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Diphenylmethyl selenocyanate attenuates malachite green induced oxidative injury through antioxidation & inhibition of DNA damage in mice

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Abstract

Background & objectives:

Malachite green (MG), an environmentally hazardous material, is used as a non permitted food colouring agent, especially in India. Selenium (Se) is an essential nutritional trace element required for animals and humans to guard against oxidative stress induced by xenobiotic compounds of diverse nature. In the present study, the role of the selenium compound diphenylmethyl selenocyanate (DMSE) was assessed on the oxidative stress (OS) induced by a food colouring agent, malachite green (MG) *in vivo* in mice.

Methods:

Swiss albino mice (*Mus musculus*) were intraperitoneally injected with MG at a standardized dose of 100 µg/mouse for 30 days. DMSE was given orally at an optimum dose of 3 mg/kg b.w. in pre (15 days) and concomitant treatment schedule throughout the experimental period. The parameters *viz*. ALT, AST, LPO, GSH, GST, SOD, CAT, GPx, TrxR, CA, MN, MI and DNA damage have been evaluated.

Results:

The DMSE showed its potential to protect against MG induced hepatotoxicity by controlling the serum alanine aminotransferase and aspartate amino transferase (ALT and AST) levels and also ameliorated oxidative stress by modulating hepatic lipid peroxidation and different detoxifying and antioxidative enzymes such as glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), and also the selenoenzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) and reduced glutathione level which in turn reduced DNA damage.

Interpretation & conclusions:

The organo-selenium compound DMSE showed significant protection against MG induced heptotoxicity and DNA damage in murine model. Better protection was observed in pretreatment group than in the concomitant group. Further studies need to be done to understand the mechanism of action.

Keywords: Diphenylmethyl selenocyanate, genotoxicity, malachite green, oxidative stress

Malachite green (MG) is an N-methylated triphenylmethane dye used worldwide as an antifungal agent in aquaculture and also for dyeing silk, wool, jute, leather, and cotton. It is also used extensively as a non permitted food colouring agent especially in India[1]. The potential for consumer exposure exists most notably through its use as an antifungal agent in commercial fish hatcheries and as food additive. MG is extremely cytotoxic to mammalian cells, as it induces free radical formation, acts as a potent liver tumour promoter and induces malignant transformation[2]. MG also induces DNA damage[3]. Though the use of this dye has been banned in several countries and not approved by US Food and Drug Administration, it is still being used in many parts of the world including India due to its low cost, ready availability and efficacy[2]. Despite its known harmful effects, information on the mechanism(s) of action of MG, its carcinogenicity and tumour promoter properties is not available. Upon absorption malachite green is reduced to leucomalachite green, which is the persistent form of the dye and the toxicity exerted by leucomalachite green has greater severity[4]. Most of the work on MG induced cellular toxicity was done *in vitro*, only a few studies have been reported in animal models. There is no report available on the effect of MG on different antioxidant and detoxifying enzymes and lipid peroxidation *in vivo*.

Selenium (Se) is an essential nutritional trace element required for animals and humans to guard against oxidative stress induced by xenobiotic compounds of diverse nature[5], protecting cell membranes from free radical damages. Studies suggest that low selenium status may increase the risk of oxidative damage and cancer[6]. It forms the active center of selenoenzymes such as glutathione peroxidase and thioredoxin reductase that catalyzes essential redox reactions and performs the function of an antioxidant in several metabolic and immunologic processes. The antioxidative as well as cancer chemopreventive properties of diphenylmethyl selenocyanate have earlier been reported from our laboratory[7,8]. The compound was found to be non toxic at the dose used in the experiments. The present study was aimed to determine the effect of MG on hepatic lipid peroxidation, antioxidant and detoxifying enzymes, serum transaminase (AST and ALT) levels and their modulation by diphenylmethyl selenocyanate (DMSE) in a mouse model (*Mus musculus*).

Material & Methods

The study was conducted in the Department of Cancer Chemoprevention, Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The study protocol was approved by the institutional ethics committee for animal experiments.

Experimental design: Adult (6 wk old 23 ± 2 g body weight) Swiss albino female mice bred in the animal colony of CNCI were used for this study. DMSE was synthesized in the laboratory by the known methodology[9]. The purity (99.9 %) was checked by HPLC. The dose of DMSE was selected based on our earlier work[7]. Animals were divided in four groups each containing six mice for the experiments.

Group I (vehicle control group) - This group received 5.5 per cent propylene glycol in water only orally for 30 days.

Group II (MG control group) - In this group MG was injected ip (in distilled water) at a dose of 100 µg/mouse (25g) b.w. for 30 days.

Group III (15D pre DMSE+ MG) - In this group animals were pretreated with oral administration of DMSE compound at a dose of 3 mg/kg b.w. in 5.5 per cent propylene glycol (200 µl) for 15 days before MG was injected ip (in distilled water) for 30 days and the DMSE compound was administered throughout the MG treatment.

Group IV (concomitant DMSE+ MG) - In this group, animals were received DMSE compound orally and MG was given ip (in distilled water) for 30 days simultaneously. After 30 days animals were killed by overdose of thiopentone sodium (100 mg/kg body weight) and blood samples, liver and bone marrow were collected.

