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Evaluation of clarithromycin pharmacokinetics after single and repeated oral administration of atorvastatin in hyperlipidemic Wistar rats

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The present study was aimed to evaluate the clarithromycin pharmacokinetics after atorvastatin single and repeated dose administration in induced hyperlipidemic Wistar rats. A high fat diet (2% cholesterol) was used to induce hyperlipidemia and feed was given to rats for 6 to 8 weeks. Group allocation was as follows: G1 to G3: induced hyperlipidemic rats pretreatment of atorvastatin as a single dose followed by clarithromycin single doses of 10, 20 and 100 mg/kg; G4 to G6: induced hyperlipidemic rats pretreatment of atorvastatin for 7 consecutive days followed by clarithromycin single doses of 10, 20 and 100 mg/kg; G7 to G8: atorvastatin treated hyperlipidemia rat livers harvested on day 1 and 7 post dose; G9 to G10: concomitant administration of atorvastatin and clarithromycin in presence and absence of hyperlipidemia for biliary and urinary excretion studies. Effect of atorvastatin on hyperlipidemia and alterations of clarithromycin kinetics due to various states of hyperlipidemia like induced, reduced and absence of hyperlipidemia was evaluated. The in vitro clarithromycin results suggested down/up regulation of CYP mediated metabolism in induced and reduced hyperlipidemia rats. Clarithromycin pharmacokinetics was considerably affected by hyperlipidemia and also recovered kinetics and metabolism in presence of atorvastatin compared on days 1 and 7 were also reported. The decreased area under the curve of clarithromycin in hyperlipidemic rats after co-administration of atorvastatin might be mainly due to up regulation of the CYP mediated metabolism by reducing the hyperlipidemia in the liver.

Key words: Clarithromycin, atorvastatin, hyperlipidemia, area under the curve (AUC), peak plasma, rats.

INTRODUCTION

Hyperlipidemia (HL) is defined as an elevation of one or more of the plasma lipids, including cholesterol, cholesteryl esters, triglycerides or phospholipids (Raasch, 1988). Despite the differences in lipoprotein distribution and metabolism between humans and rats, hyperlipidemic (HLM) rat models are used extensively in lipid research. Many hyperlipidemic rat models are in existence (Eliot et al., 1999).

Antimicrobial drugs manifest a wide variety of drug interactions which can differ greatly in their extent of severity and clinical relevance. Not only co-medication but also food and herbal medicine can interact with antimicrobial
drugs and vice versa. The nature of these interactions can be of pharmacodynamic (PD) and/or pharmacokinetic (PK) origin. Historically, the relevance of drug distribution, particularly of protein binding, has been over-emphasized in the assessment of drug interactions and nowadays the main cause of drug-drug interactions has been recognized to be modulation of the activity that is, inhibition or induction of cytochrome P450 (CYP) enzymes and transporters (Mathieu et al., 2011).

HL was recently proven to decrease the liver uptake of the more potent (-)-ketoconazole (KTZ) enantiomer 1 which raises a question about effect of HL on the CYP inhibitory potency of KTZ (Hamdy et al., 2011). Similarly, impact of HL on pharmacokinetics of many drugs evaluated included nifedipine (Eliot et al., 1999), docetaxel (Lee et al., 2011), amiodarone (Shayeganpour et al., 2005) and cyclosporine A (Brooks et al., 2006). Reported higher exposure and lower clearance of nifedipine in the HLM rats was due to the decrease in fraction unbound in plasma (Hamdy et al., 2011). Docetaxel showed significantly low unbound fraction and intrinsic hepatic metabolism probably due to the lower expression of cytochrome P 450 (CYP) 3A (Lee et al., 2011). Henceforth, clarithromycin (CLR) is a lipophilic and is extensively distributed in the body and metabolized by CYP 3A (Kim et al., 2005), which might also be changed in the HLM state. CLR showed significantly higher exposure in HL (data not shown). To further evaluate impact of HL, we have used atorvastatin (AT), a 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase inhibitor that efficiently and dose-dependently lowers both cholesterol (Nawrocki et al., 1995) and triglyceride (Bakker et al., 1996) levels. AT was administered orally as a single and repeated dose (consecutive 7 days) to HL rats after successful induction of HL. The reduced hyperlipidemia (RHL) term was used since after repeated dose of AT, HL has been reduced but not cured. This group is also named as atorvastatin-treated hyperlipidemic group (AHL). In our previous study, HL was induced successfully using standard cholesterol diet to rats which significantly increases high plasma total cholesterol (TC) and triglycerides (TG) levels (Prasanna et al., 2009). Rats which will have elevated TC and TG levels are called induced hyperlipidemic rats (IHL). CLR has been previously demonstrated that the total cytochrome P 450 (CYP) content was significantly lower in the microsomal protein of the liver of HL rats than of control rats (Shayeganpour et al., 2008) which showed significant decrease in the protein expressions of CYP2C11, CYP3A1 and CYP3A2 in the hepatic microsomes of hyperlipidemic rats compared to control rats (Joo-hyun et al., 2012). Henceforth, no studies have been conducted to evaluate CLR metabolism in presence of IHL and AT post dose (single and repeated). Therefore in view of the foregoing, we isolated induced and reduced HL rat livers and homogenized them with suitable buffers to establish CLR metabolism in various HLM conditions following AT pre-treatment.

Our main aim is to study the CLR pharmacokinetics and excretion at different phases of HL like IHL, RHL (AT treatment for consecutive 7 days) and absence of HL in Wistar rats. The scope is to present an overview of pharmacokinetic interaction studies on drug-hyperlipidemia of commonly prescribed antimicrobial drug like CLR in daily clinical practice. However, no studies have been conducted regarding the possible effects of HL and RHL on CLR pharmacokinetics and excretion in Wistar rats. Therefore we focused to investigate CLR pharmacokinetics and excretion following single and repeated dose of AT 7 consecutive days in HLM rat models to evaluate any potential interactions of CLR with HL and in RHL rat models.

