

Full Length Research Paper

# Protective effects of *Digera muricata* (L.) Mart. against carbon tetrachloride induced oxidative stress in thyroid of rat

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Protective effects of *Digera muricata* (DM) methanol extract (DME) were evaluated against the oxidative stress of carbon tetrachloride (CCl<sub>4</sub>) in the thyroid of male Sprague-Dawley rats. Treatment of CCl<sub>4</sub> 1 ml/kg bw (25% CCl<sub>4</sub>/olive oil i.p) once a week for 16 weeks significantly decreased the activities of catalase, peroxidase, superoxide dismutase, glutathione-S-transferase and glutathione peroxidase in the thyroid. Level of reduced glutathione was decreased, while lipid peroxidation (TBARS) was significantly increased with CCl<sub>4</sub> in the thyroid of rat. CCl<sub>4</sub> significantly enhanced the level of thyroidal DNA injuries and AgNORs count. Serum level of TT<sub>3</sub> and TT<sub>4</sub> was significantly decreased, while TSH level was increased with CCl<sub>4</sub> treatment. Pleiomorphic histopathological abnormalities in thyroid were caused by CCl<sub>4</sub> treatment. Co-treatment of DME (100 and 200 mg/kg bw) intragastrically once a week for 16 weeks suppressed the toxic effects of CCl<sub>4</sub> and level of thyroid hormones, the activities of oxidative enzymes, glutathione (GSH) and TBARS concentration. Histopathological injuries induced with CCl<sub>4</sub> were remarkably reduced with co-treatment of DME in a dose dependent fashion. These results suggest that the ameliorative effects of DME could be attributed to radical scavenging or antioxidant properties.

**Key words:** Thyroid hormone, carbon tetrachloride, peroxidase, lipid peroxidation (TBARS), *Digera muricata*, argyrophilic nucleolar organizer region

## INTRODUCTION

Carbon tetrachloride (CCl<sub>4</sub>) is a selective carcinogenic agent which induces reactive free radicals and initiates cell damage through oxidative stress. It has been shown that CCl<sub>4</sub> toxicity requires bioactivation by phase I cytochrome P450 system in various tissues, causes formation of reactive metabolic trichloromethyl radical ( $\cdot\text{CCl}_3$ ) and peroxy trichloromethyl radical ( $\cdot\text{OCCl}_3$ ). These free radicals can bind with polyunsaturated fatty acid (PUFA) to produce alkoxy (R $\cdot$ ) and peroxy radicals (finally induce injury or necrosis of hepatocytes (Kanter et al., 1978). Liver plays an important role in the metabolism of thyroid

hormones, being involved in their conjugation, excretion and peripheral deiodination and in the synthesis of thyroxine binding globulin (TBG) (Chopra et al., 1974; Walfish et al., 1979). Carbon tetrachloride is known to cause liver damage and numerous reports have shown an indirect effect of liver disorders on serum levels of thyroid hormones (Itoh et al., 1985; Goel et al., 1994; Yamagishi et al., 1994).

CCl<sub>4</sub> metabolites cause extensive DNA strand break in which, without prompt repair, may cause cell death and compensatory cell regeneration (Moustacchi, 2000; Jia et al., 2002). Recent studies suggested that CCl<sub>4</sub> causes toxic effects to argyrophilic nucleolar organizer regions (AgNORs) that would lead to increase in AgNORs count, size, morphology or spread in the nucleus (Bocking et al., 2001; Khanna et al., 2001). Nucleolar organizer regions (NORs) are composed of chromosomal sites endowed with ribosomal DNA (rDNA) and complexes with a set of non-histone proteins characterized by a high affinity for

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**Abbreviations:** DM, *Digera muricata*; DME, *Digera muricata* methanol extract; CCl<sub>4</sub>, carbon tetrachloride; AgNORs, argyrophilic nucleolar organizer regions.

silver (Trere et al., 1996) increased number of nucleoli (NORs) with abnormal shapes and sizes, including small dots which might have been utilized as an indicator of genotoxicity to complement other histological procedures. Quantification of AgNORs has been used to characterize neoplasias and hyperplasias, since they are markers of ribosomal gene activity and hence, markers of nucleolar activity (Kanematsu et al., 1997).

