EFFECT OF CANNABIS SATIVA L. EXTRACT IN MALE GENITAL SYSTEM FUNCTION

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Abstract Cannabis sativa leaves are traditionally used in the treatment of male dysfunction and hormonal disorder as well as in oxidative stress. The present study was designed to assess the protective effects of methanolic extract of Cannabis sativa leaves (MLC) on carbon tetrachloride (CCl4) - induced reproductive stress in male rats and bioactive constituents responsible for the activity. CCl4 was induced in 42 male rats for eight weeks and checked the protective efficacy of methanolic extract of Cannabis sativa leaves at various hormonal imbalances, alteration of antioxidant enzymes and lipid peroxidation caused testicular fibrosis in testis. CCl4 caused significant alteration in the secretion of reproductive hormones. Activity of antioxidant enzymes viz; catalase, superoxide dimutase and phase II metabolizing enzymes including glutathione peroxidase, glutathione reductase and reduced glutathione was decreased while hydrogen per oxide contents and thiobarbituric acid reactive substances (TBARS) were increased with CCl4 treatment. Co-administration of 100 mg/kg and 200 mg/kg b.w. MLC effectively ameliorated the alterations in the biochemical markers; hormonal and molecular levels. Protective effects of methanolic extract of Cannabis sativa against CCl4 – induced antioxidant and hormonal dysfunction which might be due to bioactive compound present in extract.

Keywords: CCl4, Cannabis sativa, Hormones, antioxidants, Rats

INTRODUCTION
During the last decade, considerable attention was given to the involvement of oxygen free radicals in various diseases. There is no doubt that reactive oxygen species (ROS) play an important role in pathological changes in the liver, particularly in the case of alcoholic and toxic liver diseases (Das and Vasudevan, 2007). Biological membranes are particularly prone to ROS effect. The peroxidation of unsaturated fatty acids in biological membranes leads to the decrease of membrane fluidity and the disruption of membrane integrity and function. Such peroxidation effect is implicated in serious pathological changes (Cabre et al., 2000). CCl4 is an industrial solvent causes tissue damages in various tissues of experimental animals. CCl4 requires bioactivation by phase I cytochrome P450 system to form reactive metabolic radicals. These free radicals can bind with polyunsaturated fatty acid (PUFA) of sperm membrane to generate lipid peroxides that are highly reactive, change enzyme activity and finally induce injury or necrosis (Sikka et al., 1995; Ogeturk et al., 2005). Several endogenous protective mechanisms have evolved to limit ROS effect and the damage caused by them (Sies, 1993). However, when this protection is not
complete, or when the formation of ROS is excessive, additional protective mechanisms of dietary antioxidants may be of a great importance. Therefore, many natural and synthetic agents possessing antioxidative properties have been proposed to prevent and treat infertility and reproductive hormonal imbalance induced by oxidative stress (Kandasamy et al., 2010). There is increasing evidence of the protective role of hydroxy and polyhydroxy organic compounds, particularly from vegetables, fruits and some herbs.

Plants are well-known excellent perspectives for the discovery of new therapeutical products. In recent years, an ample interest has been developed in finding natural antioxidants from commonly available wild plants, fruits and vegetables that were generally mistreated (Umamaheswari and Chatterjee, 2008; Kil et al., 2009) as well as an important role in detoxification of free radicals induced lung injuries and fibrosis in experimental animal’s model. Cannabis sativa is an annual herbaceous plant in the Cannabis genus, a species of the Cannabaceae family. People have cultivated cannabis sativa throughout recorded history as a source of industrial fibre, seed oil, food, recreation, religious and spiritual moods, and medicine. Each part of the plant is harvested differently, depending on the purpose of its use. The present study was conducted to examine the toxic upshots of CCl4 plus to compare the beneficial effects of plant extracts on reproductive hormonal disturbance and activity of antioxidant enzymes in various experimental groups. In this respect, several parameters regarding the testicular injury and fibrosis were studied.

MATERIALS AND METHODS

Plant collection

C. sativa leaves were collected in March 2013 from Arasbaran forest, Northwestern of Iran and recognized by their local names and validated by department of Plant Sciences, Tabriz University, Iran.

