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Protective effect of green tea extracts against dimethoate induced DNA damage and oxidant/antioxidant status in male rats

Objective: The present study was conducted to investigate the protective effect of crude green tea extract (GTE) against hepatic oxidative stress and brain DNA damage induced by dimethoate (DM) in male rats. Methods: Four groups containing six male Wister rats each were selected. Group I served as the control group. Group II rats were permitted free access to solubilised crude extract of green tea (1.5%w/v in water) as the sole drinking fluid. Group III rats were given a single daily oral dose of DM (38.7 mg/kg b. wt., 1/10 LD50) for 28 consecutive days. Group IV rats received oral dose of pesticide and green tea extract simultaneously. All rats were sacrificed after 28 days. **Results:** DM induced statistical reduction in body weight gain, while induced statistical increase in absolute and relative liver weights. Compared to control, oral administration of DM significantly caused increases in hepatic lipid peroxidation (LPO) and activities of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione-s-transferease (GST). In contrast, levels of antioxidant GSH, glutathione-dependent enzymes like glutathione peroxidase (GPx) and serum cholinesterase (ChE) activity were significantly lower than those of the control group. However, supplementation of GTE attenuates the activities of GPx and GSH content, while restores body weight gain, relative liver weights, LPO, ChE, CAT, SOD and GST. Moreover, the severity of brain DNA damage monitored by damage index (DI) and damage frequency % (DF) induced by DM was mitigated following GTE supplementation. **Conclusion**: The use of green tea extract appeared to be beneficial to rats, to a great extent in attenuating and restoring the damage sustained by DM exposure.

Keywords:DNA damage, Dimethoate, Green tea, Lipid peroxidation, Oxidative stress.

INTRODUCTION

For several years, the extensive use of different pesticides in agriculture and public health purposes has led to drastic effects in many of non-target species including man {1}. Organophosphate pesticides (OPs) are primarily recognized for their ability to induce toxicity in mammals through the inhibition of acethylcholinesterase (AChE) leading to the accumulation of acetylcholine and subsequent activation of cholinergic muscarnic and nicotinic receptors {2}. Also, it has been demonstrated that lipid peroxidation mediated by free radicals is one of the molecular mechanisms involved in OP-induced toxicity {3, 4} and several pesticides exert their biological effects through electrophilic attack on the cellular constituents of hepatic and brain tissues {5} with simultaneous generation of reactive oxygen species {6}. In this regard, previous studies have proven that oxidative stress is induced by OPs in rats ({3, 4, 7, 8, 9} and humans {10}. Dimethoate, DM, (O, O-dimethyl S-N-methyl carbomyl methyl phosphorodithioate) is widely used against a broad range of insects and mites and is also used for indoor control of houseflies. The extensive use of dimethoate poses a health hazard to animals and humans because of its persistence in soil and crops {1}. Previous studies have shown that acute and sub-chronic exposure to dimethoate alters the antioxidant status and the histology of liver and induce hepatic lipid peroxidation in mice {11} and rats {12, 13, 14}. Sharma et al. concluded that hepatic oxidative stress in DM-treated rats may be ascribed to inhibition of AChE and disturbance in activities of GSH and GST enzymes causing lipid peroxidation {15}. Antioxidants as vitamins, can prevent the uncontrolled formation of free radicals or inhibit their reaction with biological sites, also the destruction of most free radicals rely on the oxidation of endogenous antioxidants mainly by scavenging and reducing molecules {16}. Recently, interest has increased in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage {17}, because these natural antioxidants avoid undesired health problems that may arise from the use of synthetic antioxidants, which may have toxic effects {18}. Furthermore, natural antioxidant from plants (e.g. green tea) is generally recognized as safe in the form of extract. Green tea contains six primary catechin compounds: catechin, gallaocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate {19}. Green tea and its

major constituent polyphenols are best known for their various biological and pharmacological properties including anti-oxidative {20}. A number of polyphenolic compounds extracted from green tea leaves have been found to be good antioxidants against lipid peroxidation in phospholipid bilayers {21, 22} and against DNA damage {23}. Despite dimethoate's extensive use in crop protection and in the household, information on its health effects is still scarce. Therefore, the objective of this study is to investigate the protective effects of green tea extract against hepatic oxidative stress and brain DNA damage induced by DM in male rats.