Reactive oxygen species (ROS) such as hydroxyl ( $\text{OH}\cdot$ ) and peroxy radicals ( $\text{ROO}\cdot$ ) and the superoxide anion ( $\text{O}_2^{\cdot-}$ ) are constantly produced as a result of metabolic reactions in living systems (Recknagel et al., 1989). Superoxide dismutase and other antioxidant enzymes react with various species of ROS, while reduced glutathione (GSH) directly scavenges these free radicals.

Medicinal plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years. It has been found that some edible plants (as a whole or some of their portions) have protective effects against various disorders including oxidative stress and cancer (Aruoma et al., 2003). Several workers have reported the protective effects of different plant extracts against  $\text{CCl}_4$  induced injuries (Kanter et al., 2003; Sreelatha et al., 2009).

*Digera muricata* is a common weed found during the summer season throughout the plains of Pakistan. Antioxidant properties of *D. muricata* against the  $\text{CCl}_4$ -induced toxicity have been documented for testis and kidneys (Khan and Ahmed, 2009; Khan et al., 2009). The protective role of *D. muricata* against  $\text{CCl}_4$ -induced oxidative stress in thyroid had not been reported previously. The main objectives of this study were to determine the antioxidant effects of DME against the oxidative stress induced indirectly through hepatic injuries with  $\text{CCl}_4$  metabolites in the thyroid of rat.

## MATERIALS AND METHODS

### Preparation of methanol extract of *D. muricata* (DME)

The authenticated and freshly collected aerial parts of *D. muricata* were chopped, shade dried and 750 g powder was extracted twice with methanol for 72 days and dried under reduced pressure at 40°C to obtain a viscous greenish 9.5 g extract (DME).

### Animals and treatment

The experimental protocol and procedures used in this study were approved by the Ethics Committee of the Quaid-i-Azam University, Islamabad, Pakistan for the care and use of laboratory animals. This study was carried out on 3 months old male Sprague-Dawley rats weighing 250 to 260 g. Rats were provided by the Animal House of National Institute of Health (NIH), Islamabad and were maintained at the Primate Facility of Quaid-i-Azam University, Islamabad in conventional cages with free access to water and food at  $21 \pm 2^\circ\text{C}$  with a 12-h light/dark cycle. Animals were divided into five equal groups with eight rats in each group. Group I: Rats were injected intraperitoneally with olive oil (1 ml/kg bw) and orally with

DMSO (1 ml/kg bw) once a week for 16 weeks; Group II:  $\text{CCl}_4$  solution (25%/olive oil), 1 ml/kg bw was given intraperitoneally once a week for 16 weeks (Gonzales et al., 2007); Group III: Rats were treated with  $\text{CCl}_4$  (1 ml/kg bw) followed by DME (100 mg/kg bw) orally once a week for 16 weeks; Group IV:  $\text{CCl}_4$  (1 ml/kg bw) was injected intraperitoneally and DME (200 mg/kg bw) orally once a week for 16 weeks; Group V: Rats were treated with DME (200 mg/kg bw) orally once a week for 16 weeks.

At the end of the experimental period of 16 weeks, animal were sacrificed, blood was collected from atrium for routine biochemical test of tubes having EDTA and centrifuged at 2000  $\times$ g for 10 min at 4°C to get the serum. Thyroid glands were removed immediately and one lobe was fixed in formalin, while the other was dried in liquid nitrogen and stored at -70°C for various biochemical and enzymatic analysis. Histopathological studies of thyroid were carried out with 4 to 5  $\mu\text{m}$  thin sections.

