Chlorpromazine (CPZ), a commonly used antipsychotic drug (1) is used in the treatment of schizophrenia and is well documented to produce extrapyramidal side effects (EPS). These effects, such as bradykinesia, muscular rigidity, tremor and postural imbalance are the sign and symptoms of parkinsonism. These effects of antipsychotic drugs are due to an inhibition of dopaminergic system in the extrapyramidal motor system, however, the exact mechanism for the EPS remains far from fully understood. Catalepsy is a situation of prolonged motor inhibition distinguished by a failure to amend an externally imposed, abnormal posture over an extended period of time (2). Locomotor activity is a symbol of wakefulness (alertness) of mental activity. Most of the central nervous system (CNS) acting drugs promote the locomotor activities in man and animals. The CNS depressant drugs reduce the motor activity while the CNS stimulant drugs augment the activity.

Prolonged administration of CPZ causes generation of reactive oxygen species (ROS) from catecholamine metabolism by monoamine oxidase, ultimately leading to increased oxidative stress (3). Disturbances of antioxidant defence systems have been demonstrated in the brain, including increased lipid peroxidation product and alterations in the activities of antioxidant enzymes, such as glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and catalase (CAT) when the rats were treated with CPZ (4, 5).

The brain is highly vulnerable to the damage caused by free radicals because of its rapid oxidative metabolic activity, high polyunsaturated fatty acids content, relatively low antioxidant capacity, and inadequate neuronal cell repair activity. Therefore, brain tissues are predominantly susceptible to oxidative damage by CPZ. Increasing evidence demonstrated that dietary supplementation with nutritional antioxidants could reduce oxidative stress, limit brain damage and improve behavioral functions (6). Thymoquinone (Family: Ranunculaceae) is the main constituent of the volatile oil from Nigella sativa seed, grown in countries like Iran, Pakistan and India. The seeds of Nigella sativa have been com-
monly used for centuries as a food additive as well as for the treatment of various ailments throughout the world. Traditionally they have been used as antihypertensive, liver tonics, diuretics, antidiabetic, digestive, appetite stimulant, anti-diarrheal, antibacterial, analgesics and anti-inflammatory (7). Previous reports suggest that thymoquinone possesses varied pharmacological activities such as antioxidant (8), hepatoprotective (9), neuroprotective (10), nephroprotective (11), anti-mutagenic (12), anti-carcinogenic (13) and anti-convulsant (14). Therefore, the present study was designed to explore whether supplementation with thymoquinone has any protective effect on chlorpromazine induced catalepsy, locomotor activity and cerebral oxidative stress in rats.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats (200-300 g) were obtained from the animal house of Jazan University for this study. They were housed under standard laboratory condition and maintained at standard pellet diet and water ad libitum during the experimental period. Experiments were conducted in accordance with the Animal Ethics Committee of the University.

Drugs and chemicals

Chlorpromazine (CPZ) and thymoquinone were procured from Sigma, Chemicals Co., USA. All other chemicals used for the experiment were of analytical grade.

Experimental design

The rats were divided into four groups each containing eight animals. Group I served as normal control and received saline (2 mL/kg, p.o.) for 21 days. Group II served as negative control received CPZ (3 mg/kg, i.p.) for 21 days. Group III and IV served as TQ treated, received TQ (5 and 10 mg/kg, p.o.) 30 min after the administration of CPZ (3 mg/kg, i.p.) for 21 days. Catalepsy was induced by the administration of CPZ (3 mg/kg, i.p.). All behavioral studies were carried out at room temperature in unruffled room without any outside disturbances. On the last day of experiment, all the rats were sacrificed by cervical dislocation and the brains were excised for further investigation of biochemical parameters. The experimental study was conducted in the Pharmacology Laboratory, College of Pharmacy, Jazan University, Gizan, Saudi Arabia.

Behavioral parameter

Catalepsy was induced by CPZ (3 mg/kg, i.p.) and was assessed by block method described by

Figure 1. Effects of thymoquinone on lipid peroxidation (LPO) in the brain of CPZ treated rats. The data are expressed as the mean ± SEM; n = 6 in each group. * p < 0.001 compared with the corresponding value for normal control group. ** p < 0.05, *** p < 0.001 compared with the corresponding value for CPZ treated group.
Neuromodulatory effects of thymoquinone in extenuating oxidative stress...

Kulkarni (15). On the last day of experiment (21st day), the scores were recorded in three stages. The rotarod test was used to study the muscle coordination using the procedure of Dunham and Miya (16). The spontaneous locomotor activity of animals were calculated using an actophotometer (activity cage), which works on photoelectric cells joined in circuit with a counter (17).