Chemicals: Diphenylmethyl bromide, potassium selenocyanate (KSeCN), 1-chloro-2, 4- dinitrobenzene (CDNB), ethylene diaminetetra acetic acid (EDTA), reduced glutathione (GSH), pyrogallol, 5,5′-dithio-bis(2-nitro benzoic acid) (DTNB), bovine serum albumin (BSA), thioglycolate broth, β- nicotinamide adenine dinucleotide phosphate (reduced) (β-NADPH), glutathione reductase, RPMI 1640, N-(1-napthyl) ethylenediamine dihydrochloride, sodium azide (NaN_3), normal melting agarose (NMA), low melting point agarose (LMPA), dimethyl sulphoxide (DMSO), ethidium bromide, and thioredoxin reductase assay kit were purchased from Sigma-Aldrich Chemicals Private Limited, India. Malachite green, hydrogen peroxide 30 per cent (H_2O_2), thioberbituric acid (TBA), propylene glycol, hexane and orthophosphoric acid were purchased from Merck Specialities Limited, India. Sulphanilamide, tris-HCl and acetone were obtained from Sisco Research Laboratories Private Limited, India. Diethyl ether, dipotassium hydrogenphosphate (K_2HPO_4), potassium di-hydrogenphosphate (KH_2PO_4) were purchased from Spectrochem Private Limited, India.

Determination of alanine amino transferases (ALT) and aspartate amino transferases (AST) activity/(Hepatotoxicity assessment): After 30 days of experimental schedule blood samples were collected from mice by cardiac puncture and centrifuged for 5 min for serum separation. The serum samples were stored at -80°C. Serum ALT and AST activity levels were analyzed spectrophotometrically by standard enzymatic methods[10] using commercial kits (Span Diagnostics Ltd., India).

Determination of lipid peroxidation (LPO) and reduced glutathione (GSH): Lipid peroxidation was estimated in the liver microsomal fraction. Liver was homogenated, centrifuged and isolated as earlier reported[11]. Protein was estimated spectrophotometrically with BSA as standard[12]. The level of lipid peroxides formed was measured using thiobarbaturic acid and expressed as thiobarbituric acid reactive substances (TBARS)[13]. TBARS were determined by the absorbance at 535 nm and expressed as TBARS formed per milligram of protein using as extinction coefficient of 1.56×10^5/M/cm. GSH level was estimated in liver cytosol spectrophotometrically by determination of DTNB reduced by -SH groups by measuring the absorbance at 412 nm. The level of GSH was expressed as nm of GSH per milligram protein[13].

Determination of antioxidant enzymes, glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and thioredoxin reductase (TrxR): GST activity was measured in the liver cytosol. The enzymatic activity was determined from the increase in absorbance at 340 nm with 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate and specific activity of the enzyme expressed as the formation of 1-chloro-2, 4-dinitrobenzene (CDNB-GSH) conjugate per minute per mg of protein[14].

SOD activity after partial extraction and purification of SOD was determined[15] by quantification of pyrogallol auto-oxidation inhibition and expressed as units per milligram of protein. One unit of enzyme activity is defined as the amount of enzyme necessary for inhibiting the reaction by 50 per cent. Auto-oxidation of pyrogallol in tris -HCl buffer (50 mM, *p*H 7.5) was measured by increase in absorbance at 420 nm. Activity of CAT in liver

cytosol was determined[16] spectrophotometrically at 250 nm and expressed as units per milligram of protein where one unit is the amount of enzyme that liberates half the peroxide oxygen from H_2O_2 in seconds at 25°C.

Glutathione peroxidase (GPx) activity in liver tissue sample was estimated by NADPH oxidation using a coupled reaction system consisting of reduced glutathione and hydrogen peroxide[17]. The reactions were estimated by decrease in NADPH absorbance at 340 nm for 3 min after adding 100 µl of H_2O_2. The enzyme activity was expressed as µmol NADPH utilized/min/mg of protein using extinction coefficient of NADPH at 340 nm as 6,200 M/cm.

Thioredoxin reductase (TrxR) activity was measured by colorimetric assay (spectrophoto-metrically) based on the reaction of reduction of DTNB with NADPH to TNB and TrxR inhibitor. Briefly, liver tissue (100 mg) was homogenized in cold condition in 400 µl of 0.25 × assay buffer of commercially available kit (Sigma, USA) and the TrxR activity was measured at 412 nm by standard enzymatic method[18].

Determination of chromosome aberrations (CA), micronuclei (MN) and mitotic index (MI): The assays of important cytogenotoxicity parameters like CA, MN and MI were performed. Mice were injected (ip) with 0.03 per cent colchicine at a doze of 1 ml/100 g body weight and were kept for 1.15 h prior to sacrifice by following standard procedure[19]. In brief, marrows of the femur bone were fixed (acetic acid/ethanol 1:3) and the slides were prepared for scoring different types of chromosome aberrations by Giemsa staining. Chromosome aberrations were classified into two types: the "major" type comprising aberrations like ring, break, fragment, *etc*. and the "other" types comprising less significant aberrations like erosion, gap, *etc*. A total of 300 bone marrow cells were observed (50 from each of 6 mice/set). A part of the suspension of bone marrow cells in 1 per cent sodium citrate was smeared on clean grease free slides, fixed in methanol and subsequently stained with May-Grunwald followed by Giemsa for micronucleus preparation[20]. A total of 3000 bone marrow cells, both polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were counted and the ratios between PCE and NCE were calculated. The MI was determined from the same slide which was scanned for MN, and a total of 5000 cells were scored from each group. The non-dividing and dividing cells were recorded and their ratios ascertained.