MATERIALS AND METHODS

Chemicals and apparatus

The active pharmaceutical ingredients like CLR, erythromycin (internal standard) and AT were purchased from Sigma–Aldrich Co. (India). High performance liquid chromatography (HPLC) grade methanol and acetonitrile were obtained from Merck Co. (Darmstadt, Germany). All other chemicals in this study were of analytical grade and used without further purification. The apparatus used in this study included a MS/MS equipped with an Agilent isotropic HPLC Pump including auto sampler (Agilent, USA), an HPLC column with temperature controller (Discovery C18, Waters), a Bransonic ultrasonic cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex mixer (Scientific Industries Inc., Bohemia, NY, USA) and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

Induction of hyperlipidemia and animal experiments

The required animals male Wistar rats (weighing 250 to 300 g) were procured from the In-house, animal resource, Wockhardt Research Centre (MS, India) and were given access to a commercial rat chow diet (NutriLab, provimi pvt, Ltd, India) for control group and for induction of hyperlipidemia, in-house prepared standard cholesterol diet (Prasanna et al., 2009) was fed for 6 to 8 weeks and also rats were fed twice daily with butter (0.5 ml) separately to hasten the hyperlipidemia. After 6 to 8 weeks, lipid profiles were evaluated as per the procedure published and animals were selected based on higher levels of total cholesterol (> 150 mg/dl) and triglyceride (> 100 mg/dl) levels (Table 1). Diet and water was provided ad libitum and the selected rats were housed individually for pharmacokinetic and biliary excretion study and temperature maintained at 22 ± 3°C, with 42 to 65% relative humidity under a 12:12 h light/dark cycle. The animals were allowed 6 to 8 days for acclimation. The Institute of Animal Care and Ethics Committee of Drug metabolism pharmacokinetics (DMPK) department (Regd No.: 13/1999/CPCSEA, Wockhardt research Centre, India) approved the design and conduct of this study. Experimental animals were fasted for at least 12 h before the experiments and each animal was anesthetized with isoflurane during the catheterization. The right jugular vein, bile duct and duodenum were cannulated using polyethylene tubing PE-50 (i.d. 0.58 mm, o.d. 0.98 mm; Portex, Smiths medicals, USA) and PE-10 (i.d. 0.28 mm, o.d. 0.61 mm; Portex, Smiths medicals, USA) to allow for blood sampling (jugular vein, for pharmacokinetics) and bile sampling (Bile duct, for excretion) following oral administration.
Pharmacokinetic study

Effect of AT on CLR pharmacokinetics in presence of IHL following single and repeated administration

The rats were divided into n = 6 groups each. Doses selected are 10, 20 and 100 mg/kg (suspended in 0.5% carboxymethyl cellulose (CMC) containing 2% Tween 80, 5 ml/kg). In this study, CLR doses have been selected at three dose levels to investigate CLR dose proportionality in hyperlipidemic conditions after single AT treatment. AT dose was 20 mg/kg suspended in 0.5% CMC solution administered intra-gastrically using an oral feeding tube. AT was administered as a single and repeated dose up to days 1 and 7, respectively. Groups allocation was as scheduled, G1: IHL rats treated with CLR (10 mg/kg) and single dose of AT (20 mg/kg); G2: IHL rats treated with CLR (20 mg/kg) and single dose of AT (20 mg/kg); G3: IHL rats treated with CLR (100 mg/kg) and single dose of AT (20 mg/kg); G4: IHL rats treated with repeated dose of AT (20 mg/kg) up to day 7 consecutive days, followed by single dose of CLR (10 mg/kg); G5: IHL rats treated with repeated dose of AT (20 mg/kg) up to day 7 consecutive days, followed by single dose of CLR (20 mg/kg); G6: IHL rats treated with repeated dose of CLR (20 mg/kg) up to day 7 consecutive days, followed by single dose of CLR (100 mg/kg). After oral gavage co-administration of CLR and AT in IHL rats, blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post-dose and 0.2 ml aliquot of blood was collected (n = 6) into heparinized tubes from the jugular vein catheter and samples were centrifuged at 6,000 g for 6 min at +2 to +8°C and the separated plasma samples were stored at -70°C until analyzed by liquid chromatography–mass spectrometry (LC-MS/MS).

In vitro CLR metabolism in induced hyperlipidemic rat liver homogenates following AT single and repeated dose administration

Rat liver homogenates (n = 4) were prepared in-house as IHL rat liver homogenates were not commercially available. Liver tissues were harvested under isoflurane anesthesia after perfusion. Perfusion was performed using Dulbecco's modified eagle's medium (DMEM) at 37°C by portal vein. AHL rat livers were harvested at 2 h post dose of AT treatment of single (day 1) and repeated doses (consecutive 7 days) from G7 and G8. G7: AHL group (n = 4) on day 1 livers were harvested at 2 h AT post dose; G8: AHL group (n = 4) on day 7 livers were harvested at 2 h AT post dose. The homogenation procedure was as detailed, isolated rat liver tissues were diluted with DMEM buffer; 1 part of liver with 4 parts of DMEM buffer and homogenized at 10,000 rpm for 5 min using ultra-Turrax® homogenizer (IKA) at 37°C. Neat CLR (10 µM) was incubated with AT treated induced hyperlipidemic rat liver homogenate (IHLMLR) at 37°C. A 100 µL of IHLMLR homogenate was quenched with 200 µL of acetonitrile at 0, 0.083, 0.25, 0.5, 0.75 and 1 h. All samples were centrifuged and stored at -70°C until analyzed by LC-MS/MS.

Excretion study

Effect of AT on CLR excretion following co-administration of AT and CLR single dose

The % dose excretion of CLR following co-administration of single dose of AT and CLR formulation was investigated. Study group details are as follows: Group 9: absence of hyperlipidemia or non-hyperlipidemic (NHL) rats were co-administered with AT and CLR at doses of 20 mg/kg; Group 10: IHL rats were co-administered with AT and CLR at doses of 20 mg/kg. For both groups the common bile duct and duodenum were cannulated using PE-10 and 50 catheters under light isoflurane anesthesia. A surgical probe was used to tunnel subcutaneously to the dorsal scapular region where each catheter was then exited from the body. The exit incision and abdominal cavity were sutured as soon as the bile could be seen flowing freely through the catheter. A U-shaped coupler (stainless steel) was then connected to both catheters to maintain normal flow of bile to the intestines during the recovery period. Injectable analgesics were given as needed and provided 72 h recovery period for cannulated animals. The cannulated rats were installed into metabolic cages with utilities to separate feces and retain urine at a temperature of 4 ± 2°C. Bile and urine samples were collected at pre-determined time points after oral gavage dosing and simultaneously, blank bile was infused at a rate of 1.3 ml/h (Hess et al., 1999; Madeleine et al., 1998) through duodenum (non-vascular) catheter by using infusion pump (Model 11, Harvard apparatus). Cumulative bile and urine samples were stored at -70°C until bioanalysis.

