Protective effect of thymoquinone on chlorpromazine induced catalepsy and locomotor activity in rats

ABSTRACT

Objective: The in-vivo study was carried out to explore the possible protective effect of thymoquinone on chlorpromazine induced catalepsy and locomotor activity in rats.

Materials and Methods: Twenty four rats were used for this study and were divided into four groups, each group containing six animals. The animals were evaluated after repeated administration of chlorpromazine (CPZ) 30 minutes before the administration of thymoquinone (TQ) for 14 days. Catalepsy was assessed using block method whereas the locomotor activity was assessed using acceleratory rotarod and actophotometer (activity cage).

Results: The cataleptic scores were significantly increased in CPZ treated rats which was significantly decreased in TQ (5 mg/kg & 10 mg/kg) treated rats. The muscle coordination and spontaneous locomotor activity was significantly decreased in CPZ treated rats when compared with normal control rats. Treatment with TQ significantly improved the muscle coordination and spontaneous locomotor activity when compared with CPZ treated rats.

Conclusion: Our results clearly suggest that TQ reduced the cataleptic scores and improved locomotor activity in CPZ treated rats.

Recommendation: Based on our findings we can hypothesize that supplementation with TQ can be used to avert drug induced extrapyramidal motor side effects.

Key words: Chlorpromazine, Thymoquinone, Catalepsy, Locomotor activity
INTRODUCTION
Chlorpromazine (CPZ), a commonly used antipsychotic drug (Chong et al., 2004), used in the treatment of schizophrenia is well-known to produce extrapyramidal side effects (EPS). These effects, such as akinesia, rigidity, tremor and postural instability are Parkinson's like effect. These effects of antipsychotic drugs are due to blockade of dopamine receptors (D1/D2 and D5 receptors) in the extrapyramidal motor system. Catalepsy is a situation of delayed motor inhibition characterized by a failure to amend an externally imposed, abnormal posture over an extended period of time (Seeman et al., 1996). Cataleptic signs in animals are related to the Parkinson-like effects noticed clinically with administration of antipsychotic drugs. Most of the CNS acting drugs persuade the locomotor activities in human and animals. The CNS depressant drugs diminish the motor activity while the CNS stimulant drugs augment the activity. Furthermore the locomotor activity is a sign of wakefulness (alertness) of mental activity.
Thymoquinone (Family: Ranunculaceae), the main constituents of the volatile oil from *Nigella sativa* seed, grown in countries like Iran, Pakistan and India. The seed of *Nigella sativa* is very commonly used 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, anti-bacterial, analgesics and anti-inflammatory (Ahmad et al., 2013). Several studies on thymoquinone indicated that it possesses anticonvulsant, immunomodulatory, anticancer, scavenger of free radicals and superoxide anions (Daba and Abdel-Rahman, 1998; Badary et al., 2003; Hosseinzadeh and Parvardeh 2004). Therefore, the present study was undertaken to explore whether supplementation with thymoquinone has any protective effect on chlorpromazine induced catalepsy and locomotor activity in rats.

MATERIALS AND METHODS:

Experimental Animals
Twenty four Wistar rats (150-200 g) were used for this study. They were kept in college animal house for one week for acclimatization before the start of experiment and were given standard diet and water during the experimental period.

Drugs and Chemicals
Chlorpromazine (CPZ) and thymoquinone was procured from Sigma, Chemicals Company, USA. All other chemicals used for the experiment were of analytical grade.
Experimental design
The rats were divided into four groups each containing six animals. Group I served as normal control and received saline (2 ml/kg, p.o) for 14 days. Group II served as negative control received CPZ (3 mg/kg, i.p) for 14 days. Group III served as TQ treated received TQ (5 mg/kg, p.o) 30 minutes after the administration of CPZ (3 mg/kg, i.p) for 14 days. Group IV served as TQ treated received TQ (10 mg/kg, p.o) 30 minutes after the administration of CPZ (3 mg/kg, i.p) for 14 days. Catalepsy was induced by the administration of CPZ (3 mg/kg, i.p). All behavioural studies were carried out at room temperature in unruffled room without any outside disturbances. The experimental study was conducted in the Pharmacology laboratory, College of Pharmacy, Jazan University, Gizan, Saudi Arabia.

Evaluation of catalepsy
Catalepsy was induced by CPZ (3 mg/kg, i.p) and was assessed by block method described by Kulkarni (1999). On the last day of experiment (14th day) the scores were recorded in three stages and the scores for each stage was assigned. In first stage the rat was pushed on the back, if failed to move a score of 0.5 was given. For second stage front paws of each rat was placed alternately on 3 cm high block. If the rat failed to maintain the forced posture in 10 seconds, a score of 0.5 for each paw with a total of 1 for this stage was given. For third stage the front paws of each rat was placed alternately on 9 cm high block. If the rat failed to maintain the forced posture in 10 seconds, a score of 1 for each paw with a total of 2 was added to the scores of first and second stage. Thus for the single rat, the maximum possible score was 3.5 and that indicate the total catalepsy.

Evaluation of muscle coordination:
The rotarod test was used to study the motor coordination of muscle using procedure of Dunham and Miya (1957). The equipment has a rotating rod (3 cm diameter) connected to a motor with adjustable speed and has four holding chambers directly below each section of the rod (50 cm below). The rats were trained on the rotating rod at a speed of 20 rpm for three days until they could stay on the rotating rod for 300 seconds without falling. On the last day of experiment (14th day) the animals were kept on the rotating rod with a fixed speed of 20 rpm. The fall off time was recorded for 300 seconds. The time for each rat to stay on the rotating rod was recorded.