Single cell gel electrophoresis (SCG/comet assay) for detection of DNA damage: Possible DNA damage induced by MG was analyzed using SCG following a simplified protocol with slight modifications[21]. This method is highly sensitive for detecting the DNA damage of individual cells, expressed as single strand breaks, alkali-labile site formation and disordered DNA fragmentation. After 30 days of experimental schedule, blood (3 ml) was collected from each mouse in all groups. Lymphocytes were separated and 10^4 cells per slide were taken. The viability in each group was measured (83 ± 5%) by the trypan blue exclusion method[22]. Half frosted microscope slides were coated with 1 per cent normal melting agarose in PBS. An aliquot of 10 µl of freshly prepared single cell suspension was mixed with 75 µl of 1 per cent low melting point agarose in Milli-Q water. This mixture was layered on to the pre-coated slide and covered with a 24 × 50 mm cover slip and kept on ice to solidify the agarose. Then cover slip was gently removed and a third layer of 0.5% low melting point agarose was layered. After the agarose was solidified, the slides were immersed in ice-cold lysing solution (2.5 ml NaCl, 100 mM Na_2EDTA, 10 mM Trizma base, 1% sodium lauryl sarcosinate, 1% triton X-100, 10% DMSO, *p*H 10.0) at 4°C for overnight. Then the slides were placed into an electrophoresis unit filled with fresh electrophoresis buffer (1 mM Na_2EDTA, 0.3M NaOH, *p*H >13.1) and incubated for 20 min at 4°C to allow the unwinding of DNA. The slides were subjected to electrophoresis (25 V, 400 mA) for another 25 min. After electrophoresis, the slides were placed on a tray to remove alkali and detergents and neutralized with neutralizing buffer (0.4 M Trizma buffer, *p*H 7.5) for 10 min. The slides were stained with ethidium bromide in water (20 µg/ml; 50 µl/slide). The stained slides were examined at 400×magnification under a fluorescence microscope (Nikon Eclipse E600, Japan; excitation filter: 510-560 nm; barrier filter: 590 nm). Photomicrographs of cells were taken with a still camera (Nikon FDX-35) and the cells (150–200) were counted in each slide (4 slides/animals in each group) to determine the number of damaged cells and the percentage of damaged cells. The calculation was performed using the formula: % damaged cell = (Number of damaged cells/Total number of cells counted) × 100. Length of the comet tail was determined by using an occulometer

affixed in the eye piece of the microscope. Comet tail length was measured between the edge of the comet head and the end of the comet tail, calculated in micrometers (μm)[23]. The result was expressed as olive tail moment (OTM). The OTM is (per cent of DNA in the tail) × (distance between the center of gravity of DNA in the tail and that of center of gravity of DNA in the head). The result was expressed as percentage of cells with tail (tailed cells) in each group that was scored and was calculated for average tail length.

Statistical analysis: All statistics were performed using Vassar Stats web statistical software (release©Richard Lowry, Poughkeepsie, NY, USA). One-way analysis of variance (ANOVA) was performed to compare the differences of means between groups. Data were expressed as mean ± SD. Comparisons were made between vehicle control group vs MG control group and MG control group vs pre DMSE + MG and con DMSE+ MG treated group.

Results

Inhibition of MG induced hepatotoxicity by DMSE: The effect of Se compounds treatment on the MG induced elevation of hepatic ALT and AST activities are shown. The MG induced hepatotoxicity in mice was indicated by the sharp increase of serum ALT and AST levels significantly ($P<0.01$) by 89 and 71 per cent, respectively compared to the vehicle control animals (Fig. 1a). However, treatment with DMSE reduced this enhanced level of ALT significantly ($P<0.01$) by 38.97 per cent (15 days pretreatment) and by 16.95 per cent (concomitant treatment). Similarly the enhanced AST level was reduced significantly ($P<0.01$) by 29.53 per cent (15 days pretreatment) and by 16.31 per cent (concomitant treatment) (Fig. 1b).

To evaluate the effect of DMSE pretreatment on MG induced hepatic LPO, the levels of MDA and other reactive aldehydes were measured as TBARS. The production of TBARS in MG treated mice increased by 81 per cent compared to the non treated control mice. Consistent with the serum ALT and AST levels, pretreatment with Se compound significantly ($P<0.01$) decreased MG induced hepatic LPO level by 51 per cent (Fig. 1c), whereas concomitant treatment with the selenium compound reduced the LPO level significantly ($P<0.01$) by 11.3 per cent.

To further understand the possible mechanism of protection by DMSE the hepatic GSH level was measured. MG was given to three groups of mice with or without the Se compounds treatment and the hepatic GSH level was determined. The administration of MG alone significantly ($P<0.01$) depleted GSH level by 76 per cent. Mice, treated with this Se compound, were protected from GSH depletion induced by MG (Fig. 1d). GSH level was enhanced significantly ($P<0.01$) by 68.06 per cent (15 days pretreatment) and by 61.85 per cent (concomitant treatment) compared to the MG treated animals.