MATERIAL AND METHODS

2.1. Animals

Healthy male Wister rats weighing 150 ±10 g were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal room (23 ± 2 °C). On standard pellet diet, tap water ad libitum, and daily dark/light cycle (12/12 hrs.) the rats were acclimatized for 1 week prior to the start of experiments. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, National Research Centre, Cairo, Egypt, and international guidelines for care and use of laboratory animals.

2.2. Chemicals

Dimethoate (Perfekthion® 40% EC, BASF, Germany) and Pu-erh green tea of post-fermented tea produced in Yunnan province, China. It was a gift from The 4th International Academic conference on Environmental & occupational medicine, 16-19/10/2006, Kunming, China. Kits used for biochemical measurements were purchased from Biodiagnostic, Giza, Egypt. The assay kits used for biochemical measurements of cholinesterase (ChE), glutathione (GSH), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione-s-transferase (GST) and glutathione peroxidase (GPx) were all purchased from Biodiagnostic Company, 29 Tahreer St., Dokki, Giza, Egypt. All other chemicals were of reagent grades and were obtained from the local scientific distributors in Egypt.

2.3. Preparation of green tea extract

Likewise, the crude aqueous extract of green tea was prepared according to Maity et al. {24} and later adopted by El-Beshbishy {25} by soaking 15 g of instant green tea leaves in 1 L of distilled water with the temperature of 90 ºC or less, for 5 min to obtain soluble polyphenols dissolved in the aqueous extract. The solution was filtered to obtain the final 1.5% (w/v) green tea extract. This solution was substituted in the place of water as the sole source of drinking fluid.

2.4. Experimental design

The rats were randomly divided into 4 groups with 6 animals for each group. The route of administration selected for the study was oral gavage for 28 consecutive days. Rats in Group I served as control and were given distillate water ad libitum. Rats in Group II were given aqueous green tea extract as the sole drinking fluid during the 28 days at a concentration of 1.5% (w/v). Rats in Group III were given DM in water (0.5 ml/rat) alone at a dose of 38.7 mg a.i. /kg b. wt. daily for 28 days (1/10 LD_{50}, Tomlin {26}). Rats in Group IV were simultaneously given green tea extract as the sole source of drinking fluid and exposed daily to the pesticide dose.

2.5. Data recording and sample collection

After the completion of the treatment period, body weights were recorded. Blood samples were withdrawn from the animals under light ether anesthesia by puncturing the retro-orbital venous plexus of the animals with a fine sterilized glass capillary tube. The collected blood samples were left for 20 minutes to coagulate at room temperature, and then centrifuged at 600 x g for 10 min to separate the sera. The sera were kept in a deep freezer (-20˚C) until analyzed.

The rats were sacrificed by cervical dislocation. Immediately, brain tissues were cryopreservered at -80ºC in RPMI 1640 media (w/o L-Glutamine, w/ 25 mM Hepes) (biowest) Containing 10% DMSO and 1% Fetal Calf Serum, until processing for comet assay. Liver tissues were isolated, cleaned from of adhering matters, washed with ice-cold saline solution, weighed and stored at −70 ◦C for the biochemical studies.

2.6. Biochemical measurements

The sera and liver homogenates obtained from different treatments were subjected to certain biochemical analyses by using Shimadzu UV- VIS Recording 2401 PC (Japan).

2.6.1. Lipid peroxidation (LPO) and glutathione (GSH)

Liver tissues were separately homogenized in ice-cold 50 mM phosphate buffer (pH 7.5, 1 mM EDTA) for 2 min to yield a 10% (w/v) homogenates, using a Teflon-fitted Potter Elvehjem homogenizer. The homogenates were centrifuged at 800 x g at 4 ˚C for 15 min. The supernatant was used for the quantification of LPO and GSH according to the method of Ohkawa et al. {27} and Owens and Belcher {28}, respectively. LPO was determined based on the formation of thiobabituric acid reactive substances (TBARS) at 532 nm and

expressed as the extent of malondialdehyde (MDA) production. On the other hand, GSH is based on the reaction of DTNB (50, 5- dithiobis-(2-nitrobenzoic acid)) with GSH and yields a yellow colored chromophore; 5-thio-nitrobenzoic acid with a maximum absorbance at 412 nm.