### Estimation of serum level of total $\text{T}_3$ ( $\text{TT}_3$ ), total $\text{T}_4$ ( $\text{TT}_4$ ) and TSH

Serum level of  $\text{TT}_3$  (ng/ml),  $\text{TT}_4$  (ng/ml) and TSH (ng/dl) were estimated in duplicate using the standard protocol for ELISA with Diagnostic System Laboratories (Texas USA) kits. Inter assay variations were less than 10%.

### Assessment of thyroidal antioxidant enzymes

Ten percent solution (w/v) of thyroid tissue was made in 100 mM  $\text{KH}_2\text{PO}_4$  buffer containing 1 mM EDTA (pH 7.4) and centrifuged at 12,000  $\times$ g for 30 min at 4°C. In the supernatant, protein concentration was determined by using crystalline BSA as standard (Lowry et al., 1951). Catalase (CAT) activity was determined by using  $\text{H}_2\text{O}_2$  as substrate and change in absorbance of the reaction solution at 240 nm were determined for one minute (Chance and Maehly, 1955). Activity of peroxidase (POD) was estimated by using guaiacol and  $\text{H}_2\text{O}_2$ , and changes in absorbance of the reaction solution at 470 nm were determined for one minute (Chance and Maehly, 1955). Superoxide dismutase (SOD) activity of thyroid was estimated by recording color intensity of chromogen at 560 nm (Kakkar et al., 1984). Glutathione-S-transferase (GST) activity was assayed by using reduced glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) (Habig et al., 1974). Activity of glutathione peroxidase (GSH-Px) was assayed by the method of Mohandas et al. (1984).

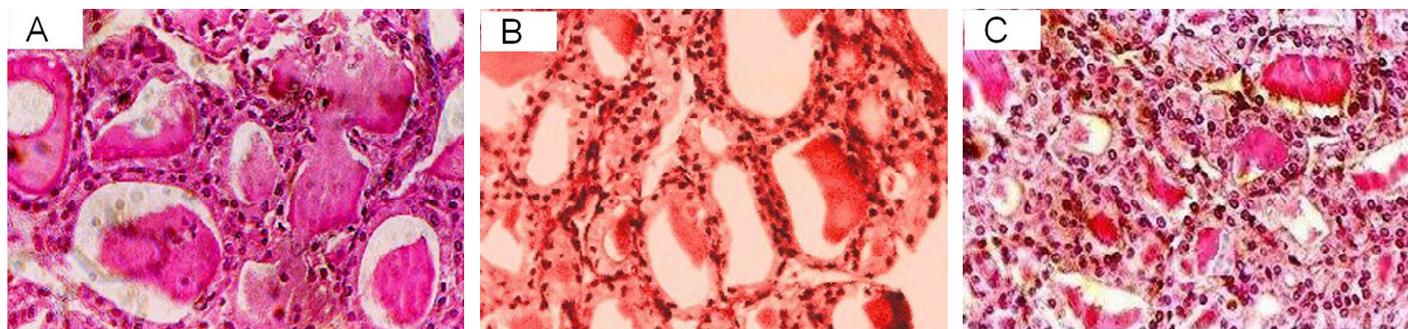
### Reduced glutathione and lipid peroxidation assay

Reduced glutathione was estimated by the method of Jollow et al. (1974), while assay for lipid peroxidation (TBARS) was carried out following the method of Iqbal et al. (1996).

### DNA fragmentation percentage assay

DNA fragmentation percentage assay was conducted using the procedure of Wu et al. (2005) with some modifications. DNA contents were assayed in the pellet and supernatant using a freshly prepared DPA (diphenylamine) solution for reaction. Optical density was read at 620 nm using (SmartSpec™ Plus) spectrophotometer. The results were expressed as amount of fragmented (%) DNA using the following formula:

$$\text{DNA fragmented (\%)} = \frac{T}{T+B} \times 100$$



**Figure 1.** Histology of thyroid; H & E stain. (A) Control thyroid tissues showing normal follicles with colloid and cuboidal cells of lumen; (B) CCl<sub>4</sub> treated thyroid tissue showing follicles paucity or absent of colloids, and columnar cells surrounding the follicles; (C) CCl<sub>4</sub> + DME (200 mg/kg bw) treated thyroid tissues showing normal lining of follicles with colloid in lumen.