DMSE modulates antioxidative enzymes against MG induced oxidative damage: The activities of the antioxidative enzymes, GST, SOD, CAT, GPx and TRx were measured in mouse liver (Fig. (Fig.2a2a--e).e). MG treatment alone significantly ($P<0.01$) depleted GST by 76 per cent, SOD by 27 per cent, CAT by 82 per cent, GPx by 71 per cent whereas TRx activity was enhanced by 46 per cent. Treatment with the Se compound enhanced the depleted activity of GST significantly ($P<0.01$) by 55 per cent (15 days pretreatment) and 37 per cent (concomitant treatment), SOD by 22 per cent significantly ($P<0.01$) for 15 days pretreatment and by 11 per cent for concomitant treatment. The CAT activity increased significantly ($P<0.01$) by 61 per cent (15 days pretreatment) and 40 per cent (concomitant treatment), GPx by 55 per cent (15 days pretreatment) and 54 per cent (concomitant treatment) significantly ($P<0.01$) in comparison to the MG control group. The TRx activity was decreased significantly ($P<0.01$) by 27 and 20 per cent by pretreatment and concomitant treatments, respectively. Our present findings also suggested that DMSE was more effective to increase the enzyme level in pretreatment group compared to concomitant treatment group.

Protective effect of DMSE against MG induced CA, MN and MI: To determine the protective effect of DMSE against MG induced genotoxicity in mice, CA was measured from bone marrow cells from animals received

MG with or without the Se compound (Fig. 3a). The result showed that MG alone significantly increased the percentage of CA (70%) compared to vehicle control mice ($P<0.01$). The percentage of CA in bone marrow cells was significantly ($P<0.01$) reduced by 20.57 per cent (15 days pretreatment) and 20.41 per cent (concomitant treatment) with the Se compound. The observed result showed significant ($P<0.01$) increase of MN by 75 per cent in the MG treated mice compared to the vehicle control. Treatment with DMSE showed an inhibition of MN formation by 21 per cent (15 days pretreatment) and 12 per cent (concomitant treatment) but the inhibition was statistically significant ($P<0.01$ and $P<0.05$, respectively). MG treated animals showed an enhancement of MI significantly by 68 per cent ($P<0.01$) compared to the vehicle control animals. The DMSE treated mice also showed a decreased level of MI compared to the MG treated control (Fig. 3c). MI was decreased significantly ($P<0.01$) by 67.24 and 20.68 per cent in the pretreated and concomitant treated animals, respectively when compared with the MG treated animals.

Protection of MG induced DNA damage by DMSE; comet assay findings: The comet assay was performed to ascertain the protective efficacy of the Se-compound against MG induced DNA damage. Lymphocytes were isolated from the blood of mice from experimental animals. The microscopical images of comets are with large round head and no tail in vehicle control (Fig. 4a) compared to MG treated mice. The maximum damaged cell with distinct comet tail was observed in MG treated mice (Fig. 4b). Pretreatment with compounds lowered the lymphocyte damage in compound specific manner. The large head with almost no prominent tail was observed in lymphocytes resulting from pretreatment with DMSE compound (Fig. 4c) than concomitant treatment (Fig. 4d). The findings of DNA damage in the head, tail and Olive tail moment (OTM) are presented in the Table. Consistent with this observation, DMSE pretreatment significantly protected MG induced DNA damage by means of lowering the comet tail formation than concomitant treatment group.

Discussion

The AST and ALT are known important biomarkers for assessment of hepatotoxicity[18]. These enzymes exist in liver in large quantities, and increase in serum by leakage from liver cytosol into blood-stream due to cellular damage of liver and these enzymes are useful quantitative markers to assess the extent and type of hepatocellular damage[24]. In our experiment, the activity of AST and ALT increased in MG treated mice indicating acute liver injury. However, treatment with DMSE restored the activities of AST and ALT towards normal. Pretreatment with DMSE exhibited better preventive activity against hepatocellular damage than concomitant treatment group.

LPO is used as a biomarker to show the index of oxidative stress, and cell membrane damage resulting in gradual loss of membrane fluidity, decreased membrane potential and increased permeability to ions[25]. The present investigation revealed that there was a significant increase in LPO in the liver of the MG treated mice indicating oxidative stress. Significant reduction of LPO level was observed in 15 days pretreatment group with DMSE. The LPO levels indicated the protective potential of DMSE against MG induced toxicity through modulation of oxidative stress. The results also indicated that pretreatment with DMSE had more effect compared to the concomitant treatment.

GSH depletion decreases the GSH/GSSG ratio leading the production of free radicals[26] and facilitating LPO. Previous studies on the mechanism of action of MG have shown that GSH plays a key role in eliminating MG from the animal body[27]. In particular, liver necrosis commences when the GSH stores are substantially depleted and this depletion of GSH is a crucial determinant for cell survival or death in oxidative stress conditions[28]. Therefore, it appears that GSH conjugation is critical to reduce the toxic effect of MG. Our results showed that DMSE was able to significantly inhibit the MG -induced depletion of hepatic GSH indicating potential improvement of the host defense system.

GST is a phase II enzyme that plays an important role in detoxifying /transport of many DNA alkylating agents, carcinogens and environmentally hazardous chemicals[29]. In our experiment lowering of hepatic GST activity

was seen in MG treated animals indicating susceptibility of hepatocytes to attack by the toxic electrophilic compound generated from MG metabolism. The overproduction of free radicals as indicated by increased level of LPO by MG may be associated with the depletion of GST activity. Reduction of GST markedly promotes formation of free radicals by MG. In our experiment, pretreatment with the DMSE enhanced the GST activity in a significant manner than concomitant treatment.