Evaluation of spontaneous locomotor activity:
The spontaneous locomotor activity was calculated using an actophotometer (activity cage), which works on photoelectric cells joined in circuit with a counter. When a beam of light falls on the photocell is cut off by the rat, a count is recorded. On the last day of experiment (14th
day) the rats from each group were placed individually in the activity cage for 10 mins and the scores of each rat was recorded (Bhattacharya and Haldar, 2012).

**Statistical analysis:** All values were expressed as the mean ± standard error of mean (S.E.M). The results were compared by one-way analysis of variance (ANOVA) followed by Tukey-Kramer test, which was used to identify differences between groups. \( P < 0.05 \) was considered as statistically significant.

**RESULTS:**

**Effect of thymoquinone on CPZ induced catalepsy:**

Table 1 shows the effect of TQ on CPZ induced catalepsy. Cataleptic scores were significantly \( (p < 0.001) \) increased in CPZ treated rats as compared to normal control rats. Oral administration of TQ at two doses (5 and 10 mg/kg) reduced the cataleptic scores significantly \( (p < 0.05 - p < 0.01) \) as compared to 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 fall off time from rotating rod (an index of muscle coordination) in rats as compared to normal control rats. Treatment with TQ at two doses (5 and 10 mg/kg) significantly \( (p < 0.05 - p < 0.01) \) decreased the fall off time from rotating rod as compared to CPZ treated rats.

**Effect of thymoquinone on CPZ induced spontaneous locomotor activity:**

Table 2 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 as compared to normal control rats. Oral administration of TQ at two doses (5 and 10 mg/kg) significantly \( (p < 0.05 - p < 0.001) \) increased both horizontal and vertical locomotor activity in rats as compared to CPZ treated rats.

**DISCUSSION:**

Antipsychotic drugs are used for the treatment of schizophrenia. The drugs which are used to treat this mental illness are typical and atypical antipsychotics. Typical antipsychotic drugs such as chlorpromazine, haloperidol is effective in controlling positive symptoms of schizophrenia but at the same time they produces both beneficial effects as well as untoward side effects in humans such as extrapyramidal disturbances, sedation, endocrine and autonomic effects (Nsimba, 2009). Antipsychotic drugs, which block central dopamine receptors, create a behavioral condition in which the animals fail to amend externally imposed
posture (Rao et al., 2005). The 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 (Bisong et al., 2010). Hence the present study was undertaken to find out whether supplementation with thymoquinone has any protective effect on chlorpromazine induced catalepsy, motor coordination and spontaneous locomotor activity in rats.

Our results clearly demonstrate that, the animals that 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 rats. We found that oral administration of TQ (5 and 10 mg/kg) significantly reduced the cataleptic scores. In the present study CPZ treatment significantly impaired muscle coordination as evidenced by increase in the fall off time from rotating rod. The fall off time from rotating rod was significantly decreased by oral administration of TQ (5 and 10 mg/kg) which suggest improvement in motor coordination. Furthermore, the CPZ treatment significantly decreased spontaneous locomotor activity indicating CNS depressant activity. Treatment with TQ (5 and 10 mg/kg) significantly improved spontaneous locomotor activity hence indicating its CNS stimulant activity. Based on our findings we can hypothesize that supplementation with TQ can be used to avert drug induced extrapyramidal side effects. Further clinical studies are necessary to confirm these effects.
REFERENCES:


2. Badary OA, Taha RA, Gamal el-Din AM, Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol 2003; 26: 87-98.


Table 1: Effect of thymoquinone on CPZ induced catalepsy and muscle coordination in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Catalepsy score</th>
<th>Fall off time (sec) (index of muscle coordination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>0</td>
<td>187.17±17.59</td>
</tr>
<tr>
<td>II</td>
<td>CPZ treated (Negative control)</td>
<td>2.25±0.21</td>
<td>22.66±5.46</td>
</tr>
<tr>
<td>III</td>
<td>CPZ + Thymoquinone (5 mg/kg)</td>
<td>1.41±0.15</td>
<td>69.33±6.80</td>
</tr>
<tr>
<td>IV</td>
<td>CPZ + Thymoquinone (10 mg/kg)</td>
<td>1.08±0.23</td>
<td>95±9.55</td>
</tr>
</tbody>
</table>

The data are expressed in mean ± S.E.; n=6 in each group. *p<0.01 compared with the corresponding value for normal control group. *p < 0.05, **p<0.01 compared with the corresponding value for CPZ treated group.

Table 2: Effect of thymoquinone on CPZ induced spontaneous locomotor activity in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Spontaneous locomotor activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Horizontal activity</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>178.50±12.17</td>
</tr>
<tr>
<td>II</td>
<td>CPZ treated (Negative control)</td>
<td>63.83±3.79</td>
</tr>
<tr>
<td>III</td>
<td>CPZ + Thymoquinone (5 mg/kg)</td>
<td>98.16±5.53</td>
</tr>
<tr>
<td>IV</td>
<td>CPZ + Thymoquinone (10 mg/kg)</td>
<td>129.23±7.58</td>
</tr>
</tbody>
</table>

The data are expressed in 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.