**Table 1.** Effects of DME (*Digera muricata*) on thyroid hormones in serum of rat

Group	TT <sub>3</sub> (ng/ml)	TT <sub>4</sub> (ng/ml)	TT <sub>4</sub> /TT <sub>3</sub> ratio	TSH (ng/dl)
Control (olive oil + DMSO)	1.21±0.14	25.1±4.2	20.6±3.6	14.3±2.2
CCl <sub>4</sub> (1 ml/kg bw)	0.82±0.13**	16.2±3.1**	19.8±3.4	24.6±4.2**
CCl <sub>4</sub> + DME (100 mg/kg bw)	0.93±0.12**	19.2±4.4**	19.3±4.1	21.2±3.4**
CCl <sub>4</sub> + DME (200 mg/kg bw)	1.16±0.15*	23.6±4.5*	19.8±3.8	23.6±4.5**
DME (200 mg/kg bw)	1.22±0.16	24.2±5.6	20.3±3.6	17.2±2.3

Mean ±SD (n = 08). \* = P<0.05; \*\* = P<0.005 from control group.

### AgNORs count

AgNORs count was conducted according to the method of Romão-Corrêa et al. (2005). After the slides were fixed, they were dewaxed with xylene for three minutes, rehydrated by grading of alcohol (90 and 50%) for 3 min followed by hydration in distilled water for 10 min and were dried in the oven. To visualize the AgNORs, one drop of colloidal solution (2% gelatin and 1% formic acid) was added along with two drops of 50% AgNO<sub>3</sub> solution onto the hydrated slides and incubated at 37°C for 10 min in the dark. The progressive staining was followed under the microscope to get gold color nuclei and brown/black NORs. Then, the slides were washed in distilled water followed by 1% sodium thiosulfate treatment at room temperature to stop the reaction, and washed with distilled water. The cells were examined under light microscope (DIALUX 20 EB) to note the number of nucleoli.

### Statistical analysis

Parametric data, expressed as mean and standard deviation (SD), were analyzed through one way ANOVA, followed by the *post hoc* Fisher least significant difference (LSD) for comparison of various treatments using the SPSS 13.0. Differences were considered statistically significant when P < 0.05.

## RESULTS

### Histopathology of thyroid

Mortality of rats was not recorded during this experiment. Histopathology of thyroid in group I (control: Figure 1)

showed follicles containing amorphous eosinophilic colloid and were lined by cuboidal follicular cells.

Follicles of CCl<sub>4</sub>-treated rats revealed marked variability in follicles (macro- as well as microfollicles) containing no or small amounts of colloid and lined by columnar follicular cells in certain areas of thyroid in all the organisms. Co-treatment of DME protected the thyroid of rat and histopathology showed normal follicles with colloid and cuboidal follicular cells (Figure 1).

### Serum level of TT<sub>3</sub>, TT<sub>4</sub> and TSH

No significant difference was found in serum TT<sub>3</sub>, TT<sub>4</sub> and TSH levels between the control (group I) and DME alone treated rats. Serum level of TT<sub>3</sub> and TT<sub>4</sub> were decreased (P<0.005), while TSH level was elevated in all CCl<sub>4</sub> treated rats with or without DME. However, TT<sub>4</sub>/TT<sub>3</sub> ratio was not effected (P<0.05) with CCl<sub>4</sub> treatment. Treatment of rats with DME alone (group V) had no significant effect on serum TT<sub>3</sub>, TT<sub>4</sub> or TSH (Table 1). In rats given CCl<sub>4</sub> plus DME, serum TT<sub>3</sub> and TT<sub>4</sub> were higher in group IV than in group III with lower DME dosage, however, TSH was also surprisingly higher in group IV also.