SOD and CAT are the two other antioxidant enzymes that play an important role in eliminating free radicals in hepatic tissues[30]. There was a significant reduction in SOD and CAT activities in MG treated mice. However, activities of these enzymes were elevated significantly by treatment with DMSE compared to the MG control group, suggesting a protective effect exerted by DMSE against MG induced oxidative stress. Pretreatment with DMSE was found to be a more potent inducer of SOD and CAT than concomitant treatment with DMSE in reducing oxidative stress.

The selenium containing enzyme GPx forms the first line of antioxidant defense of the host by scavenging ROS[31]. The increased level of ROS is correlated with the decline in GPx activity, a crucial enzyme that plays an important role in protection against hydrogen and lipid peroxides[32]. In the present study, the GPx activity was decreased significantly in MG treated mice. However, a significant increase of GPx activity was observed in animals treated with the DMSE as compared to MG treated group where the effect was more prominent with pretreatment with DMSE than the concomitant treatment.

TrxR is another major enzyme that protects cell against oxidative damage. The thioredoxin system, composed of TrxR, thioredoxin (Tr), and NADPH regulates a multiple range of activities for cellular redox control, antioxidant function, cell viability and proliferation. Recently, mammalian TrxR has become a new target for anticancer drug development as cancer cells have been shown to contain higher levels of TrxR[33]. In the present study, enhanced TrxR activity was observed in MG treated mice compared to vehicle control which was reduced towards normal value when treated with DMSE. Generally redox state of the hepatocytes affects the cascade of events related to the onset of carcinogenesis. TrxR plays a central role in maintaining the redox homeostasis. The higher level of TrxR will reduce the cell's ability to defend against Reactive oxygen species/Reactive nitrogen species (ROS/RNS)[34].

The present study revealed that MG treated mice exhibited genotoxic effects as evidenced from the increased numbers of chromosomal aberrations (CA). Pretreatment with DMSE showed better effect than concomitant treatment with DMSE. There was an elevated frequency of micronuclei (MN) and an increase in the MI of the bone marrow cells in MG treated mice in comparison to the vehicle control animals. All micronuclei originate from either broken or intact chromosomes that fail to incorporate into daughter nuclei following mitosis, and thus is an indirect measurement of the induction of structural chromosome aberrations. There was a decrease in MN and MI in DMSE pretreated groups. Occurrence of increased MN in the MG treated mice could be due to their effect on the chromosomes in different stages of cell cycle or due to their clastogenic effect.

In the present study, significant DNA damage was observed in MG treated animals exhibiting acute hepatotoxicity as compared to the vehicle control. However, the DNA damage was reduced significantly when the animals were treated with DMSE as compared with the MG treated mice.

In conclusion, our results showed that DMSE exerted its chemoprotective effect against MG induced hepatotoxicity and DNA damage in part by modulating the antioxidant and detoxifying systems. The greater efficacy shown by pretreatment with DMSE might be the result of the compound providing some added protection to the target cells before exposure to MG. Therefore, pretreatment with DMSE may be considered for better chemopreventive effects than concomitant treatment with DMSE + MG. However, further studies are required to elucidate the underlying molecular mechanism exerted by DMSE against MG induced cellular damage due to environmental exposure.

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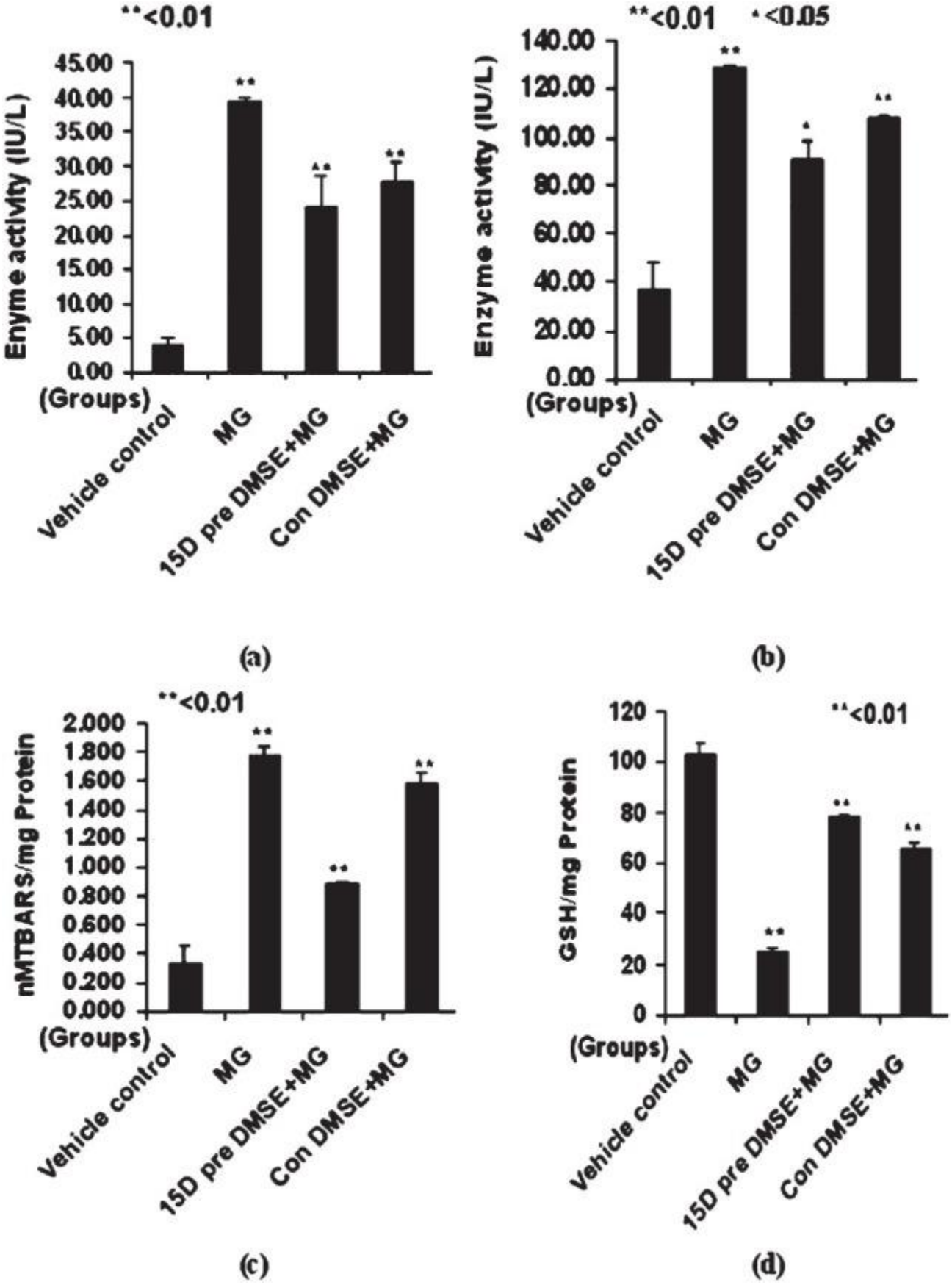
Figures and Tables

