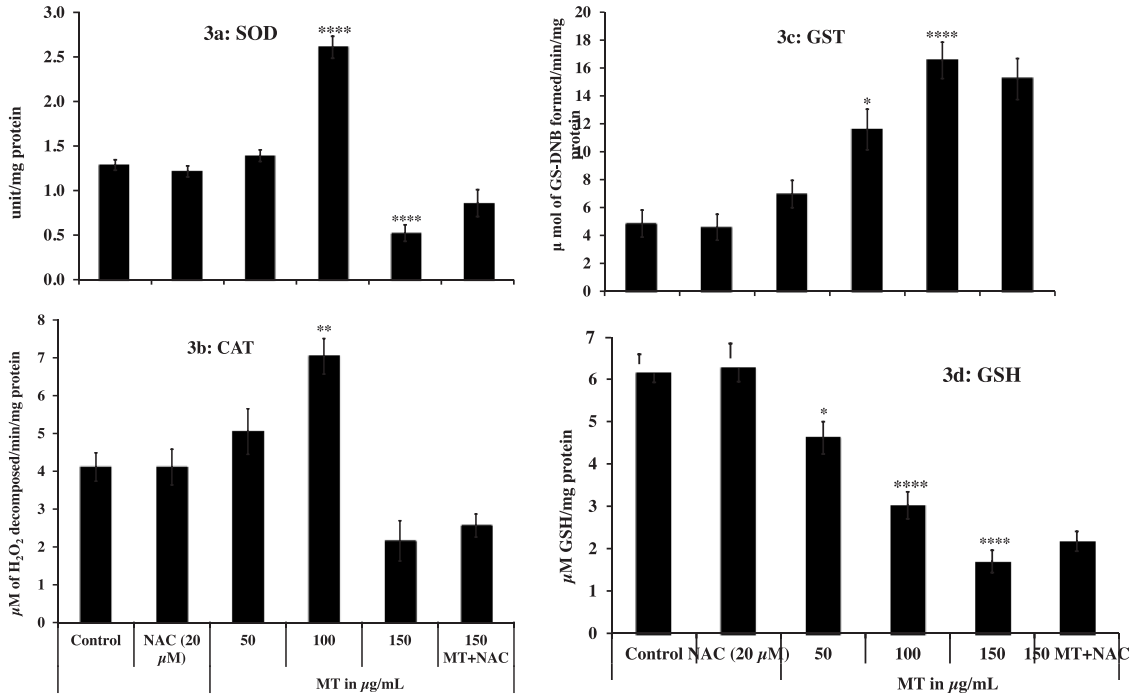

Fig. 3. Superoxide dismutase (SOD; a), catalase (CAT; b), and glutathione transferase (GST; c) activities and glutathione (GSH) content (d). Values without (*) are not significantly different from controls; significance from control indicated at *p < 0.05; **p < 0.01; **** p < 0.0001 levels.

Table 1
DNA damage (comet assay) results.

Treatment	% DNA in tail				
	Alkaline comet	Modified alkaline comet assay			
		Endo III buffer control	Endo III	Fpg buffer control	Fpg
Control	0.63 ± 0.05	1.27 ± 0.1	2.02 ± 0.2	1.24 ± 0.15	2.16 ± 0.1
NAC	0.55 ± 0.04	0.85 ± 0.1	1.30 ± 0.2	0.86 ± 0.1	1.14 ± 0.1
EMS	26.9 ± 1.7****	27.3 ± 3.7****	30.0 ± 2.0****	28.9 ± 1.6****	42.2 ± 3.3****#
MT, 50 μg/mL	3.5 ± 0 .6	3.5 ± 0.5	5.7 ± 0.8	3.2 ± 0.4	4.6 ± 0.8
MT, 100 μg/mL	15.1 ± 1.5***	15.8 ± 1.4***	29.1 ± 1.8****##	15.9 ± 1.4**	17.7 ± 2.1****
MT, 150 μg/mL	21.0 ± 2.3****	21.8 ± 2.4****	38.9 ± 3.3****##	21.0 ± 1.4****	22.0 ± 1.2****
MT, 150 μg/mL + NAC	19.9 ± 2.0****	20.7 ± 1.4****	28.3 ± 2.5****#††	18.8 ± 1.8**	20.6 ± 2.3****

All results are means ± S.E.M of three independent experiments.
EMS, 0.15 mM; positive control); NAC, 20 μM; MT: Malathion.
**p < 0.01, ***p < 0.001, ****p < 0.0001 between control and treatment groups.
#p < 0.05; ##p < 0.01 % between buffer and corresponding enzyme.
††p < 0.01 MT without *vs* with NAC.

The increased DNA damage seen in the Endo-III modified comet assay at higher concentrations of malathion shows that reactive free radicals produced by malathion treatment may damage DNA pyrimidine bases and contribute to formation of single-strand breaks. Malathion has been reported to cause DNA lesions/breakage in oncogenes or tumor suppressor genes and to induce malignancies in exposed persons [68,26]. Navarrete-Meneses et al. [69] showed that exposure to malathion induces aberrations in genes involved in the etiology of hematological malignancies. Epidemiological and experimental studies have shown that exposure to malathion may increase risk of lung and breast cancers, leukemia, and lymphoma in both humans and rodents [18,19,70,71]. Koutros et al. [72] reported that use of malathion increased risk of non-Hodgkin's and B-cell lymphomas.

5. Conclusions

Malathion exposure increases LPO, causes enhanced production of ROS, and affects SOD, CAT, GSH and GST antioxidant activities. Alkaline comet assay data showed that malathion is genotoxic. The enzyme-modified comet assay results suggest that malathion-induced oxidative stress may cause oxidative DNA damage in human lymphocytes. Oxidative stress may play an important role in malathion-induced genotoxicity and might contribute to initiation and progression of haematological malignancies, supporting the possibility that malathion is a mutagen and carcinogen.

Declaration of Competing Interest

None declared.

Acknowledgments

The authors are thankful to the authorities of the Central University of Kerala for providing facilities and to the Kerala State Council for Science, Technology, and Environment (KSCSTE) for the research fellowships. The authors also thank UGC (No. F.30-25/2014 (BSR)/27-

Jan- 2016), DBT (6242-P-36/RGCB/PMD/DBT/HPGS/2015/29-July-2015) and DST-SERB (SB/YS/LS-387/2013/ 31-Oct-2014) for funding. Dr. V. Vasudev, Emeritus Professor, University of Mysore, Karnataka, India and Professor M. Arunachalam, Department of Zoology, Central University of Kerala, Periya, India generously provided encouragement and critical readings of the manuscript.

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