Calculation of % dose excreted from bile and urine

After oral administration of CLR, the % CLR recovered (IHL Vs NHL: CLR alone and co-administration of AT) in bile and urine was calculated by cumulative dose excreted at respective time points/the actual dose administered to rat × 100 for bile and urine.

LC-MS/MS analysis

The plasma concentrations of CLR were determined using a LC-MS/MS method. Briefly, 10 µl of erythromycin, as an internal standard and a 0.2 ml aliquot of acetonitrile were mixed with a 50 µl of plasma sample and same method was employed for bile and urine. The resulting mixture was then vortex mixed for 2 min and centrifuged at 10,000 rpm for 10 min. A 100 µl aliquot of the supernatant was injected (injection volume, 10 µl) into the LC-MS/MS system. The chromatographic separations were achieved using Waters, discovery C18 column (4.6 × 50 mm, 5 µm). The mobile
phase consisted of 30% of 5 mM ammonium formate and 70% of acetonitrile containing 0.1% formic acid. The flow rate of the mobile phase was maintained at 0.5 ml/min. Chromatography was performed at 40°C, which was set by an HPLC column temperature controller. CLR and internal standard were eluted with retention times of 3.47 and 1.87 min, respectively. A linear correlation coefficient of ≥0.99 was obtained with calibration range of 20 to 3000 ng/mL. A 10-fold dilution was applied for the calibration range. The operational parameters for the tandem mass spectrum of analyte were obtained after running in quantitative optimization mode. The turbo ion spray setting and collision gas pressure were obtained (IS voltage: 5500 V, temperature: 550°C, nebulizer gas: 37 psi, heater gas: 45 psi, curtain gas: 20 psi). The clean chromatogram of matrix blank obtained from the injection of a matrix blank extract immediately after an upper limit of quantitation (ULOQ) sample demonstrated that this method had neither injector carryover nor analytical column carryover.

Pharmacokinetic analysis

The plasma concentration data were analyzed using a noncompartmental method on WinNonlin software version 2.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (Kd) was calculated by the log linear regression of CLR concentration data during the elimination phase, and the terminal half-life (T1/2) was calculated by 0.693/Kd. The peak plasma concentration (Cmax) and time to reach the peak concentration (Tmax) of CLR in the plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration–time curve (AUC0–t) from time zero to the time of the last measured concentration (Clast) was calculated using the linear trapezoidal rule. The AUC zero to infinite (AUC0–∞) was obtained by adding AUC0–t and the extrapolated area as determined by Clast/Kd.

Statistical analysis

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by a student’s paired t-test with a two tailed distribution (performed by Graph pad prism 6.0). The differences were considered significant at a level of P < 0.05 for 10, 20, and 100 mg/kg. All mean values are presented with their standard deviation (Mean ± SD).

RESULTS

The main objective of this work was to investigate the severity of IHL on CLR pharmacokinetics and the severity of IHL was evaluated by AT, following single (day 1) dose and repeated (up to day 7) dose for 7 consecutive days by oral gavage administration, followed by single oral CLR administration to evaluate CLR pharmacokinetics in IHL rats. Hyperlipidemia was successfully induced by standard cholesterol diet along with butter, twice a day administration. Considered and confirmed successful induction of hyperlipidemia by examination of TC levels (TC > 150 mg/dl) and TG levels (TG > 100 mg/dl) levels and results are presented in Table 1.

Effect of AT on CLR pharmacokinetics in IHL following single AT (day 1) and repeated AT (up to day 7) dose administration followed by single dose of CLR

The mean plasma concentration–time profiles of CLR after concomitant oral administration of AT (20 mg/kg; dosed for single as day 1 and repeated up to day 7) following CLR (10, 20 and 100 mg/kg; only single dose) to IHL rats are shown in Figures 1 to 3. The corresponding pharmacokinetic parameters are shown in Table 2. The effect of AT on IHL found that CLR pharmacokinetic parameters were significantly recovered and the same was re-evaluated by administration of AT on day 1 and up to day 7 (negative control for hyperlipidemia). The Cmax and AUC0–t of clarithromycin on day 1 were found to be 0.56 ± 0.06 μg/ml and 2.4 ± 0.59 μg h/ml for 10 mg/kg; 1.6 ± 0.20 μg/ml and 9.48 ± 3.05 μg h/ml for 20 mg/kg; 14.90 ± 3.87 μg/ml and 113.00 ± 41.40 μg h/ml for 100 mg/ml, respectively and similarly on day 7, clarithromycin Cmax and AUC0–t was 0.47 ± 0.08 μg/ml and 1.81 ± 0.44 μg h/ml for 10 mg/kg; 1.34 ± 0.12 μg/ml and 5.61 ± 0.66 μg h/ml for 20 mg/kg; 12.10 ± 3.30 μg/ml and 67.40 ± 16.9 μg h/ml for 100 mg/kg, respectively. The AUC0–t and the AUC0–∞ of CLR were significantly (P < 0.05, 20 and 100 mg/kg) decreased. The percentage differences of Cmax and AUC0–t are 84 to 69%, 84 to 65% and 81 to 59%, respectively, in IHL AT day 7 group (given oral AT repeated dose up to 7 days followed by CLR single dose) as compared with the IHL AT day 1 group (given oral co-administration of CLR and AT on day 1). IHL rats treated with AT up to day 7 followed by single dose of CLR exposures were similar to NHL group (data was not shown). There was no significant difference in the Tmax, MRT and the T1/2 across the groups. CLR kinetic exposure was high in IHL rats and after single AT treatment, it was slightly recovered to normal and completely recovered on day 7 AT post dose.

In vitro CLR metabolism in induced hyperlipidemic rat liver homogenates following AT single and repeated dose administration

AT effect on CLR metabolism was evaluated in IHLMRL homogenates and livers were harvested at 2 h post dose of AT as a single (day 1) and repeated (up to day 7) oral administration. Thereafter livers were homogenized and incubated at 37°C with CLR at 10 μM. Results were summarized in Table 3. The CLR metabolism at 5 and 15 min was 6 and 18% for IHLMRL homogenates on day 1, similarly 17 and 33% for IHLMRL homogenates on day 7, respectively. At 0.5 and 0.75 h was reported 40 and 66% for day 1 and 58 and 81% for day 7, respectively and finally at 1 h post dose the % metabolism was noted as 80% for day 1 and 87% for day 7.