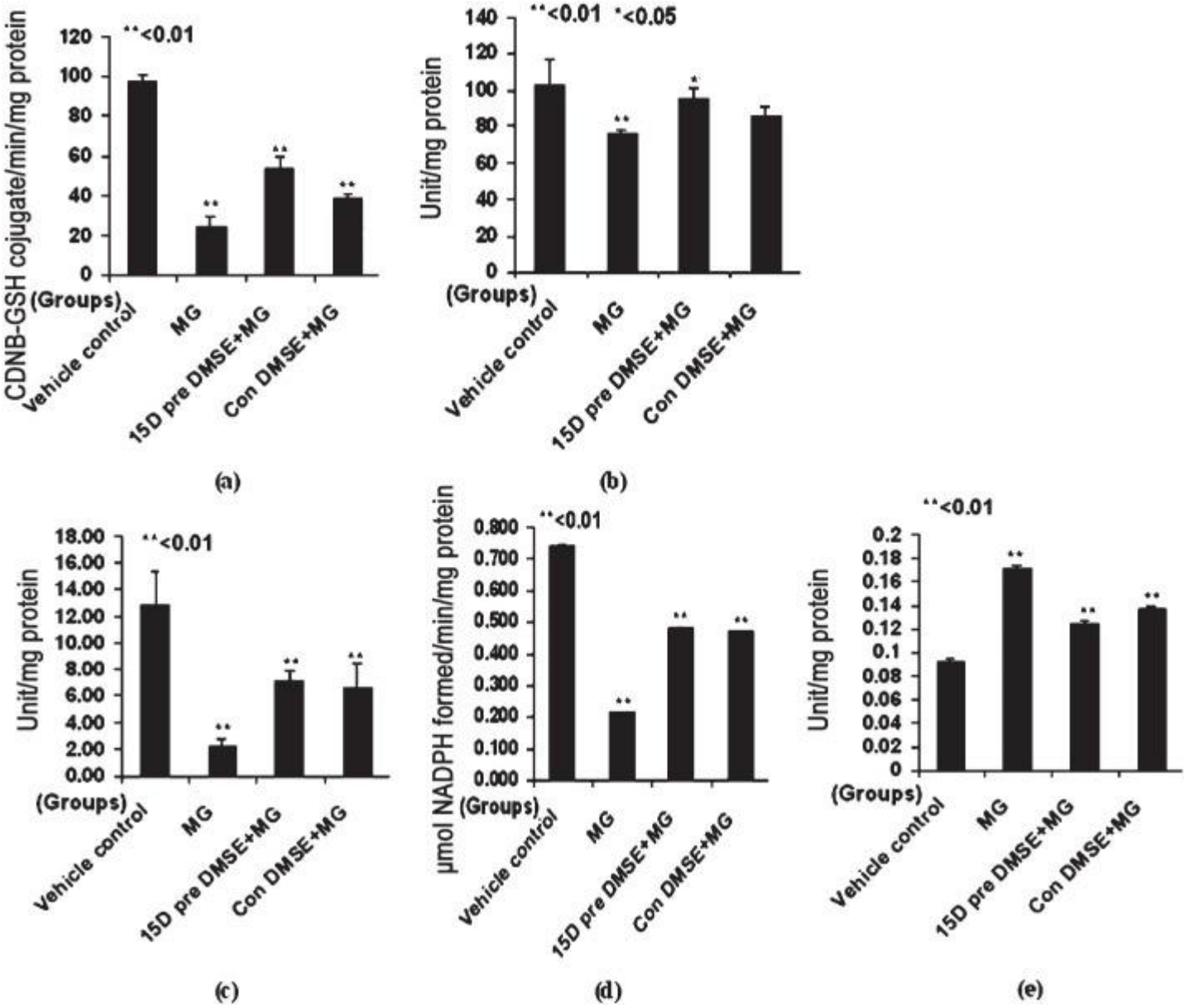
Fig. 1



Effects of DMSE on **(a)** ALT activity **(b)**, AST activity **(c)**, LPO level, and **(d)** GSH level in liver of *Mus musculus*. Data were analyzed by ANOVA followed by Tukey's HSD test for multiple comparisons. Data are expressed as mean ± SD (n=6). (ALT & AST n=6, LPO & GSH n=6). Comparisons were made between vehicle