Extract preparation

The collected plant leaves were cleaned and dried under shade for fifteen days. Willy Mill of 60-mesh size was used to prepare powder of dried samples and then 5 kg powdered plant sample was extracted twice with 10 L of 95% methanol at 25°C for 48 h. For filtration Whatman No. 1 filter paper was used and then filtrate was concentrated on rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan) under reduced pressure at 40°C and dry extract was stored at 4°C for further in vivo investigation.

Experimental plan

Six-week-old male Sprague Dawley rats weighing 180 ± 10 g were provided with food and water ad libitum and kept at 20–22°C on a 12-h light–dark cycle. The study protocol was approved by Ethical committee of Tabriz University, Iran. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. For chronic toxicity eight week experiment was designed. 42 male albino rats were randomly divided into seven groups (6 rats of each group). Administration of CCl4 (0.5 ml/kg b.w., 20% CCl4/olive oil) was intraperitoneally (i.p.) twice a week for eight weeks. At the same time, the rats were administered individually silymarin (50 mg/kg b.w.) and extract (100, 200 mg/kg b.w.) orally twice a week for eight weeks.

Experimental protocol

Following dosing plan was adapted for the study.

Group I: the normal control received only feed

Group II: Olive oil (0.5 ml/kg b.w., i.p.) + DMSO (0.5 ml/kg b.w. orally)

Group III: CCl4 twice a week (0.5 ml/kg b.w., i.p., 20% CCl4/olive oil)
Group IV: CCl₄ twice a week (0.5 ml/kg b.w., i.p.) + sylimarin (50 mg/kg b.w., orally)  
Group V: CCl₄ twice a week (0.5 ml/kg b.w., i.p.) + MLC (100 mg/kg b.w., orally)  
Group VI: CCl₄ twice a week (0.5 ml/kg b.w., i.p.) + MLC (200 mg/kg b.w., orally)  

At the end of eight weeks, after 24 h of the last treatment, Urine was collected and stored at −70°C for further analysis, and then animals were given chloroform anesthesia and dissected from ventral side. All the animals were sacrificed; blood was drawn prior to the excision of organ tissues. The serum was stored at −80°C after separation until it was assayed as described below.

Assessment of reproductive hormones and lipid profile of serum
Serum analysis of testicular hormones like FSH, LH, testosterone, prolactin and esteradiol were radioimmunoassayed by using Marseille Cedax 9 France Kits and Czch Republic Kits from Immunotech Company. Then again, lipid profile such as Triglycerides, total cholesterol, LDL and LDH were estimated by using standard AMP diagnostic kits (Stattogetter Strasse 31b 8045 Graz, Austria).

Assessment of antioxidant enzymes
10% homogenate of tissue was prepared in 100 mM KH₂PO₄ buffer containing 1 mM EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for the following parameters as described below.

Catalase assay (CAT)
CAT activities were determined by the method of Chance and Maehly, (1955) with some modifications. The reaction solution contained: 2.5 ml of 50 mM phosphate buffer (pH 5.0), 0.1 ml of 20 mM guaiacol, 0.3 ml of 40 mM H₂O₂ and 0.1 ml enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined after one minute. One unit of POD activity was defined as an absorbance change of 0.01 units/min.

Peroxidase assay (POD)
Activities of POD were determined by the method of Chance and Maehly, (1955) with some modifications. The POD reaction solution contained: 2.5 ml of 50 mM phosphate buffer (pH 5.0), 0.1 ml of 20 mM guaiacol, 0.3 ml of 40 mM H₂O₂ and 0.1 ml enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined after one minute. One unit of POD activity was defined as an absorbance change of 0.01 units/min.

Superoxide dismutase assay (SOD)
SOD activity was estimated by the method of Kakkar et al. (1984). Reaction mixture of this method contained: 0.1 ml of phenazine methosulphate (186 μM), 1.2 ml of sodium pyrophosphate buffer (pH 7.0), 0.3 ml of supernatant after centrifugation (1500 × g for 10 min followed by 10000 × g for 15 min) of testis homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 μM) and stopped after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed in units/mg protein.