**Oxidative stress parameters**

**Homogenate and post-mitochondrial supernatant (PMS) preparation**

The brains tissue was homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing potassium chloride. The homogenate was centrifuged at 825 ◊ g for 5 min at 4°C in a refrigerated centrifuge to separate the nuclear debris. The supernatant-1 (S1) was used for the assay of LPO and the rest was again centrifuged at 10500 ◊ g for 15 min at 4°C to separate post mitochondrial supernatant (PMS) which was used to assay GSH, GPx, GR, GST and CAT activity.

**Biochemical parameters**

Thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation (LPO) were estimated according to the procedure of Utley et al. (18) as modified by Islam et al. (19). Glutathione-S-transferase activity was estimated according to the method of Habig et al. (23) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. CAT was estimated following the method of Claiborne (24) based on a decrease in absorbance at 240 nm due to consumption of hydrogen peroxide (H₂O₂).

**Statistical analysis**

Data were expressed as the mean ± standard error (S.E.) of 6 animals. For statistical analysis, group means were compared by one-way analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison test to identify significance among groups; p < 0.05 was considered to be statistically significant.

**RESULTS**

**Effect of thymoquinone on CPZ induced catalepsy**

Table 1 shows the effect of TQ on CPZ induced catalepsy. Significant (p < 0.001) increase in the cataleptic scores was observed in CPZ treated rats when compared with normal control rats. Oral administration of TQ at two doses (5 and 10 mg/kg, p.o.) reduced the cataleptic scores significantly (p < 0.05 and p < 0.01) when compared with CPZ treated rats.

**Effect of thymoquinone on CPZ induced muscle coordination**

Table 1 shows the effect of TQ on CPZ induced muscle coordination. CPZ significantly (p < 0.001) increased the latency of falling from rotat-
ing rod (an index of muscle coordination) in rats when compared with normal control rats. Treatment with TQ at two doses (5 and 10 mg/kg, p.o.) significantly (p < 0.05 and p < 0.01) decreased the latency of falling from rotating rod when compared with CPZ treated rats.

Effect of thymoquinone on CPZ induced spontaneous locomotor activity

Table 1 shows the effect of TQ on CPZ induced spontaneous locomotor activity. CPZ significantly (p < 0.001) decreased both horizontal and vertical locomotor activity in rats when compared with normal control rats. Oral administration of TQ at two doses (5 mg/kg and 10 mg/kg, p.o.) significantly increased both horizontal (p < 0.05 and p < 0.001) and vertical (p < 0.05 and p < 0.001) locomotor activity in rats when compared with CPZ treated rats.

Effect of thymoquinone on TBARS content

Figure 1 shows the contents of thiobarbituric acid reactive substances (TBARS) in brain tissue homogenate. CPZ treatment resulted in a significant (p < 0.001) increase in TBARS contents when compared with normal control group rats. Thymoquinone treatment significantly (p < 0.05 and p < 0.001) decreased the contents of TBARS when compared with CPZ treated group rats.

Effect of thymoquinone on GSH content

Figure 2 shows the contents of GSH in CPZ treated control rats which was significantly (p < 0.001) decreased in brain tissue homogenate when compared to the normal control group. Thymoquinone treatment has protected GSH levels significantly (p < 0.05 and p < 0.01) in CPZ + TQ group as compared to CPZ treated group.

Effect of thymoquinone on antioxidant enzyme (AOE) activities

Table 2 shows the activities of antioxidant enzymes namely GPx, GR, GST and CAT in the brain of normal control, CPZ control and TQ treated group rats. CPZ treatment significantly (p < 0.001) decreased the activities of GPx, GR and CAT when compared with normal control group rats. Administration of TQ significantly (p < 0.05, p < 0.001) restored the activities of GPx, GR, GST and CAT in CPZ + TQ group when compared with CPZ treated group rats.

DISCUSSION

Antipsychotic drugs are used for the treatment of schizophrenia. The drugs which are used to treat schizophrenia are typical and atypical antipsychotics. Typical antipsychotic drugs such as chlorpromazine, haloperidol are effective in controlling
positive symptoms of schizophrenia but at the same time they produce both therapeutic effects as well as untoward side effects in humans such as extrapyramidal disturbances, sedation, endocrine and autonomic effects (25). Antipsychotic drugs, which block central dopamine receptors, produce a behavioral condition in animals in which they fail to amend externally imposed posture (26). This behavioral condition in which the animals fail to amend externally imposed posture is known as catalepsy. Previous studies showed that chlorpromazine causes disturbances in motor coordination and reduced spontaneous locomotor activity in animals (27).

The results obtained clearly revealed that the animals who were treated with CPZ showed severe cataleptic response as confirmed by significant increase in the time spent on the block as compared to the normal control group rats. Previous studies showed that chlorpromazine causes disturbances in motor coordination and reduced spontaneous locomotor activity in animals (27).