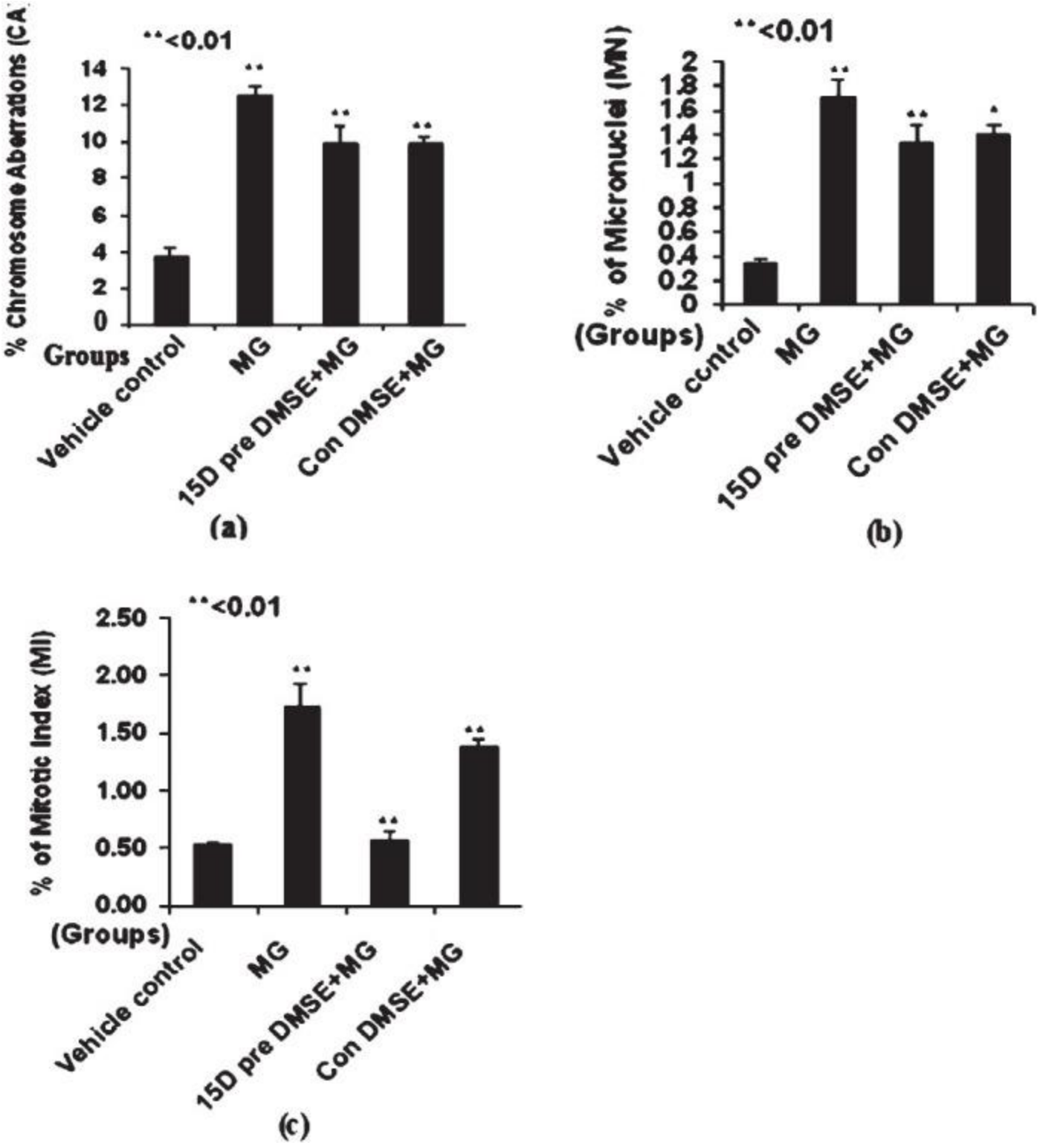
control group vs MG control group and MG control group vs pre DMSE + MG and con DMSE+ MG treated group (*P* *<0.05, **<0.01).