2.6.2. Antioxidant enzymes

Another part of liver tissues were separately homogenized in ice-cold 50 mM phosphate buffer (pH 7.4, 1 mM EDTA and 1 mL/L Triton X-100) for 2 min to yield a 10% (w/v) homogenates. The homogenates were centrifuged at 800 x g at 4˚C for 15 min. The supernatant was used for assaying catalase (CAT) activity by the method of Aebi {29}. The activity of CAT was expressed as nmoles min^{-1} mg^{-1} protein and the change in absorbance was measured at 510 nm.

Parts of liver tissues were separately homogenized in ice-cold 0.25 M sucrose for 2 min to yield a 10% (w/v) homogenates. The homogenates were centrifuged at 800 x g at 4˚C for 15 min. The supernatant was used for assaying superoxide dismutase (SOD) activity by the method of Nishikimi et al. {30}. The activity of SOD was expressed as units of SOD min^{-1} mg^{-1} protein and the change in absorbance was measured at 560 nm.

Glutathione-S-transferase (GST) activity was assayed by the method of Habig et al. {31} in 0.1 M phosphate buffer (pH 7.0, 2 mM EDTA). The activity was expressed as µmol of 1-chloro-2, 4-dinitrobenzene (CDNB)-GSH conjugate min^{-1} mg^{-1} protein and the change in absorbance at 340 nm was monitored in a UV-visible spectrophotometer.

2.6.3. Total protein and Cholinesterase (CHE)

The protein concentrations were measured as described by Lowry et al. {32} in liver homogenate. According to the method described by Ellman et al., cholinesterase (ChE; EC 3.1.1.8) activity was determined in the sera of rats {33}.

2.7. Comet assay

The alkaline comet assay (or single cell gel electrophoresis—SCGE) was carried out as described by Singh et al. {34} and Tice et al. {35}. Pre-aliquot cyropreserved brain cells were thawed in water bath at 37°C, wash with phosphate-buffered saline (PBS) containing 20 mM EDTA (Ca^{++} and Mg^{++} free), then weigh. Each piece of 0.2 g brain tissue was placed in 1 ml of cold PBS phosphate-buffered saline containing 20 mM EDTA (Ca^{++} and Mg^{++} free) and minced into fine pieces in order to obtain a cellular suspension. These cells from brain (10 µl) were embedded in 95 µl of 0.75% low melting agarose (Gibco–BRL). The mixture (cell/agarose) was added to a fully frosted microscope slide coated with a layer of 300 µl of normal melting agarose (1%) (Gibco–BRL). After solidification, the slides were placed in lysis buffer (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0, with freshly added 1% Triton X-100 (Sigma) and 10% DMSO for one h at 4˚C. Subsequently, the slides were incubated in freshly made alkaline buffer (300 mM NaOH and 1 mM EDTA, pH>13) for 20 min, at 4˚C to allow DNA to unwind. An electric current of 300 mA and 25 V (0.90 V/cm) was applied for 15 min to electrophoreses the DNA. After electrophoresis, the slides were placed in a staining tray and covered with neutralizing solution (0.4M Tris, pH 7.5) for 5 min and washed three times. The slides were drained and 50 µl of ethidium bromide solution (20 µg/ml) (Sigma) were added. Slides were placed in a humid dark box at 4˚C until analysis, which was done immediately.

The presence of comets was examined in cells using Leica epifluorescent microscope (Green filter), the images for the brain cell nucleoids were digitalized with Leica DFC camera. DFC 280 supplied with Leica DFC Twain software with Host application program Imaging® to view and capture digital images.

Scoring of the slide was done visually according to tail size into five grades (0, 1, 2, 3, 4) ranging from undamaged (Grade 0) to maximum damage (Grade 4) (Figure 1), resulting in a single DNA damage score for each animal, and consequently each studied group. Coded slides were scored blindly and 100 brain cell nucleoids were scored from each animal (50 per slide). Therefore, the composite score (damage index) can range from 0 (completely undamaged, 100 cells x 0) to 400 (with maximum damage, 100 x 4) as described by Collins et al. {36}. The damage frequency (%) was calculated based on a number of cells with tail versus those with no tails.