Glutathione-S-transferase assay (GST)
Glutathione-S-transferase activity was assayed by the method of Habig et al. (1974). The reaction mixture consisted of 1.475 ml phosphate buffer (0.1 mol, pH 6.5), 0.2 ml reduced glutathione (1 mM), 0.025 ml (CDNB) (1 mM) and 0.3 ml of homogenate in a total volume of 2.0 ml. The changes in the absorbance were recorded at 340 nm and enzymes activity was calculated as nM CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 × 10³ M⁻¹cm⁻¹.
Glutathione reductase activity was determined by method of Carlberg and Mannervik, (1975). The reaction mixture consisted of 1.65 ml phosphate buffer: (0.1 mol; pH 7.6), 0.1 ml EDTA (0.5 mM), 0.05 ml oxidized glutathione (1 mM), 0.1 ml NADPH (0.1 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. Enzyme activity was quantitated at 25°C by measuring disappearance of NADPH at 340 nm and was calculated as nM NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22×10³ M⁻¹cm⁻¹.

**Glutathione peroxidase assay (GPx)**

Glutathione peroxidase activity was assayed by the method of Mohandas et al. (1984). The reaction mixture consisted of 1.49 ml phosphate buffer (0.1 M; pH 7.4), 0.1 ml EDTA (1 mM), 0.1 ml sodium azide (1 mM), 0.05 ml glutathione reductase (1 IU/ml), 0.05 ml GSH (1 mM), 0.1 ml NADPH (0.2 mM), 0.01 ml H₂O₂ (0.25 mM) and 0.1 ml of homogenate in a total volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at 25°C. Enzyme activity was calculated as nM NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22×10³ M⁻¹cm⁻¹.

**Quinone reductase assay (QR)**

The activity of quinone reductase was determined by the method of Benson et al. (1990). The 3.0 ml reaction mixture consisted of 2.13 ml Tris–HCl buffer (25 mM; pH 7.4), 0.7 ml BSA, 0.1 ml FAD, 0.02 ml NADPH (0.1 mM), and 0.1 ml of homogenate. The reduction of dichlorophenolindophenol (DCPIP) was recorded at 600 nm and enzyme activity was calculated as nM of DCPIP reduced/min/mg protein using molar extinction coefficient of 2.1×10⁴ M⁻¹cm⁻¹.

**Reduced glutathione assay (GSH)**

Reduced glutathione was estimated by the method of Jollow et al. (1974). 1.0 ml sample of homogenate was precipitated with 1.0 ml of (4%) sulfosalicylic acid. The samples were kept at 4°C for 1 h and then centrifuged at 1200×g for 20 min at 4°C. The total volume of 3.0 ml assay mixture contained 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1 M; pH 7.4) and 0.2 ml DTNB (100 mM). The yellow color developed was read immediately at 412 nm on a SmartSpecTM plus Spectrophotometer. It was expressed as μM GSH/g tissue.

**Estimation of lipid peroxidation assay (TBARS/LPO)**

The assay for lipid peroxidation was carried out following the modified method of Iqbal et al. (1996). The reaction mixture in a total volume of 1.0 ml contained 0.58 ml phosphate buffer (0.1 M; pH 7.4), 0.2 ml homogenate sample, 0.2 ml ascorbic acid (100 mM), and 0.02 ml ferric chloride (100 mM). The reaction mixture was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by addition of 1.0 ml 10% trichloroacetic acid. Following addition of 1.0 ml 0.67% thiobarbituric acid, all the tubes were placed in boiling water bath for 20 min and then shifted to crushed ice-bath before centrifuging at 2500×g for 10 min. The amount of TBARS formed in each of the samples was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer against a reagent blank. The results were expressed as nM TBARS/min/mg tissue at 37°C using molar extinction coefficient of 1.56×10⁵ M⁻¹cm⁻¹.

**Hydrogen peroxide assay (H₂O₂)**

Hydrogen peroxide (H₂O₂) was assayed by H₂O₂-mediated horseradish peroxidase-dependent oxidation of phenol red by the method of Pick and Keisari, (1981). 2.0 ml of homogenate sample was suspended in 1.0 ml of solution containing phenol red (0.28 nM), horse radish peroxidase (8.5 units), dextrose (5.5 nM) and phosphate buffer (0.05 M; pH 7.0) and were incubated at 37°C for 60 min. The reaction was stopped by the addition of 0.01 ml of
NaOH (10 N) and then centrifuged at 800 × g for 5 min. The absorbance of the supernatant was recorded at 610 nm against a reagent blank. The quantity of H₂O₂ produced was expressed as nM H₂O₂/min/mg tissue based on the standard curve of H₂O₂ oxidized phenol red.