The outcome of in vitro study on CLR metabolism in
IHLMLR homogenates supports that hyperlipidemia was a rate limiting step for CLR metabolism and also evaluated the CLR metabolism with AT post dose treatment on day 1 and 7. IHLMLR were harvested at respective days of AT post dose treatment at 2 h and homogenized. The same was incubated with CLR to know the % remaining and % metabolism at respective intervals results states that at 0.083 h, the % metabolism was very rapid in all treated groups that is, almost 50% was higher in IHL group on day 7 than day 1 and later on significantly decreased to 25 to 30% of CLR metabolism in day 7 as compared to day 1 group. Therefore, the difference in metabolism was low among all groups. So, based on the results, we can also conclude that hyperlipidemia have an impact on CLR pharmacokinetics and same was confirmed with AT single dose (day 1) and repeated dose administration (day 7). AT concludes that CLR metabolism was recovered and up-regulated on day 7 as compared with day 1 in induced HLM rat liver homogenates.

Effect of IHL and AT on the CLR biliary excretion

As in vivo is a metabolically intact system than in vitro, we have performed a study to support the severity of hyperlipidemia on the CLR pharmacokinetics in awakening rats cannulated with bile duct to study the biliary excretion of CLR. The study was performed in presence and absence of HL and AT (single administration) was used as negative control for hyperlipidemia. We also reported the CLR percentage dose recovered in rat bile after co-administration of single AT with CLR in presence and absence of hyperlipidemia. Table 4 represents the mean cumulative biliary concentration (ng/ml) for 0 to 6 h, mean % biliary excretion of CLR (20 mg/kg) after oral co-administration of AT (20 mg/kg, only single dose) and CLR in presence and absence of hyperlipidemia. The cumulative biliary excretion of CLR in NHI (non-hyperlipidemic) rats treated with CLR was 123 ± 16.8 µg/ml and similarly in IHL rats was 172 ± 14.2 µg/ml which demonstrated higher significant (P > 0.05, 20 mg/kg)

Table 2. Pharmacokinetic parameters of clarithromycin after the oral concomitant administration of atorvastatin (20 mg/kg, single and repeated up to day 7) following clarithromycin single dose (10, 20, and 100 mg/kg; n=6, mean ± SD) to rats in the presence of induced hyperlipidemia.

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>Induced hyperlipidemia + CLR + AT day 1</th>
<th>Induced hyperlipidemia + CLR + AT day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>0.56±0.026</td>
<td>1.60±0.201</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.67±0.261</td>
<td>0.67±0.261</td>
</tr>
<tr>
<td>AUC0-1 (µg*h/ml)</td>
<td>2.40±0.590</td>
<td>9.48±3.05</td>
</tr>
<tr>
<td>AUC0-∞ (µg*h/ml)</td>
<td>3.13±0.82</td>
<td>10.6±3.25</td>
</tr>
<tr>
<td>AUC % extrapolated</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.16±0.250</td>
<td>4.33±1.10</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>3.74±0.402</td>
<td>4.10±0.911</td>
</tr>
</tbody>
</table>

AUC0-∞ Area under the plasma concentration–time curve from 0 h to infinity, Cmax peak plasma concentration, Tmax time to reach Cmax, T1/2 terminal half-life, MRT Mean resident time calculated by AUMC/AUC; AUMC Area under the first movementam curve ; * P<0.05 significant difference compared with day 1 of respective groups.

Table 3. Clarithromycin % remaining and metabolism in induced hyperlipidemic rat liver (IHLMLR) tissue homogenates after oral co administration of Atorvastatin (dose: 20 mg/kg) on day 1 and day 7.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IHL_RLH treated AT day 1</th>
<th>IHL_RLH treated AT up to day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% remaining</td>
<td>% metabolism</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>0.083</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>0.25</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>0.5</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>0.75</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

*P<0.05, significant difference compared with day 1; IHLMLR homogenates treated AT day 1: hyperlipidemic rat liver homogenates (n=4) were harvested 2h post dose of atorvastatin treatment as a single dose on day 1; IHLMLR homogenates treated AT up to day 7: induced hyperlipidemic rat liver homogenates (n=4) were harvested 2 h post dose of atorvastatin treatment for consecutive 7 days. CLR incubated at 10 µM.
Table 4. Biliary excretion of clarithromycin after the oral co-administration of atorvastatin (20 mg/kg) to rats in the presence or absence of hyperlipidemia.

<table>
<thead>
<tr>
<th>Mean parameter</th>
<th>NHL+CLR+AT</th>
<th>IHL+CLR+AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative concentration (µg/ml)</td>
<td>123±16.8</td>
<td>172±14.2*</td>
</tr>
<tr>
<td>Total bile (ml)</td>
<td>12.0±0.131</td>
<td>16.3±0.382*</td>
</tr>
<tr>
<td>Total Amount (mg)</td>
<td>1.47±0.210</td>
<td>2.80±0.191</td>
</tr>
<tr>
<td>Rat body weight (kg)</td>
<td>0.290±0.01</td>
<td>0.272±0.01</td>
</tr>
<tr>
<td>Dose per rat (mg)</td>
<td>5.80±0.182</td>
<td>5.44±0.251</td>
</tr>
<tr>
<td>% Dose excreted</td>
<td>25.1±3.80</td>
<td>51.1±1.62*</td>
</tr>
</tbody>
</table>

NHL: non-hyperlipidemia, CLR: clarithromycin, IHL: induced hyperlipidemia, AT: atorvastatin; * P < 0.05, significant difference compared with controls (NHL+CLR+AT).

Table 5. Urinary excretion of clarithromycin after the oral administration of clarithromycin alone (20 mg/kg; n = 4, mean ± SD) and concomitant administration of atorvastatin (20 mg/kg) and clarithromycin (20 mg/kg) to rats in the presence or absence of hyperlipidemia.

<table>
<thead>
<tr>
<th>Mean parameter</th>
<th>NHL+CLR+AT</th>
<th>IHL+CLR+AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative concentration (µg/ml)</td>
<td>189±16.2</td>
<td>206±30.6</td>
</tr>
<tr>
<td>Total Urine (ml)</td>
<td>13.6±0.64</td>
<td>12.0±0.29</td>
</tr>
<tr>
<td>Total Amount (mg)</td>
<td>2.56±0.15</td>
<td>2.47±0.36</td>
</tr>
<tr>
<td>Rat body weight (kg)</td>
<td>0.290±0.01</td>
<td>0.272±0.01</td>
</tr>
<tr>
<td>Dose per rat (mg)</td>
<td>5.80±0.18</td>
<td>5.44±0.25</td>
</tr>
<tr>
<td>% Dose Excreted</td>
<td>44.0±3.32</td>
<td>46.0±6.15</td>
</tr>
</tbody>
</table>


Effect of IHL and AT on the CLR urinary excretion

Table 5 represents the mean cumulative urinary concentration (ng/ml) for 0 to 6 h and mean % urinary excretion of CLR (20 mg/kg) followed by co-administration of AT single dose (20 mg/kg) in presence and absence of IHL. The cumulative urine concentrations found in NHL and IHL groups were 189 ± 16.2 and 206 ± 30.6 µg/ml, respectively. NHL rats treated with CLR and AT exhibited no significant difference in mean percentage urinary excretion as compared with IHL rats treated with CLR and AT (44 and 46%, respectively).