2.8. Statistical analysis

The results of body weight and biochemical measurement were analyzed by using SPSS (version 11.0) for Windows and expressed as means ± SE. Paired samples t test was used to compare between the data of the control and the treatment data.

RESULTS

3.1. Body and organ weights

No death was observed in any of the experimental groups. At the end of the experimental period, body weight gain and relative liver weights did not significantly differ in GT-treated and DM+GT-treated groups compared to the control group, while statistical ($P < 0.01$) difference changes were observed in DM-treated group (Table I).

3.2. Cholinesterase activity (ChE)

Compared to control value, the activity of ChE was inhibited by 31.9%, in DM-treated group. However, this inhibition was attenuated to 4.8% after the supplementation of GT (Figure 2).

3.3. Lipid peroxidation and glutathione (LPO & GSH)

Compared to the control group, LPO in hepatic tissue was increased significantly (Figure 3), while GSH content was decreased significantly (Figure 4), in DM-treated group. However, the supplementation of GT attenuated the significant changes in both LPO and GSH content.

3.4. Antioxidant enzymes

Table II shows the influence of DM exposure and treatment with GTE on the activities of the enzymes CAT, SOD, GST and GPx. Subacute levels of DM resulted in a state of liver injury and extensive oxidative damage in rats as manifested by the significant increases ($p < 0.01$) in SOD, CAT and GST enzyme levels by 67%, 22% and 27% and 85%, respectively, compared to control rats. While, a significant decline ($p < 0.01$) in the activity of GPx enzyme was observed in DM-treated group. In contrast, the treatment with GTE resulted in amelioration in the activities of enzymes CAT, SOD, GST and GPx. A small but significant decrease of activity, notably in CAT and SOD was observed in rats fed crude GTE alone.

3.5. Comet assay result

Table III shows the effects of DM and crude GTE treatments on damage index (DI) and damage frequency percent (DF), as measured by DNA damage in rat brain tissue, using the comet assay. Compared to the control values for DI (58.0) and DF (38.25), DM induced a significant elevation ($P< 0.01$) in the levels of the tested parameters, regardless the repairing and/or the ameliorating effect of GTE supplementation.

Figure 5 shows the extent of DNA damage in brain of rat treated with DM and GTE, analyzed by using a SCG assay (grades 0-4). In DM-treated group, the percentage of maximum damage cells (grade 4) was 23.5% compared to the untreated group (3.75%). However, supplementations of GTE changed the percent of maximum damage cells to 18%. Also, the undamaged cells (grade 0) was 72.5 % in control group, which in turn changed to 40% in DM-treated group, however this percentage was modulated to 52.5% in DM+GTE-treated group.

DISCUSSION

Dimethoate is one of the most important Organophosphate insecticides (OPI) and it is frequently used in agriculture against a wide range of insects and mites. OPI are primarily recognized for their ability to induce toxicity in mammals through inhibition of AChE and/or induction of oxidative stress through the overproduction of ROS {3, 4, 7, 8, 9, 10}.

The present study investigated the protective effects of green tea extract against hepatic oxidative stress and brain DNA damage in DM-treated rats. Aqueous green tea extract (GTE) prepared from the leaves of Camellia sinensis is particularly rich in flavonoids, which are strong antioxidants {37}. In general, GTE seems to be effective against hepatic oxidative stress {25, 38, 39}.

The strong health-promoting effect of green tea can be attributed to the antioxidant activity of (-)-epigallocate epigallocatechin- 3-gallate (EGCG), its main polyphenolic catechin constituent {40}. EGCG, is a member of a group of constituents collectively referred to as green tea epicatechins {41}. The present study was not designed to determine the role of individual catechin constituents present in the green tea extract. Rather, it was designed as a pilot study to determine whether the crude green tea extract has any protective role in general, in DM-intoxicated rats.

In the present study, oral administration of DM resulted in a significant reduction in the body weight gain, and an increase in the relative liver weight. Reduction in body weight gain in experimental animals due to OPI intoxication is a commonly reported phenomenon {4, 12}. This occurs probably due to the decreased food intake in these animals {42, 43}. The increase in relative liver weight is consistent with previous studies {44}. This increase could be attributed to the relationship between liver weight increase and various toxicological effects or to the reduction in body weight gain of experimental animals {45}.