Statistical analysis
To find the different treatment effects of in vivo studies one way analysis of variance was carried by computer software SPSS 19.0. Level of significance among the various treatments was determined by LSD at 0.05% level of probability.

RESULTS
Effects of MLC against CCl₄ induced testicular toxicity in rat
The current study was paying attention on the estimation of ameliorating potential of C. sativa leaves against testicular toxicity provoked by CCl₄. The biomarkers for testicul toxicity evaluation were based on serological studies, antioxidant enzyme levels of tissue, genotoxicity and histological variation of testis.

Effects of MLC on male reproductive hormones of rats
Data from the serological markers for reproductive status such as testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin and estradiol is summarized in Table 1. CCl₄ intoxication drastically (p < 0.05) reduced the serum level of testosterone, LH and FSH, while notably (p < 0.05) increased the intensity of prolactin and estradiol. The serum level of above said reproductive hormones was re-established (p < 0.05) by oral administration of 100 mg/kg b.w., 200 mg/kg b.w., MLC near to control group. 50 mg/kg body weight of silymarin treatment erased CCl₄ intoxication and restored the level of all tested reproductive hormones in serum of rats.

Table 1: Effects of MLC on male reproductive hormonal level

<table>
<thead>
<tr>
<th>Group</th>
<th>Testosterone (ng/ml)</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
<th>Prolactin (ng/ml)</th>
<th>Estradiol (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.2±0.07d</td>
<td>2.28±0.09c</td>
<td>40.37±0.14h</td>
<td>10.43±0.34e</td>
<td>17.43±0.32g</td>
</tr>
<tr>
<td>Oil+DMSO</td>
<td>4.1±0.13d</td>
<td>2.23±0.01c</td>
<td>39.12±0.36h</td>
<td>11.23±0.32e</td>
<td>15.24±0.43g</td>
</tr>
<tr>
<td>CCl₄</td>
<td>1.9±0.08a</td>
<td>1.10±0.08a</td>
<td>16.68±0.21a</td>
<td>22.11±0.44a</td>
<td>30.16±0.65a</td>
</tr>
<tr>
<td>Silymarin+CCl₄</td>
<td>3.5±0.10b</td>
<td>1.89±0.12b</td>
<td>32.87±0.48g</td>
<td>14.46±0.22d</td>
<td>20.23±0.63f</td>
</tr>
<tr>
<td>100 mg/kg b.w. MLC + CCl₄</td>
<td>2.7±0.03c</td>
<td>1.74±0.05b</td>
<td>23.04±0.35e</td>
<td>16.01±0.50c</td>
<td>24.18±0.29c</td>
</tr>
<tr>
<td>200 mg/kg b.w. MLC + CCl₄</td>
<td>3.3±0.23c</td>
<td>2.01±0.13b</td>
<td>28.15±0.49f</td>
<td>16.23±0.21c</td>
<td>22.43±0.25d</td>
</tr>
<tr>
<td>MLC alone</td>
<td>4.0±0.07d</td>
<td>2.36±0.09c</td>
<td>40.85±0.62h</td>
<td>12.27±0.30e</td>
<td>14.37±0.30g</td>
</tr>
</tbody>
</table>

a-f (Means with different letters) indicate significance at p < 0.05.

Effects of MLC on lipid profile changes
Table 2 summarizes protective effects of MLC against CCl₄ induced toxicity in lipids profile of serum. For lipid parameters total triglycerides, total cholesterol, HDL, and LDL were investigated. CCl₄ disputation markedly increased the levels of triglycerides, total cholesterol, and LDL cholesterol while decreased (p < 0.01) HDL cholesterol as against the control group. Treatment of MCL cancelled the toxicity of CCl₄ thus, restoring the serum level of total triglycerides, total cholesterol, HDL, and LDL towards the control group. Treatment with silymarin also produced similar results.