Substantial evidences showed generation of reactive oxygen free radicals in schizophrenic patients when treated with chlorpromazine (28, 29), resulting in LPO and protein oxidation which leads to cellular disintegrity. Lipid peroxides and protein oxidation are the secondary products of oxidative stress causing secondary injury by further generating relatively more stable and diffusible cytotoxic agents such as malondialdehyde, which is responsible for magnifying oxidative stress (30). Recent study have demonstrated that thymoquinone have considerable antioxidant activity against free radicals and lipid peroxidation (31). In the present study, it was observed a significant increase in the contents of TBARS, a secondary product of LPO in the brain of CPZ treated rats. Thymoquinone treatments to such rats have shown significant decrease in the contents of TBARS suggesting its possible role in scavenging hydroxyl and peroxyl radicals generated by CPZ.

Biochemical analysis of brain homogenate showed significant reduction in GSH which is a natural reservoir of the reductive capacity of cell (4). GSH is a well known non-enzymatic endogenous antioxidant against ROS to protect the cellular system against the toxic effects of lipid peroxidation. The low content of GSH may be directly related to increased ROS, lipid peroxides and highly reactive hydroxyl radicals (32). The detoxification pathway of ROS involves oxidation of GSH to glutathione disulfide (GSSG), resulting in a decrease of GSH content. Reduction of GSH content enhances the cellular damage caused by oxidative stress. A marked decrease in the GSH content was observed in CPZ treated rats. Furthermore, thymoquinone treatment showed a significant increase in GSH content in the brain of CPZ treated rats.

Furthermore, the reduced activities of enzymatic antioxidants led to the decreased scavenging

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>GPx (nmol NADPH oxidized/min/mg protein)</th>
<th>GR (nmol NADPH oxidized/min/mg protein)</th>
<th>GST (nmol CDNB conjugates formed/min/mg protein)</th>
<th>CAT (nmol H2O2 consumed/ min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>251.07 ± 8.35</td>
<td>288.57 ± 13.20</td>
<td>156.08 ± 6.91</td>
<td>39.53 ± 1.56</td>
</tr>
<tr>
<td>II</td>
<td>CPZ treated (Negative control)</td>
<td>167.34 ± 6.76</td>
<td>189.61 ± 11.55</td>
<td>108.13 ± 4.81</td>
<td>23.20 ± 0.87</td>
</tr>
<tr>
<td>III</td>
<td>CPZ + Thymoquinone (5 mg/kg)</td>
<td>205.86 ± 9.40</td>
<td>233.64 ± 9.97</td>
<td>130.92 ± 2.76</td>
<td>28.50 ± 1.45</td>
</tr>
<tr>
<td>IV</td>
<td>CPZ + Thymoquinone (10 mg/kg)</td>
<td>223.35 ± 13.19</td>
<td>254.27 ± 7.50</td>
<td>140.45 ± 6.22</td>
<td>32.97 ± 1.18</td>
</tr>
</tbody>
</table>

GPx (nmol NADPH oxidized/min/mg protein); GR (nmol NADPH oxidized/min/mg protein); GST (nmol CDNB conjugates formed/min/mg protein); CAT (nmol H2O2 consumed/ min/mg protein). The data are expressed as the mean ± S.E.; n = 6 in each group. * p < 0.001 compared with the corresponding value for normal control group. ** p < 0.05, *** p < 0.01, **** p < 0.001 compared with the corresponding value for CPZ treated group.
activities against ROS, eventually causing oxidative stress. The concentration of ROS is modulated by antioxidant enzymes such as GPx, GR, GST and CAT. All antioxidant enzymes are interconnected, disturbance of one would disrupt the whole enzymatic microenvironment. CPZ treatment resulted in a significant decrease in the activities of enzymatic antioxidants viz., GPx, GR, GST and CAT. GPx has been reported to reduce hydroperoxides to water by using GSH as a hydrogen donor (33), whereas GR is able to regenerate its GSH from GSSG. The observed decreased activities of GPx, GR and GST in this study might be due to decreased content of GSH. In the present study, it was established that thymoquinone potentiates the antioxidative system, and increases the activities of GSH dependent enzymes such as GPx, GR and GST in CPZ treated rats. The decreased activity of CAT in tissue is due to excess availability of hydrogen peroxide (H2O2) in the biological systems, which, in turn, generate hydroxyl and peroxyl radicals, resulting in the initiation and propagation of lipid peroxidation. Thymoquinone treatment showed a significant augmentation in the activities of CAT in the brain of CPZ treated rats. The results clearly suggest that treatment with TQ reduced the cataleptic scores, improved locomotor activity and normalized antioxidant enzyme activities in the brain of CPZ treated rats. Based on the findings it is postulated that supplementation with TQ can be used to prevent CPZ induced extrapyramidal side effects and may find a role in reducing the oxidative stress. Further clinical studies are necessary to confirm these effects.

REFERENCES


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