The principal objective of the present study was to assess the oxidative damage sustained by the liver following subacute exposure to DM. Liver plays a central role in detoxification and is chronically exposed to xenobiotics and their toxic derivatives. It has been previously reported that oxidative stress has been implicated to be an important component of the mechanism of toxicity of several OPI {46, 47}.

A significant decrease in the GSH level and a concomitant increase in lipid peroxidation (LPO) level following administration of the DM dose were observed in the present study. The decrease in GSH level leads to a net suppression in the total antioxidant capacity since it plays a key role as a substrate for the enzyme glutathione S-transferase (GST) and as a cofactor for a variety of enzymes including glutathione peroxidase (GPx). Also, GSH depletion has been shown to intensify lipid peroxidation and predispose cells to further oxidant damage {48}. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and its increase is an important indicator of LPO {49}. In fact, DM is a lipophilic substance and therefore it can interact with cellular liver membrane. Similar

increases in MDA levels in different tissues have been reported in rats after OPI administration {46, 47}.

Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. These radicals are removed either non-enzymatically or by antioxidant enzymes such as SOD, CAT, GST and GPx. In our model, DM induced a significant increase in the activities of the antioxidant enzymes CAT, SOD and GST. Also, a significant decrease in activity of GPx was noted in the present study.

SOD and CAT are the most important defense mechanisms against toxic effects of oxygen metabolism. SOD accelerates the dismutation of H2O2, also termed as a primary defense, as it prevents further generation of free radicals. CAT helps in the removal of H2O2 formed during the reaction catalyzed by SOD {50}. CAT is present in all major organs in the body of animals and human beings and is especially concentrated in the liver. The elevated activity of CAT in DM-treated rats may be due to an adaptive response to the generated free radicals {51}. SOD is a ubiquitous chain breaking antioxidant and is found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and plays a protective role against ROS-induced oxidative damage. The increased activity of SOD in DM treated rats probably indicates an activation of the compensatory mechanism through the effects of DM on progenitor cells {42}.

In our study, activities of the GSH dependent phase II detoxifying enzymes in liver, GST and GPx were altered significantly in DM-treated rats. GST are detoxifying enzymes that catalyze the conjugation of variety of electrophilic substrate to the thiol group of GSH, producing less toxic forms {52}.

The increase in GST activity in the liver tissue among DM treated rats indicates the role played by this system in the detoxification of DM. Our results are consistent with previous researchers {11, 12, 15, 53, 54}.

Apart of lipid peroxidation and glutathione content, another major question addressed was whether DM induced brain DNA damage is mediated through oxidative stress. In the present study, DM treatment induced brain DNA damage measured by comet assay {36}. The types of DNA damage produced by radiation and/or chemicals are many and varied, including single- and double-strand breaks. The single cell gel electrophoreses test (SCG), or comet assay, adopted in our study is a rather new test with a widespread potential applications in genotoxicity testing and biomonitoring {34, 52}. The increase of DNA damage in our result is consistent with previous investigators, Yu et al. {54}, who reported similar result with chlorpyrifos. It is well documented that xenobiotic chemicals, including OPI, induce oxidative stress {46, 47}, which in turn, participate for more extent to the DNA damage in our study. ROS left unbalanced by antioxidants (enzymatic) can result in damage to cellular macromolecules. In DNA, ROS can produce single-double strand DNA breaks, purine, pyrimidine, or deoxyribose modifications and DNA crosslink {55, 56}.

Green tea contains polyphenols that have been shown to selectively induce phase I and phase II metabolic enzymes which increase the formation and excretion of detoxified metabolites resulting from xenobiotic metabolism {57}. It appears that supplementation with 1.5% crude green tea extract in rats exposed to DM leads to a partial reversal of oxidative damage.

This is shown by a marked, but not complete recovery in terms of oxidative stress parameters e.g., a significant reduction in level of lipid peroxidation by 36.3% in group IV rats when compared to group III, highlighting their protective role against pesticide-induced toxicity. Similarly, green tea supplementation resulted in a small but significant amelioration of 24% in the activity of enzyme GPx. However, green tea supplementation resulted in complete recovery in the activities of enzymes CAT, SOD and GST. In this respect, a partial reversal of DNA damage is shown by a marked, but not complete recovery in terms of DNA damage parameters e.g., a significant reduction in level of damage index (DI) and damage frequency % (DF) in group IV rats when compared to group III, highlighting their protective role against pesticide-induced toxicity.