Table 2: Effects of MLC on lipid profile

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglycerides (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>130.15±2.97e</td>
<td>39.15±1.43e</td>
<td>32.3±1.08d</td>
<td>15.34±1.34d</td>
</tr>
<tr>
<td>Oil+DMSO</td>
<td>128.76±2.45e</td>
<td>40.24±1.34e</td>
<td>30.32±1.73d</td>
<td>13.56±1.11d</td>
</tr>
<tr>
<td>CCl₄</td>
<td>230.19±2.08a</td>
<td>78.99±1.05a</td>
<td>52.36±1.04a</td>
<td>30.61±2.04a</td>
</tr>
<tr>
<td>Silymarin+CCl₄</td>
<td>159.32±2.42d</td>
<td>49.23±2.33d</td>
<td>40.33±1.27c</td>
<td>20.76±1.73c</td>
</tr>
<tr>
<td>100 mg/kg b.w. MLC + CCl₄</td>
<td>190.06±4.11b</td>
<td>60.73±1.72b</td>
<td>48.23±1.12b</td>
<td>25.26±1.95b</td>
</tr>
<tr>
<td>200 mg/kg b.w. MLC + CCl₄</td>
<td>168.99±2.22c</td>
<td>52.84±2.62c</td>
<td>44.84±1.69c</td>
<td>22.33±1.41c</td>
</tr>
<tr>
<td>MLC alone</td>
<td>127.19±1.33e</td>
<td>39.65±1.28e</td>
<td>29.47±1.36d</td>
<td>12.15±1.11d</td>
</tr>
</tbody>
</table>

a-e (Means with different letters) indicate significance at p < 0.05.
Effects of MLC on testis enzymatic antioxidant levels

Oxidative stress produced by CCl4 upsets the cellular antioxidant defense system. The protective effects of MLC against CCl4 toxicity on the antioxidant profile are presented in Table 3. Administration of CCl4 for eight weeks caused noteworthy (p < 0.05) decrease in the tissue soluble protein and CAT, POD and SOD activities as opposed to control group. Post-treatment of 100 mg/kg b.w., 200 mg/kg b.w., MLC markedly ameliorated the effects of CCl4 intoxication, and distinctly enhanced (p < 0.05) testicular protein and CAT, POD and SOD levels of testicular tissue. Lipid peroxidation is umpired via free radicals produced by CCl4 intoxication. The significant decline in H2O2 and TBARS level of testicular tissue corroborates with protective power 100 mg/kg b.w., 200 mg/kg b.w., MLC against testicular CCl4 induced lipid peroxidation in testis tissue. Similar protective effects were reported, while treating with silymarin.

### Table 3: Effects of MLC on tissue proteins and antioxidant enzyme levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein (μg/mg tissue)</th>
<th>CAT (U/min)</th>
<th>POD (U/min)</th>
<th>SOD (μM/g protein)</th>
<th>TBARS (nM/Min/mg protein)</th>
<th>H2O2 (μM/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.421 ± 0.023d</td>
<td>6.92 ± 0.22d</td>
<td>9.38 ± 0.13c</td>
<td>3.08 ± 0.23d</td>
<td>3.10 ± 0.14d</td>
<td>1.244 ± 0.012c</td>
</tr>
<tr>
<td>CCl4</td>
<td>0.834 ± 0.029a</td>
<td>4.02 ± 0.55d</td>
<td>9.04 ± 0.34c</td>
<td>3.12 ± 0.14d</td>
<td>3.10 ± 0.27d</td>
<td>1.244 ± 0.012c</td>
</tr>
<tr>
<td>Silymarin+CCl4</td>
<td>1.312 ± 0.008c</td>
<td>2.02 ± 0.11a</td>
<td>4.76 ± 0.51a</td>
<td>0.88 ± 0.07a</td>
<td>5.71 ± 0.48a</td>
<td>2.536 ± 0.023a</td>
</tr>
<tr>
<td>100 mg/kg b.w. MLC+CCl4</td>
<td>1.27 ± 0.06c</td>
<td>7.79 ± 0.85c</td>
<td>1.95 ± 0.13c</td>
<td>3.74 ± 0.70b</td>
<td>1.30 ± 0.069c</td>
<td>1.560 ± 0.091b</td>
</tr>
<tr>
<td>200 mg/kg b.w. MLC+CCl4</td>
<td>1.344 ± 0.073c</td>
<td>8.37 ± 0.41b</td>
<td>2.29 ± 0.11b</td>
<td>4.01 ± 0.25c</td>
<td>1.644 ± 0.086b</td>
<td>1.644 ± 0.086b</td>
</tr>
<tr>
<td>MLC alone</td>
<td>1.214 ± 0.012d</td>
<td>3.95 ± 0.68d</td>
<td>10.00 ± 0.61c</td>
<td>3.63 ± 0.13d</td>
<td>2.91 ± 0.33d</td>
<td>1.121 ± 0.045c</td>
</tr>
</tbody>
</table>

a-h (Means with different letters) indicate significance at p < 0.05.