### Antioxidant enzymes studies

Treatment of CCl<sub>4</sub> caused substantial injuries to thyroid

**Table 2.** Effects of DME on antioxidant enzymes in thyroid of rat.

Group	CAT (units/min)	POD (units/min)	SOD (units/mg protein)	GST (nM/mg protein)	GSH-Px (nM/mg protein)
Control (olive oil + DMSO)	0.65±0.023	1.90±0.04	0.66±0.02	1.42±0.08	19.97±1.5
CCl <sub>4</sub> (1 ml/kg bw)	0.18±0.006**	0.86±0.07**	0.06±0.003**	0.56±0.05**	11.12±1.2**
CCl <sub>4</sub> + DME (100 mg/kg bw)	0.29±0.060**	1.06±0.08**	0.09±0.005**	0.67±0.07**	12.90±1.0**
CCl <sub>4</sub> + DME (200 mg/kg bw)	0.64±0.035	1.60±0.07**	0.56±0.061**	1.12±0.05**	20.07±0.9
DME (200 mg/kg bw)	0.61±0.023*	1.86±0.07*	0.63±0.03*	1.46±0.07	20.53±1.6

Mean ± SD (n = 08). \* = P<0.05; \*\* = P<0.005 from control group. CAT, Catalase; POD, peroxidase; GST, glutathione-s- transferase, GSH-Px, glutathione peroxidase

**Table 3.** Effects of DME on GSH, TBARS, AgNORs and DNA injuries in thyroid of rat

Groups	GSH (µM/g tissue)	TBARS (nM/mg protein)	DNA Injuries (%)	AgNORs (count/cell)
Control (olive oil + DMSO)	20.4±3.6	28.3±3.2	0.14±0.06	1.3±0.23
CCl <sub>4</sub> (1 ml/kg bw)	8.8±1.3**	36.8±4.5**	0.18±0.08**	11.8±2.2**
CCl <sub>4</sub> + DME (100 mg/kg bw)	11.2±2.2**	33.1±4.6**	0.16±0.04*	6.4±1.1**
CCl <sub>4</sub> + DME (200 mg/kg bw)	16.1±3.5**	29.6±3.7*	0.15±0.04	2.6±0.35**
DME (200 mg/kg bw)	18.2±3.1	27.2±4.4	0.15±0.05	1.4±0.13

Mean ± SD (n = 08). \* = P<0.05; \*\* = P<0.005 from control group. GSH, Glutathione; TBARS, lipid peroxidation; AgNORs, argyrophilic nucleolar organizer regions.

tissues in rat and decreased the enzyme activity of catalase, peroxidase, superoxide dismutase, glutathione-S-transferase and glutathione peroxidase (Table 2). Activity of these enzymes was restored by the treatment of DME. Higher dose of DME (200 mg/kg body weight) produced most remarkable ameliorating effects and the activity of catalase, peroxidase, superoxide dismutase, glutathione-S-transferase and glutathione peroxidase reversed towards the control level. Activity of catalase, peroxidase and superoxide dismutase was decreased (P<0.05) while glutathione-S-transferase and glutathione peroxidase remained unchanged with the DME treatment alone.

### Studies on thyroid GSH contents

CCl<sub>4</sub> treatment in rats decreased (P<0.005) the contents of reduced glutathione in the thyroid tissues (Table 3). Reduced glutathione contents were dose dependently recovered with the treatment of DME.