CONCLUSION

In summary, our results demonstrate that subacute exposure to DM leads to a significant oxidative damage as shown by increase in lipid peroxidation and decrease in GSH content. However, the activity of key antioxidant enzymes like CAT, SOD, GST and GPx was significantly altered. In addition, the brain DNA damage is demonstrated as shown by the increase in DI and DF parameters. The administration of crude aqueous green tea extract has a protective role against the oxidative stress in rat liver and brain DNA damage following the pesticide toxicity. Further molecular studies should unravel the detailed role played by the individual components present in the green tea extract.

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Table I. Body weight, liver weight, and relative liver weight of control and experimental rats.

Groups	*Body weight*			*Absolute liver weight (g)*	*Relative liver weight (g/100g body weight)*
	Initial (g)	*Final (g)*	*% Change*		
Group I (Control)	***149.1±6.37***	***195.5±12.81***	***34.1±2.23***	***7.22±0.32***	***3.70±0.11***
Group II (GT)	***153.3±3.51***	***203.2±5.29***	***33.4±1.97***	***7.45±0.20***	***3.67±0.09***
Group III (DM)	***155.6±2.21***	***191.5±4.56***	***23.0±1.38*****	***8.88±0.31*****	***4.64±0.07*****
Group IV (DM+GT)	***159.1±5.25***	***205.1±2.43***	***29.9±2.88***	***7.80±0.26*****	***3.89±0.14***

*Each value is a mean of 6 rats ± SD; Statistical difference from the control: *significant at P< 0.05 & **highly significant at P< 0.01; % of body weight change = [(final b .w t. – initial b .wt.)/ initial b .wt.] X 100. DM: dimethoate; GT: green tea.*

Table II. Effect of green tea on hepatic antioxidant enzymes of control and experimental rats.

Groups	*CAT (nmoles/min/mg protein)*	*SOD (u /mg Protein)*	*GST (µmoles/min/mg protein)*	*GPx (nmoles/min/mg protein)*
Group I (Control)	***337.3±8.9***	***10.61±0.33***	***0.021±0.002***	***23.73±2.02***
Group II (GT)	***315.0±4.3*****	***8.99±0.65****	***0.018±0.002***	***21.33±3.39***
Group III (DM)	***413.3±18.9*****	***17.76±1.23*****	***0.039±0.002*****	***12.59±2.17*****
Group IV (DM+GT)	***334.0±9.9***	***10.94±1.07***	***0.023±0.002***	***18.29±1.31****

*Each value is a mean of 6 rats ± SD; Statistical difference from the control: * significant at P< 0.05& ** highly significant at P< 0.01; DM: dimethoate; GT: green tea. CAT: Catalase; SOD: Superoxide dismutase; GST: Gutathione-S-transferase; GPx: Glutathione peroxidase.*

Table III. Effect of green tea on brain DNA damage of control and experimental rats.

Groups	*Damage index (DI)*	*Damage frequency % (DF)*
Group I (Control)	***58.00±12.36***	***38.25±7.80***
Group II (GT)	***53.25±11.33***	***32.78±7.56***
Group III (DM)	***173.00±18.42*****	***153.93±35.41*****
Group IV (DM+GT)	***132.00±14.31*****	***92.33±21.56****

Each value is a mean of 4 rats ± SD; Statistical difference from the control: * significant at P< 0.05& ** highly significant at P< 0.01; DM: dimethoate; GT: green tea. Damage index (DI) = cells number of each class X number of classes (O-4), maximum value = 400 arbitrary unit); Damage frequency % (DF) = (number of cells with tail/ number of cells without tail) X 100.