Alterations in phase II antioxidant metabolizing enzymes viz; GST, GPx, GR, GSH and QR testicular tissues of rat are demonstrated in Table 4. Chronic administration of CCl4 extensively (p < 0.05) abridged the glutathione status of GST, GPx, GR, GSH and QR. Post-treatment with 100 mg/kg b.w., 200 mg/kg b.w., MLC attenuated the intoxication of CCl4 and restored the enzymes activity near to control rats. Silymarin treatment markedly increased the GST, GSH, GR, GPx and QR activation similar to the effects of 100 mg/kg b.w., 200 mg/kg b.w., MLC.

### Table 4: Effects of MLC on phase II antioxidant enzymes

<table>
<thead>
<tr>
<th>Group</th>
<th>GST (nM/g protein)</th>
<th>GPx (nM/g protein)</th>
<th>GR (nM/g protein)</th>
<th>GSH (μM/g tissue)</th>
<th>QR (nM/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>150.23 ± 5.34a</td>
<td>110.17 ± 4.46f</td>
<td>198.34 ± 5.78e</td>
<td>16.25 ± 1.11c</td>
<td>99.90 ± 4.97f</td>
</tr>
<tr>
<td>Oli+DMSO</td>
<td>157.17 ± 4.45f</td>
<td>105.55 ± 4.55f</td>
<td>204.73 ± 6.47e</td>
<td>15.74 ± 1.31c</td>
<td>107.03 ± 4.42f</td>
</tr>
<tr>
<td>CCl4</td>
<td>78.37 ± 4.33a</td>
<td>63.33 ± 3.11a</td>
<td>103.14 ± 4.66a</td>
<td>9.60 ± 1.34a</td>
<td>52.40 ± 4.16a</td>
</tr>
<tr>
<td>Silymarin+CCl4</td>
<td>133.67 ± 2.43f</td>
<td>98.46 ± 2.22e</td>
<td>179.36 ± 4.45d</td>
<td>13.75 ± 0.32b</td>
<td>89.14 ± 2.56e</td>
</tr>
<tr>
<td>100 mg/kg b.w. MLC+CCl4</td>
<td>120.54 ± 1.98c</td>
<td>77.39 ± 2.92b</td>
<td>166.07 ± 2.21c</td>
<td>20.05 ± 0.90c</td>
<td>75.25 ± 3.12d</td>
</tr>
<tr>
<td>200 mg/kg b.w. MLC+CCl4</td>
<td>124.12 ± 2.81e</td>
<td>94.65 ± 2.16e</td>
<td>170.68 ± 4.97d</td>
<td>13.29 ± 0.46b</td>
<td>82.56 ± 3.67d</td>
</tr>
<tr>
<td>MLC alone</td>
<td>159.31 ± 3.21g</td>
<td>112.40 ± 3.26f</td>
<td>201.25 ± 4.12e</td>
<td>17.59 ± 1.45c</td>
<td>109.34 ± 2.23f</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSION

Oxidative stress induced by an increase in free radicals and/or decrease in antioxidant defenses is well documented in animal model (Botsoglou et al., 2009; Marañón et al., 2008). CCl4, a typical toxic agent, exerts its toxic effects by the generation of free radicals. By the activation of liver cytochromes P450, CCl4 is converted into free radicals which immediately react with cell membrane (Dashti et al., 1989). This free radical not only targets liver but it can also causes free radical generation in other tissues like kidneys, heart, lung, testis, brain and blood (Ozturk et al., 2003; Preethi and Kuttan, 2009). In the current study, the proposed plan aimed to assess and examine the possibility of MLC to protect and reduce the lipid peroxidation and oxidative damages caused by CCl4 in
testis tissue homogenate of male Sprague Dawley rats. The reduction of testosterone levels in serum indicates either direct effects of CCl₄ at Leydig cell level or indirect effects by disturbing the hormonal environment at hypothalamo-pituitary axis (Latif et al., 2008) due to oxidative trauma in CCl₄ treated rats. It was reported that abnormal level of intra testicular testosterones inhibits spermatogenesis (Tohda et al., 2001). The production of testosterone in Leydig cells is stimulated by LH, which activates FSH to bind with sertoli cells to stimulate spermatogenesis (Conn, 1986). CCl₄ intoxicated rats show the malfunctioning of pituitary to secrete FSH and LH indicating testicular dysfunction leading to infertility as was reported by previous results (Khan and Ahmed, 2009). GSH levels are dependent upon the activities of glutathione reductase (GR) and NADH (Meister and Anderson, 1983). Glutathione system including GPx, GR, GST, as well as SOD and CAT represent a mutually loyal team of defense against ROS (Bandhopedhy et al., 1999). Enhanced lipid peroxidations expressed in terms of TBARS determine structural and functional alterations of cellular membranes (Halliwell et al., 1990). In the present study, administration of various fractions of plant samples in different experimental groups improved the activities of SOD, CAT, POD, GPx, GST, GR and QR as well as non-enzymatic (GSH, TBARS and H₂O₂) levels of CCl₄-intoxicated testis towards normalcy in warfare of oxidative trauma in vivo. Hence, the present results regarding chronic toxicity of CCl₄ are in accordance with previous reports (Khan et al., 2012; Khan, 2012), while studying the protective effects of Sonchus asper and Launaea procumbens on testis against oxidative stress of CCl₄. Present study revealed that the activities of antioxidant enzymes were significantly reduced the toxication of chemical which might be due to the presence of bioactive elements like myricetin, kaempferol, isoquercetin, hyperoside and vitexin, propagating free radicals like peroxy radicals and converting the reactive free radicals to inactive products.

It was reported that CCl₄ resulted in the oxidative damage to testicular proteins in rats. Oxidative damage to proteins is very important as it can contribute secondary damage resulting in hampering the DNA repair enzymes and loss of reliability of damage polymerases during DNA replication. The DNA damage in various tissues like brain, testis and liver was reported by Manierea et al. (2005). From the present study, it can be assumed that chronic exposure of CCl₄ may cause accumulation of many toxic species in cells thus damaging both DNA and lipids.

In fact, treatment with various fractions of plant samples ameliorated the toxic effects on DNA as revealed by DNA fragmentation % and ladder assay. The present study clearly augments the defensive mechanism of various samples against oxidative stress induced by CCl₄ and provides confirmation about its therapeutic use in reproductive abnormalities.

Previous studies on histomorphology of testis showed shrinkage of the tubular diameter and testicular atrophy leading to degenerative changes in the germinal epithelium (Debnath and Mandal, 2005) after exposure to toxic chemical. Similar destructive effects were also accounted in CCl₄ treated groups. The CCl₄ challenge revealed testicular destruction and degeneration in histological architecture like that of profenofos that was recorded by Moustafa et al. (2007) who represented damaged columnar epithelial layer, vacuolated spermatogonial cells, oedematous alterations in the seminiferous tubules and extra elongated Leydig cells. Data of the present study revealed that CCl₄ may hamper continuing proliferative behavior of testicular cells thus obstruct reproduction. Deformities in spermatogenesis and partial degeneration of germ and Leydig cells have been
displayed by CCl₄-treated rats. However, groups administered various fractions of plant samples in different experiments demonstrated a quality active spermatogenesis, thin basement membranes and normal seminiferous tubules in most of the part of testis. Same histopathology was noticed by Manjrekar et al. (2008), while evaluating the protective effects of Phyllanthus niruri Linn treatment on testis against CCl₄ intoxication. This paper is in continuation of our previous studies Sahreen et al. (2011) in which hepatoprotective effect of methanolic extract of leaves were assessed.

It can be concluded from the current study that bioactive components of MLC especially flavonoids (myricetin, kaempherol, isoquercetin, hyperoside and vitexin) have the ability to recover the metabolic enzymatic activities and repair cellular injuries, thus providing scientific evidence in favor of its pharmacological use in testicular dysfunctioning.

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REFERENCES