DISCUSSION

We aimed to explore CLR pharmacokinetics in presence of IHL and compared with RHL which was achieved by AT repeated dose. IHL was successfully achieved by high fat standard cholesterol diet (Prasanna et al., 2009). Hyperlipidemia is a state of elevated levels of lipoproteins like total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL) etc. Hyperlipidemia proved alteration of unbound fraction clearance and volume of distribution of ketoconazole to be a high potent CYP inhibitor (Kobayashi et al., 2002).

In disease state, physiological changes can influence drug pharmacokinetics, although the mechanism remains to be elucidated. In many critically ill patients, extracellular fluids have increased, possibly resulting in a higher volume of distribution that might affect pharmacokinetics (Gomez et al., 1999). One should bear in mind that findings in pharmacokinetics interaction studies performed in healthy volunteers might not be observed in clinical practice in specific patient populations. Furthermore, pharmacokinetics interaction studies administering both single doses and multiple doses to study subjects were used in this overview. It need hardly be mentioned that multiple-dose studies will reflect best clinical practice. This is particularly true for pharmacokinetics interaction studies with biotransformation as possible underlying mechanism, since induction of enzyme systems might require days to weeks to develop fully (Stockley, 2011). The interaction may also persist at a similar length of time when the inducing agent is stopped. Unlike induction, inhibition of enzyme systems can occur within 2 to 3 days.
Figure 1. Mean plasma concentration-time profiles of clarithromycin (10 mg/kg) following co-administration of atorvastatin (20 mg/kg) in the presence of induced and reduced hyperlipidemia in rats. Concomitant administration of clarithromycin and atorvastatin on day 1 in induced hyperlipidemic (HL) rats at 10 mg/kg (▲), repeated administration of atorvastatin up to 7 days followed by clarithromycin single dose in reduced hyperlipidemic (HL) rats at 10 mg/kg (△).

Figure 2. Mean plasma concentration-time profiles of clarithromycin (20 mg/kg) following co-administration of atorvastatin (20 mg/kg) in the presence of induced and reduced hyperlipidemia in rats. Concomitant administration of clarithromycin and atorvastatin on day 1 in induced hyperlipidemic rats at 20 mg/kg (♦), repeated administration of atorvastatin up to 7 days followed by clarithromycin single dose in reduced hyperlipidemic rats at 20 mg/kg (◊).
Figure 3. Mean plasma concentration-time profiles of clarithromycin (100 mg/kg) following co-administration of atorvastatin (20 mg/kg) in the presence of induced and reduced hyperlipidemia in rats. Concomitant administration of clarithromycin and atorvastatin on day 1 in induced hyperlipidemic rats at 100 mg/kg (■). Repeated administration of atorvastatin up to 7 days followed by clarithromycin single dose in reduced hyperlipidemic rats at 100 mg/kg (□).

The current study was planned, as they have reported that hyperlipidemia showed a significant impact on PK including potent CYP inhibitors. Henceforth, we have selected CLR which is a potent CYP inhibitor and its kinetics have been evaluated in IHL rat models following single and repeated dose for consecutive 7 days of AT. Our previous study demonstrated that hyperlipidemia has an impact on CLR kinetics as compared with presence and absence of HL (data not shown). This study is concentrated only to monitor the reversal effect of hyperlipidemia following AT treatment as a single and repeated dose for 7 consecutive days.

AT is an anti-hyperlipidemic drug used to reduce hyperlipidemia and compared clarithromycin pharmacokinetics with hyperlipidemic (single post dose) and reduced hyperlipidemic (7 days consecutive dose) rat models. Since no studies were reported on CLR pharmacokinetics in presence of IHL and RHL rat models, we performed the experiment in rat as a model to study the effect of AT on CLR pharmacokinetics in IHL following single AT (dose: 20 mg/kg; day 1) and repeated dose of AT (dose: 20 mg/kg; up to day 7) administration followed by single dose of CLR at three dose levels (Dose: 10, 20 and 100 mg/kg) to monitor dose proportionality in hyperlipidemic groups. The current study revealed that CLR AUC₀₋₄ at the doses of 20 and 100 mg/kg was significantly low (P < 0.05) on day 7 than day 1 but no significant different was observed at lower dose (10 mg/kg) within the days (day 7 and 1). The MRT and T₁/₂ was not altered in induced and reduced hyperlipidemic rat models. Hyperlipidemia potentiates the anti-microbial effect by increasing the plasma concentration of CLR and same hyperlipidemic effect was supported by publication (Eliot et al., 1999; Shayeganpour et al., 2005; Prasanna et al., 2014).

Our current study clearly shows that in vitro metabolism of CLR in IHLRRL homogenates significantly lower (P < 0.05) at 10 µM on day 1 as compared with day 7. Current data supporting CLR metabolism was altered in the presence of IHL which might be down regulation of CYP mediated metabolism and changes of unbound drug concentrations. However, previous publications (Eliot et al., 1999; Joo-Hyun et al., 2012) substantially emphasized that hyperlipidemia altered the liver and intestine metabolism and unbound drug concentrations which supports our observations. In addition to this, we reported CLR biliary and urinary cumulative excretion and percentage of dose excreted in bile and urine after co-administration of AT and CLR in presence and absence of induced HL. The groups without AT treatment exhibited higher levels of CLR excretion through bile (data not shown). The reported data concludes that the % dose...
excretion of CLR in IHL rat was significantly higher ($P < 0.05$) as compared with NHL rats. Increased levels of CLR cumulative urine concentrations were observed in IHL rats but not significantly different from NHL rats.