Fig. 2



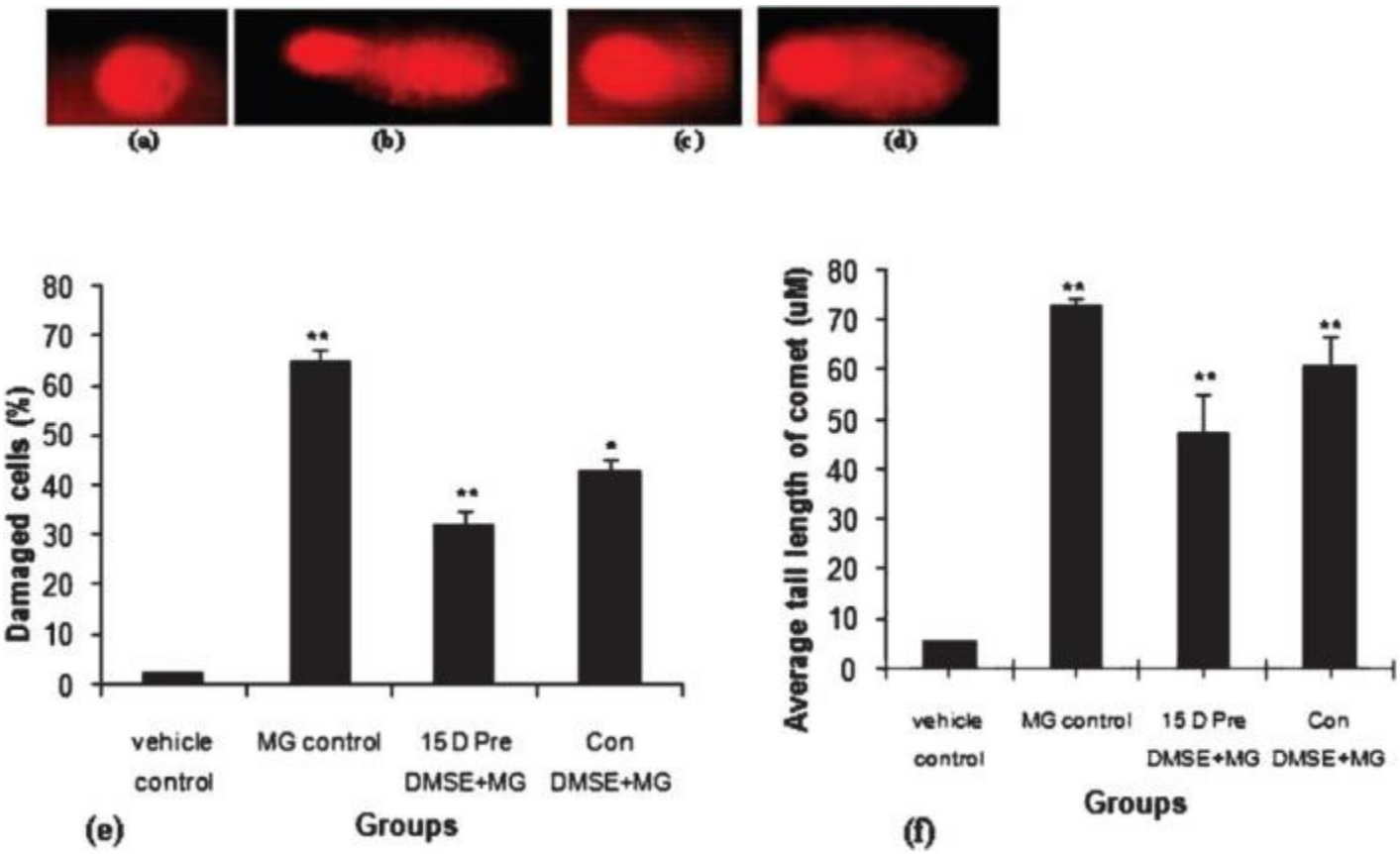
Effects of DMSE on **(a)** GST activity, **(b)** SOD activity, **(c)** CAT activity, **(d)** GPx activity and **(e)** TrxR activity in liver of *Mus musculus*. Data were analyzed by ANOVA followed by Tukey's HSD test for multiple comparisons. Data are expressed as mean ± SD (n=6). Comparisons were made between vehicle control group vs MG control group and MG control group vs pre DMSE + MG and con DMSE+ MG treated group (*P* *<0.05, **<0.01).

Fig. 3



Effects of DMSE on percentage (%) of **(a)** CA, **(b)** MN, and **(c)** MI in *Mus musculus*. Data were analyzed by ANOVA followed by Tukey's HSD test for multiple comparisons. Data are expressed as mean ± SD (n=6). Comparisons were made between vehicle control group vs MG control group and MG control group vs pre DMSE + MG and con DMSE+ MG treated group (*P* *<0.05, **<0.01).

Fig. 4



Comet assay from lymphocytes **(a)** no significant DNA damage in vehicle control group, **(b)** significant DNA damage in MG control group. **(c)** slight DNA damage in pre DMSE + MG, **(d)** concomitant DMSE+ MG, **(e)** percentage (%) of DNA damage, and **(f)** the average tail lengths of comet. Data were analyzed by ANOVA followed by Tukey's test for multiple comparisons. Data are expressed as mean ± SD (n=6). Comparisons are made between vehicle control group with MG control group and MG control group with pre DMSE + MG and con DMSE+ MG treated group (*P* *<0.05, **<0.01).

Table

Group	Head DNA (%)	Tail DNA (%)	OTM
Vehicle control	82.35 ± 2.06	2.80 ± 1.00	2.21 ± 0.083
MG control	35.86 ± 5.20**	69.53 ± 3.40**	23.63 ± 2.73**
15 day pre DMSE + MG	83.90 ± 1.60**	9.84 ± 2.77**	14.99 ± 2.30**
Con DMSE + MG	60.78 ± 6.00**	29.81 ± 4.01	19.16 ± 2.89*

OTM, Olive tail moment; data were analyzed by ANOVA followed by Tukey HSD test for multiple comparisons. Data are expressed as mean ± SD (n=6)
Comparisons were made between vehicle control group vs MG control group and MG control group vs pre DMSE + MG and con DMSE + MG treated group (*P**<0.05, **<0.01)

Index of the DNA damage based on Comet assay