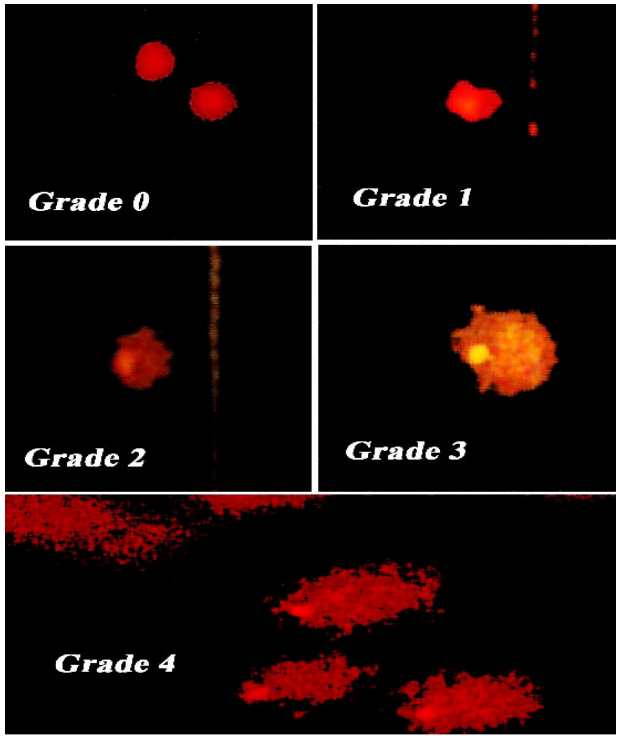

Figure 1. Representative images of the comet assay grades according to tail size, from no tail (Grade 0), to maximally long tail (Grade 4)

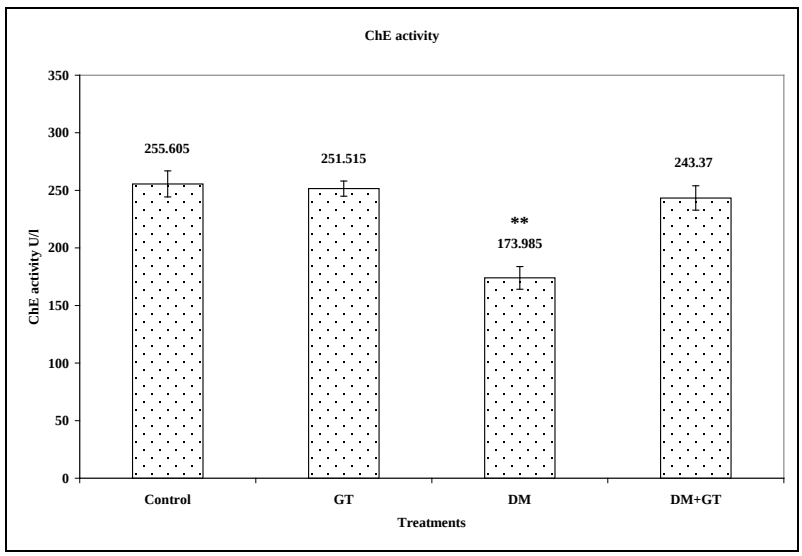

Figure 2. The effect of repetitive doses for 28 consecutive days, of DM and GT on ChE activity in sera of rat. Values are expressed as mean ± S.D. of 6 rats; significantly different from control (* P < 0.05, **P< 0.01); **ChE**: cholinesterase; **DM**: dimethoate; **GT**: green tea.

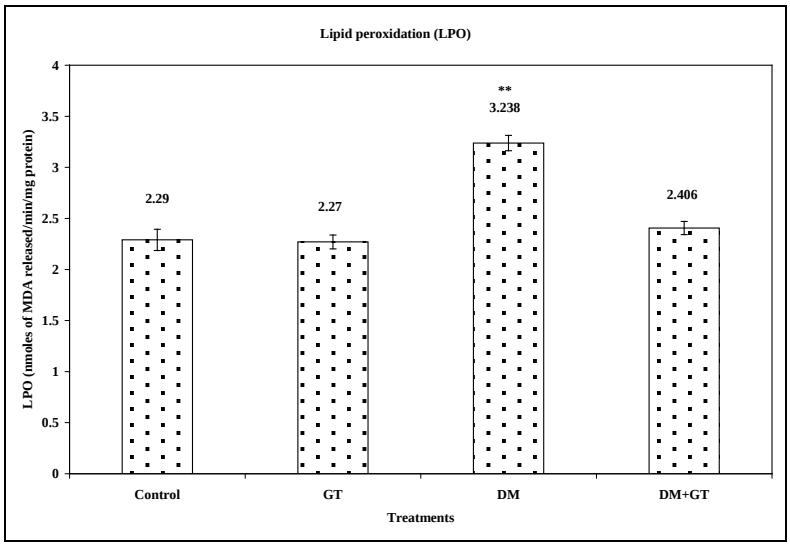

Figure 3. The effect of repetitive doses for 28 consecutive days, of DM and GT on LPO in rat liver. Values are expressed as mean ± S.D. of 6 rats; significantly different from control (* P < 0.05, **P< 0.01); **DM**: dimethoate; **LPO**: lipid peroxidation; **GT**: green tea.