Overall, results demonstrated that CLR pharmacokinetics was significantly altered in plasma and bile by induced hyperlipidemia and the same was concluded from in vitro rat induced liver homogenates. So, we need to further evaluate or focus on particular transporters involved or responsible for the uptake or efflux in the presence of hyperlipidemia. Results obtained from in vitro and in vivo studies were co-related and concludes that CLR pharmacokinetics in induced hyperlipidemic rats models after AT post dose (Day 1 and 7) are significantly altered and might be the reason of change of fraction unbound drug concentration, increased volume of distribution by lowering the clearance and alteration of transport mechanisms.

**Conclusion**

It can be concluded that the hyperlipidemic stages like induced hyperlipidemia, reduced hyperlipidemia and absence of hyperlipidemia causes down/up regulations of CYP mediated metabolism in the liver (AT post treatment) which leads in term to high/low systemic availability of CLR. Hyperlipidemia might have an impact on CLR free drug concentrations. Therefore, a more detailed investigation is required to get a clearer understanding of transporters involved and affected in presence of induced hyperlipidemia in rat and other species as well as for safety perspective.

**ACKNOWLEDGMENTS**

The author was thankful to Wockhardt Research Centre for providing the necessary laboratory facilities to carry out the present research work.

**Conflict of Interest**

The author(s) have not declared any conflict of interests

**REFERENCES**


Discriminating power of dissolution medium in comparative study of solid dispersion tablets of Biopharmaceutics Classification System class 2 drug

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**In vitro** dissolution testing should discriminate between the impact of excipients on release profile of Biopharmaceutics Classification System class 2 drugs. Among the four batches of carvedilol solid dispersion tablets, SDT-2-L containing 30% w/w mannitol and 2.5% w/w L-hydroxypropyl cellulose LH-11 exhibited least mean dissolution time in gastric buffer (pH = 1.2) and phosphate buffer (pH = 6.8). However, inter-batch differences in release parameters from phosphate buffer were usually higher and also statistically significant at p < 0.05. Drug release from an individual formulation exhibited different kinetics in two media. Therefore, improper selection of dissolution medium may fail to discriminate between product performances.

**Key words:** Solid dispersion tablet, dissolution efficiency, mean dissolution time.

INTRODUCTION

For drugs belonging to Biopharmaceutics Classification System (BCS) Class 2, strong correlation exists between **in vitro** dissolution and **in vivo** absorption. Dissolution procedure should be able to detect, discriminate and predict the effect of minor changes in product composition on the **in vivo** performance. The discriminatory power of the dissolution medium is affected by its pH, speed of agitation, presence of surfactants in the medium etc. Selection of the most appropriate dissolution condition depends on the discriminatory ability, accuracy, reproducibility, robustness and level of correlation with **in vivo** performance. Products possessing different pharmaceutical attributes (for example, formulation variables or process variables) can be appropriately differentiated with the help of discriminatory dissolution profiles (Qureshi, 2006; Bajerski et al., 2010; Hurtado et al., 2012; Lagace et al., 2004).

Carvedilol, an anti-hypertensive drug and a member of BCS Class 2 suffers from low bioavailability following oral administration due to its poor aqueous solubility (Shete et al., 2012). Solubility improvement can be achieved by fabrication of tablets from polyethylene glycol 6000 (PEG 6000)-hydroxypropylmethyl cellulose (HPMC)-Tween 80(T-80) based solid dispersion of carvedilol using...
various excipients in definite proportions by direct compression (Dannenfelser et al., 2004). The objective of the present study was to compare the dissolution profiles of the various batches of carvedilol solid dispersion tablets and to identify the discriminatory power of the dissolution medium based on pH, in assessment of the effect of excipients like mannitol and L-hydroxypropyl cellulose LH-11 (L-HPC LH11) on dissolution process related parameters. For treatment and analysis of data, both model-dependent and model-independent approaches were adopted and parameters like dissolution efficiency, mean dissolution time, $Q_{\text{av},\text{mins}}$ and $T_{95\%}$ were determined.

METHODOLOGY

Formulation of carvedilol solid dispersion tablets

The quaternary solid dispersion (Carvedilol: Polyethylene glycol 6000: HPMC: T-80= 1.8:675:0.075:0.25) was processed by melting-solvent evaporation technique. The ratios are expressed as weight/weight. Enhancement in solubility and improvement in dissolution characteristics of the solid dispersion (SD) compared to pure drug was investigated in water and the probable mechanism was also studied with the help of X-ray diffraction, differential scanning calorimetry and scanning electron microscopy. Four batches of carvedilol tablets were fabricated from quaternary solid dispersion by direct-compression technique using Avicel PH102, dicalcium phosphate dihydrate, mannitol, magnesium stearate as excipients. The batches differed in the percentages of mannitol and L-HPC LH-11 and were represented as SDT-1 [Mannitol: 19.75% w/w], SDT-1-L [Mannitol: 19.75% w/w; L-HPC: 2.5% w/w], SDT-2 [Mannitol: 30% w/w] and SDT-2-L [Mannitol: 30% w/w; L-HPC: 2.5% w/w]. Carvedilol and HPMC were obtained as gift samples from Zydus Cadilla, Mumbai, India and Colorcon, respectively. L-HPC LH-11 was generously provided by Mylan Laboratories, Hyderabad. All the reagents and chemicals used were procured from Merck, India. Prior to compression, drug-excipient compatibility study was carried out. Powder flow behavior was characterized by angle of repose, compressibility index and Hausner ratio. Excipients were dried and sieved through mesh no. 60. Solid dispersion tablet batches were prepared by mixing the various ingredients and finally compressed in 10-station Minipress single punch tablet machine (Karnavati Engg. Pvt. Ltd., India) to produce round, flat-faced tablets, each designed to weigh around 180 mg ± 5% and contain 12.5 mg of carvedilol. The tablet shape, size, thickness and hardness were held constant for all the batches.

Evaluation of carvedilol solid dispersion tablets

Determination of wetting time

A twice-folded tissue paper (10.75 mm × 12 mm) was placed in two separate 6.5 cm diameter culture dishes containing definite volume of gastric buffer (pH = 1.2) and simulated saliva (phosphate buffer, pH = 6.8). Two drops of water soluble dye eosin was added to both the media and observations carried out at 37°C. Two tablets were carefully placed on the surface of tissue paper in the two dishes and the time required for the two buffers to reach the upper surface of the tablet was noted as the wetting time. The experiments were repeated thrice in both cases.

Determination of in vitro disintegration time

Disintegration time for the tablets was determined using digital US Pharmacopeial (USP) disintegration test apparatus (Voego India Pvt. Ltd., India) with 900 ml of gastric buffer (pH = 1.2) and simulated saliva (phosphate buffer, pH = 6.8) as the disintegrating medium. Temperature was maintained at 37°C.