### Studies on thyroid TBARS contents

Level of TBARS in the thyroid tissues obtained with various treatments is shown in Table 3. Treatment of CCl<sub>4</sub> on rats caused severe injuries in thyroid tissues and increased (P<0.005) the TBARS contents as compared to

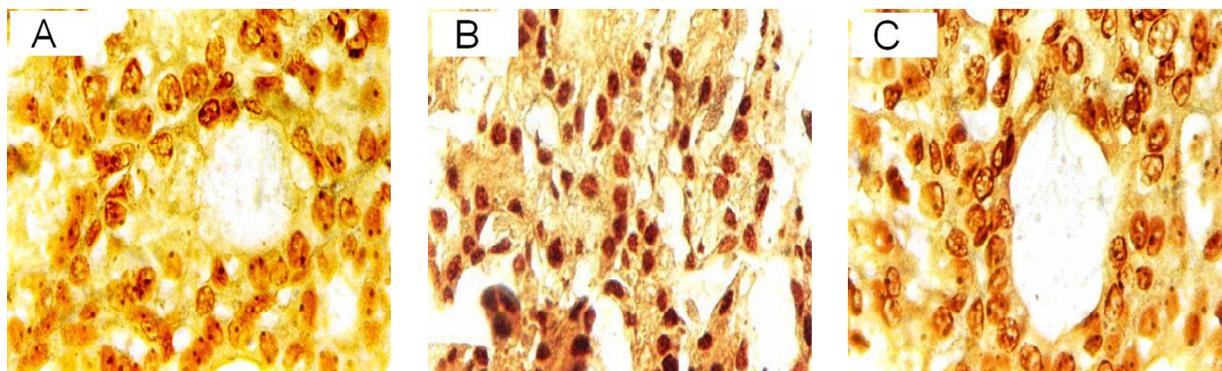
the control. Co-treatment of DME ameliorated the oxidative stress induced by CCl<sub>4</sub> metabolites and the level of TABRS reversed towards the normal level in a dose dependent way.

### Assessment of AgNORs count

Number of AgNORs in the thyroid cells was markedly increased (P<0.005) with the toxicity of CCl<sub>4</sub> metabolites. However, co-treatment of rats with DME decreased the toxicity of CCl<sub>4</sub> metabolites and the AgNORs count was decreased in a dose dependent manner. The ameliorating effects were more pronounced at the higher dose of DME (200 mg/kg body weight) and it did not differ statistically (P>0.05) from the control group of rat. Statistically similar AgNORs count was observed in the treatment of DME alone versus the control group (Table 3 and Figure 2).

### Thyroid DNA fragmentation studies

Toxicity of CCl<sub>4</sub> metabolites significantly increased the percentage of DNA fragmentation (P<0.005) in the thyroid tissues of rat. Treatment of DME in combination with CCl<sub>4</sub> reduced the toxicity of metabolites and percentage of DNA fragmentation was decreased dose dependently in thyroid tissues. Percentage of DNA



**Figure 2.** Silver staining of thyroid tissues. (A) Control thyroid tissues showing normal morphology and number of AgNORs in the cells; (B) CCl<sub>4</sub> treated thyroid tissues showing deformed and dot like AgNORs in the cells; (C) CCl<sub>4</sub> + DME (200 mg/kg) treated thyroid tissues showing protection against CCl<sub>4</sub> and AgNORs reduced to near normal level in the cells.

fragmentation obtained at 200 mg/kg bw dose was statistically ( $P > 0.05$ ) not different from the control group.

## DISCUSSION

In this study, treatment with CCl<sub>4</sub> for 16 weeks led to pleiomorphic structural abnormalities of follicles in thyroid of rats. Epithelial cells of follicles changed from squamous to columnar which was associated with paucity or even complete loss of colloid (Khan et al., 1999). Exposure to a low dose of halogenated aromatic hydrocarbons appears to disturb thyroid morphology and function, leading to hyperplasia with a modulation of negative feed back mechanism through hypothalamic pituitary thyroid axis (HPTA) (Nishimura et al., 2003). Histopathology observed in thyroid tissues from CCl<sub>4</sub>-treated animals suggests that hepatotoxicity leading to decreased metabolism of TT<sub>4</sub> to TT<sub>3</sub> and a resultant increase in TSH concentration might lead to observed pleiomorphic changes of the thyroid tissues. Treatment of rats with DME ameliorated the toxic effects of CCl<sub>4</sub> and normal histopathology of thyroid was observed. Bioactive compounds present in the DME possibly prevented the CCl<sub>4</sub> metabolism leading to a reduction in the levels of reactive metabolites being formed, thereby restoring the structure of thyroid tissues.