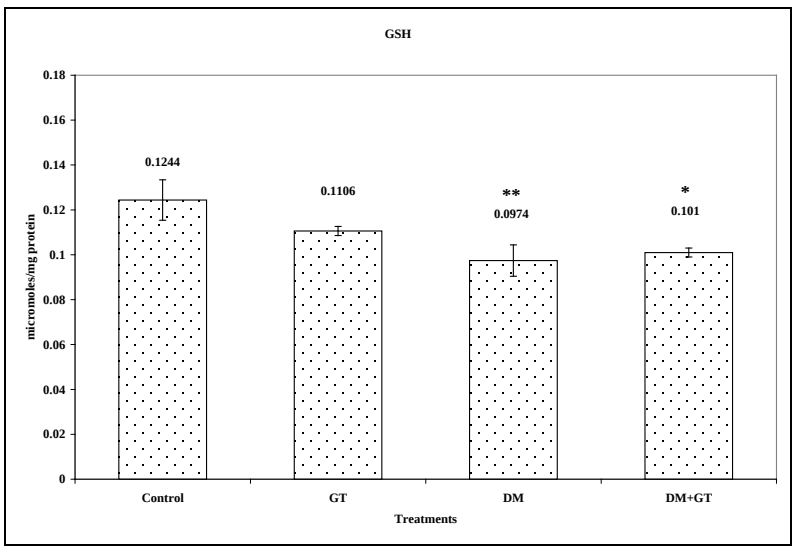

Figure 4. The effect of repetitive doses for 28 consecutive days, of DM and GT on GSH content in rat liver. Values are expressed as mean ± S.D. of 6 rats; significantly different from control (* P < 0.05, **P< 0.01); **DM**: dimethoate; **GSH**: glutathione; **GT**: green tea.

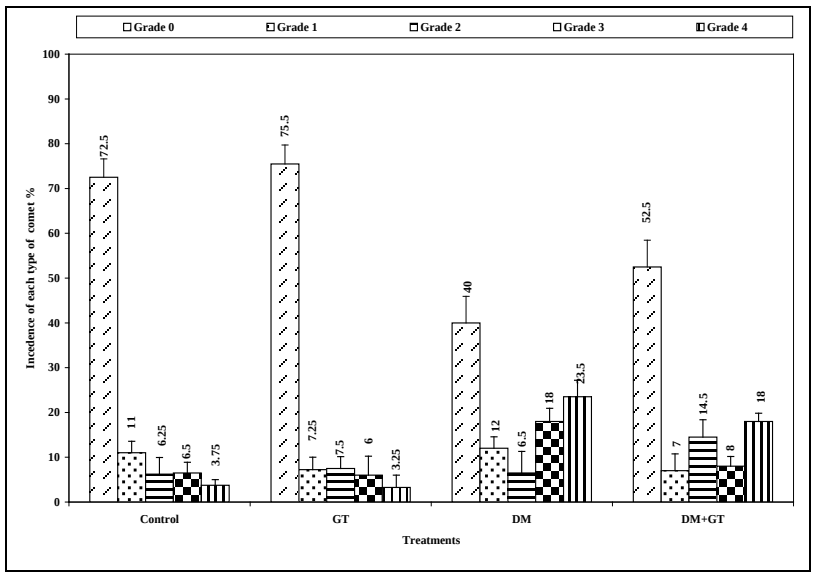

Figure 6. The distribution of damage grades (0—undamaged to 4—maximum damage) in brain of rats treated with DM and GT for 28 consecutive days. Values are expressed as mean ± S.D. of 4 rats; **DM**: dimethoate; **GT**: Green Tea.