In vitro dissolution study

In vitro drug dissolution of all tablet batches was carried out using USP-type II dissolution apparatus (paddle type) (8-station dissolution test apparatus, LABINDIA Model No. DS-8000). The dissolution medium [900 ml gastric buffer (pH = 1.2) or phosphate buffer (pH = 6.8)] was placed into the dissolution vessel maintained at 37 ± 0.5°C and 50 rpm and dissolution studies were carried out for 180 and 300 mins, respectively. Aliquot of 10 ml was withdrawn, replenished with fresh medium, filtered and analysed spectrophotometrically at 240 nm in both cases. The absorbance values were transformed to concentration using calibration curves in corresponding medium obtained experimentally ($r^2 = 0.9878$ and 0.996, respectively). All tests were done in triplicate.

Comparison of in vitro dissolution data

For comparison of dissolution profiles, model-independent approaches based on the ratio of area under the dissolution curve (dissolution efficiency) or mean dissolution time were adopted. The differences between the drug release data of the tablet batches were compared using Tukey test. The significance level ($\alpha = 0.05$) was based on the 95% probability value ($p < 0.05$). The dissolution Efficiency (DE %) was used to evaluate the dissolution performance of the batches in comparison to the marketed formulation. DE was calculated as follows (Costa et al., 2001).

$$\text{% DE} = \frac{\int_0^t Y \, dt}{Y_{100} t} \times 100$$

Where $y$ is the percentage of drug dissolved at time $t$. DE was determined for the entire time period of release study for each batch. The mean in vitro drug release data (n = 3) from 0 to 85% release were fitted to different kinetic models (first order, Higuchi and Hixon-Crowell). The value of the coefficient of determination ($R^2$) was selected as the criterion to identify the best-fit model of drug release from the tablets. The mean dissolution time (MDT) for each batch has been determined with the help of the following equation (Costa et al., 2001).

$$\text{Mean Dissolution Time (MDT)} = \frac{\sum_{j=1}^{n} t_{j} \cdot \Delta M_{j}}{\sum_{j=1}^{n} \Delta M_{j}}$$

Where $j$ is the sample number, n is the number of dissolution sampling points, $t_{j}$ is the time at midpoint between $t_{j-1}$ and $t_{j}$, [calculated as $(t_{j-1}+t_{j})/2$] and $\Delta M_{j}$ is the additional percentage of drug released in the time interval between $t_{j-1}$ and $t_{j}$ Other release parameters used to characterize and compare dissolution profiles.
for tablet batches include cumulative percent released at x mins [Qx(mins)%] and time taken for a fixed percentage of drug to be released [TQx% (mins)]. The results obtained in two buffers are displayed in Table 1.

**RESULTS**

Presence of mannitol in higher percentage (30% w/w) and simultaneous addition of 2.5% w/w L-HPC LH-11 in SDT-2-L promotes minimum wetting time and fastest disintegration in 7 mins 29.44 ± 14.91 s in phosphate buffer (pH = 6.8) (Table 1). Similar pattern in wetting time and disintegration time was also observed with the study in gastric buffer (pH = 1.2) Table 1. It is to be noted that wetting and disintegration occurred comparatively faster in acidic pH. Positive influence of L-HPC LH-11 as well as synergistic effect of optimum percentages of mannitol and L-HPC on wetting and disintegration is clearly evident from the data. Compared to the batches containing both mannitol and L-HPC LH-11 (that is, SDT-1-L and SDT-2-L), the batches containing mannitol only (that is, SDT-1 and SDT-2) were found to perform poorly with respect to the aforementioned parameters in both the buffers. Lower percentage of mannitol along with absence of L-HPC in the batch SDT-1 resulted in slow wetting and disintegration in the buffers tested. However, increasing the percentage of mannitol to 30% in the batch SDT-2 improved the wetting and disintegration profile.

Analysis of dissolution profiles in two media with respect to Q45(mins), T75%, and mean dissolution time (MDT) showed that the differences observed among the batches were higher in phosphate buffer (Costa et al., 2001). There was 5-fold difference in MDT and 7-fold difference in T75% between SDT-2-L and SDT-1 in phosphate buffer. However, the difference in both of the aforementioned parameters between the same pair of formulations was 3-fold, respectively in gastric buffer (pH = 1.2) (Figures 1 and 2). Comparison of the drug release data for all the four batches in the buffers studied revealed that with SDT-2-L, MDT was least, Q45(mins) was highest and T75% could be achieved fastest and just the reverse was observed with SDT-1. SDT-1 was found to possess exceptionally high MDT of 139.26 min in phosphate buffer. This indicated that comparable disintegration time between the formulations may not always predict comparable dissolution profiles. It can be concluded that presence of mannitol and L-HPC LH-11 in the batch SDT-2-L in optimum percentage promoted not only rapid wetting and disintegration but also comparatively faster and complete dissolution in contrast to other batches. Therefore, SDT-2-L can be considered as the best among the studied batches.

Tukey test procedure on data sets from phosphate buffer revealed that differences in release profiles among the four formulation batches were statistically significant at p < 0.05. But the differences between the means of cumulative release data in gastric buffer were statistically insignificant. All the formulations, except SDT-1, were found to possess similar values of dissolution efficiency (DE) in both the media, with slightly higher magnitudes in phosphate buffer. SDT-1 possessed less than 40% DE in gastric buffer, indicating incomplete and very poor drug release. Since SDT-1 was found to have least DE (%) in both the buffers, it can be concluded that inclusion of 19.75% w/w mannitol in the formulation failed to produce fast disintegration and dissolution. Summing up and comparing the results in both the media showed that mannitol percentage as well as presence of L-HPC in the formulations played a crucial role in dispersion tablets and can be successfully used to improve solubility-limited

### Table 1. Data for determination of wetting, disintegration time and drug release studies in phosphate buffer (pH 6.8) at 37°C (Values in the parentheses indicate mean ± standard deviation; n=3).