As regards to serum concentration of thyroid hormones, we observed that animals injected with CCl<sub>4</sub> for 16 weeks showed significant decrease in TT<sub>3</sub> and TT<sub>4</sub> level. Our results are partially consistent with other reports where decrease in TT<sub>3</sub> was recorded, while TT<sub>4</sub> level was not significantly changed (Itoh et al., 1987; Goel et al., 1994). However, other studies are completely comparable to these findings where treatment of rats with CCl<sub>4</sub> and allyl alcohol significantly decreased TT<sub>3</sub> and TT<sub>4</sub> level (Itoh et al., 1989; Moustafa, 2001). Significant decrease in TT<sub>3</sub> level was also reported in patients with various hepatic disorders (Itoh et al., 1986; Kano et al., 1987). According to Costa et al. (2001) and Torlak et al.

(2007), serum TT<sub>4</sub> entirely originate from thyroid gland, while more than 80% of TT<sub>3</sub> is produced by deiodination of TT<sub>4</sub> in other tissues, especially the liver and kidney through iodothyronine 5'-monodeiodinase (5'-activity) enzyme. In particular, the hepatic enzyme is thought to be responsible for the major part of peripheral TT<sub>3</sub> production. It was established that CCl<sub>4</sub>-induces liver injuries in rat (Kanter et al., 2003; Sreelatha et al., 2009), reduction in serum TT<sub>3</sub> level could partly be due to the decreased conversion of TT<sub>4</sub> to TT<sub>3</sub> on account of low activity of iodothyronine 5'-monodeiodinase (5'-activity) enzyme (Jatwa and Kar, 2008). However, injuries of CCl<sub>4</sub> metabolites leading to dysfunction of thyroid cannot be excluded.

Oxidative stress is characterized by an imbalance between pro-oxidant and antioxidant in the favor of the pro-oxidants (Halliwell and Whiteman, 2004). Various enzymatic and non-enzymatic systems have been developed by mammalian cells to cope with ROS and free radicals. In this study, thyroid toxicity induced by CCl<sub>4</sub> metabolites caused marked deterioration of endogenous antioxidant profile as evidenced by decrease in CAT, POD and SOD activities. These enzymes constitute the main defenses against ROS in the cell (Sreelatha et al., 2009). These results are consistent with other reports where CCl<sub>4</sub> treatment had decreased the activity of earlier mentioned enzymes in liver, testis and kidneys of rat (Kanter et al., 2003; Khan and Ahmed, 2009; Khan et al., 2009; Sreelatha et al., 2009). Accumulation of CCl<sub>4</sub> metabolites in thyroid tissues possibly led to excessive oxidative stress, thereby decreasing the activity of CAT, POD and SOD in the thyroid. It could be suggested that the toxic effects observed in the thyroid following CCl<sub>4</sub> administration may be secondary to hepatotoxicity of CCl<sub>4</sub>. Low level of CAT, POD and SOD activity obtained with the treatment of DME alone in this experiment indicated that the bioactive compounds in DME might increase the reducing capacity of hepatocytes and thyroid that eventually led to decrease in the level of antioxidant enzymes. Treatment of various doses of DME possibly

inhibited the conversion of  $\text{CCl}_4$  into its reactive metabolites, decreased the oxidative stress and protected the antioxidant enzymes of thyroid as revealed by the enhanced level of CAT, POD and SOD in this experiment.

Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyzes the GSH-dependant reduction of  $\text{H}_2\text{O}_2$  and other peroxides and protects the organism from oxidative damage. In addition, the glutathione-S-transferases showed high activity with lipid peroxides. Glutathione detoxifies toxic metabolites of drugs, regulates gene expression, apoptosis and transmembrane transport of organic solutes. We obtained significant depletion of GST and GSH-Px in thyroid tissues of rat with  $\text{CCl}_4$  treatment. These results support the view that extensive oxidative stress takes place in the thyroid with injurious  $\text{CCl}_4$  metabolites. Decrease in the activity of GST might be due to the decreased availability of GSH contents as a result of lipid peroxidation. GST binds to lipophilic compounds and act as an enzyme for GSH conjugation reactions.

Reduced GSH is an important protein thiol which coordinates body defense system against oxidative stress. Reduced GSH effectively scavenge free radicals and other reactive free oxygen species. In such reactions, GSH is oxidized to form GSSG, which is then reduced to GSH by the NADPH-dependent glutathione reductase. It is highly imperative to maintain the reduced status of the cell/tissues. GSH contents were significantly decreased by treatment of  $\text{CCl}_4$ , while TBARS contents were increased as compared to the control group in this experiment. TBARS are produced during the peroxidation of polyunsaturated fatty acids (Ohkawa et al., 1979). Thyroid toxicities induced with  $\text{CCl}_4$  metabolites is mediated by free radicals or by depletion of endogenous pool of antioxidants such as GSH. Treatment of DME to rats treated with  $\text{CCl}_4$  increased the GSH contents and decreased the TBARS contents as compared to the  $\text{CCl}_4$  treated group. Similar protective effects were also reported with the treatment of *Coriandrum sativum* and *Digera muricata* extracts against oxidative stress induced with  $\text{CCl}_4$  (Khan et al., 2009; Sreelatha et al., 2009).

TBARS react with DNA to form the adduct M1G; the mutagenic pirimedopurinone adduct of deoxyguanosine. In addition to free radical attack of lipids, DNA is also continuously subjected to oxidative damage. Among the products of oxidative DNA damage are base propenals; structural analogs of malondialdehyde would react with DNA to form M1G (Marnett, 2000). Treatment of DME reduced the DNA fragmentation percentage, possibly through its ameliorative/preventive properties by suppressing the metabolism of  $\text{CCl}_4$  into reactive species.

Cellular activity and cell proliferation have a direct relationship with AgNORs count and this criterion could be useful to estimate the overall structural and functional integrity of a cell (Bocking et al., 2001; Khan et al., 2009). Quantitative evaluation of AgNORs allowed us to nucleoli (NORs) with abnormal shapes and sizes, including small dots, may be used to evaluate the cellular damage.  $\text{CCl}_4$

treatment in this experiment significantly increased the AgNORs count per cell in thyroid tissues of rat and were scattered throughout the nucleus. Higher number of AgNORs with  $\text{CCl}_4$  treatment indicated the presence of invasive neoplasia (Trere et al., 1996). Treatment of rats with DME reduced the number of AgNORs possibly through the ameliorating effects of DME on the metabolism of  $\text{CCl}_4$ . Similar results of AgNORs count have been determined with  $\text{CCl}_4$  treatment and its protection with *D. muricata* in kidneys of rat (Khan et al., 2009).

## Conclusion

This study indicated the protective role of *D. muricata* on levels of antioxidants in rat's thyroid against the toxicity of  $\text{CCl}_4$ . However, *D. muricata* did not prevent aberrations in serum thyroid hormones levels. The protective potential may involve the preventive/ameliorative effects of DME by the inhibition of  $\text{CCl}_4$  metabolism, leading to a reduction in the level of reactive species being formed. This study further supports the scientific evidence in favor of its pharmacological use in oxidative stress diseases.

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