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Wetting time (min: 0.0 s)</th>
<th>Disintegration time (min: 0.0 s)</th>
<th>In gastric buffer (pH = 1.2)</th>
<th>Drug release data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DE (%)</td>
<td>MDT (mins)</td>
<td>Q45(mins) (%)</td>
<td>T75% (mins)</td>
</tr>
<tr>
<td>SDT-1</td>
<td>34.92</td>
<td>56.84</td>
<td>60.66</td>
<td>75</td>
</tr>
<tr>
<td>SDT-1-L</td>
<td>54.2</td>
<td>19.81</td>
<td>97.99</td>
<td>30.5</td>
</tr>
<tr>
<td>SDT-2</td>
<td>47.96</td>
<td>20.41</td>
<td>88.45</td>
<td>33</td>
</tr>
<tr>
<td>SDT-2-L</td>
<td>46.13</td>
<td>17.13</td>
<td>100% in 41 mins</td>
<td>28</td>
</tr>
</tbody>
</table>
Figure 1. Drug release profiles of four batches of Carvedilol solid dispersion tablets in gastric buffer (pH1.2) and comparative study of DE and MDT of the formulations in the same medium.

In gastric buffer, all the tablet batches exhibited Higuchi kinetics. This indicates that drug release occurred from constant area planar surface and tablet is granular in nature, which is overruled by the direct compression method of manufacture used in the study as well as the rupture of tablets into smaller free particles during dissolution. The batches SDT-1, SDT-2 and SDT-1-L obeyed first-order kinetics in phosphate buffer whereas the best formulation, SDT-2-L followed Hixon-Crowell kinetics assuming dissolution-limited drug release rate, as is expected from a tablet of BCS Class 2 drug. Based
on the in vitro drug release data of the four batches in the two buffers, the order of the formulations may be represented as SDT-2-L > SDT-1-L > SDT-2 > SDT-1. But the magnitude of difference among the parameters differs greatly. Therefore, dissolution medium is found to affect the rate, kinetics and performance efficiency of dissolution process of carvedilol tablets. Unless a proper dissolution medium is selected, it is not possible to explain and characterize the influence of varying percentages of excipients on the performance of the various batches of solid dosage forms. Moreover, improper selection of dissolution medium may also misinterpret drug release kinetics from the formulations.

DISCUSSION

Mannitol is used in tablet manufacture to improve mouth-feel properties and promote swelling-induced faster disintegration and/or dissolution whose action is further accentuated by addition of low percentage of hydrophilic low-substituted cellulose ether, like L-hydroxypropylcelluloses (L-HPC). Synergistic effect of increasing percentage of mannitol and constant level of L-HPC on wetting time and oral disintegration time is observed progressively in the four tablet batches. Similar pattern was followed during in vitro release study from the tablets in phosphate buffer. However, gastric buffer as the dissolution medium failed to discriminate between the effects of the excipients on the in vitro performance of the tablet batches studied. Therefore, characterization and proper gradation of different tablet formulations of a BCS Class 2 drug essentially depends on the selection of a dissolution medium possessing high discriminatory power.

REFERENCES

Full Length Research Paper

Ameliorating effects of thymoquinone in rodent models of schizophrenia

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The present study was carried out to evaluate the role of thymoquinone (TQ) in animal models of schizophrenia. TQ (20 mg/kg, intraperitoneally) was administered daily for 28 days in mice. Different models of schizophrenia such as haloperidol-induced catalepsy, apomorphine-induced climbing behaviour and elevated plus maze test were used. After the last dose of TQ on the 28th day, behavioural tests were performed followed by biochemical estimations. Pre-treatment of TQ alone and in combination with haloperidol observed cataleptic behaviour. In apomorphine induced climbing behaviour model, administration of TQ reduced maximum time of single climb and climbing index (p < 0.001). Scopolamine-induced prolongation of transfer latency (TL) was reduced by TQ (p < 0.001). There was no change in the percentage alternation in TQ pre-treated group of animals in elevated plus maze test. However, a significant increase in possible alternation was observed (p < 0.001), suggesting its anti-amnesic effect. The anti-amnesic effect of TQ was further confirmed with a decrease in acetyl cholinesterase (AChE) enzymatic activity in mice brain. A decrease in thiobarbituric acid reactive substance (TBARS) levels and increase in glutathione (GSH) and catalase levels were observed in all models used, thus, confirming its antioxidant properties. TQ administration also showed reduction in dopamine levels indicating the involvement of dopamine receptors in all three models; hence, demonstrating its antipsychotic like activities. The present study observed antipsychotic like actions in different animal models of schizophrenia and also improved memory. Our results are preliminary, further research is warranted to establish its role as a new candidate in schizophrenia.

Key words: Schizophrenia, thymoquinone, dopamine, antioxidant markers.

INTRODUCTION

Schizophrenia has been described as a psychotic disorder, characterized by impaired thinking, emotions and behaviour (Hans-Gert Bernstein et al., 2005; Akhtar et al., 2006a; Mahmood et al., 2012a). It is associated with anxiety and depression in about 10% of cases and is the leading cause of suicide (Range and Dale, 2008). The large majority of people with schizophrenia show substantial improvement when treated with antipsychotic
drugs. Better antipsychotic drugs were developed in recent years, producing much lesser side effects than the older traditional antipsychotic drugs.

Despite the availability of so many newer compounds, the refractory cases and adverse effects of antipsychotic drugs are the major concern in schizophrenic therapy. So, there is a need of new treatment strategy for schizophrenia with fewer side effects.

Thymoquinone (TQ) is the major bioactive constituent isolated from *Nigella sativa* seeds as the principle active ingredient from the volatile oil. The plant seeds also contain fixed oil (>30% wt/wt) and volatile oil (0.40 to 0.45%). The volatile oil contains 18.4 to 24.0% of TQ (Hosseinzadeh et al., 2007). TQ has demonstrated several pharmacological activities such as protection against chemical-induced carcinogenesis (Hassan et al., 1992), inhibition of eicosanoid generation and membrane lipid peroxidation (Houghton et al., 1995), analgesic and anti-inflammatory activity (Abdel-Fattah et al., 2000), anticonvulsant activity (Hosseinzadeh et al., 2004), down regulating tumor necrosis factor (El Mahmoudy et al., 2005), suppression of nuclear factor kappa B (NF-B) activation in brain and spinal cord (Mammad et al., 2005), neuroprotection (Al-Majed et al., 2006) and suppression of oxidative stress-induced neuropathy (Hamdy et al., 2009). *N. sativa* seed oil also showed anti-anxiety effects in rats due to an increase in brain serotonin levels (Perveen et al., 2009).

Nitric oxide (NO), an intercellular messenger in the brain generated from L-arginine by different isoforms of nitric oxide synthase (nNOS, iNOS and eNOS), plays an important role in various physiological and pathological processes (Hans-Gert Bernstein et al., 2005). El Mahmoudy et al. (2002) reported that TQ significantly suppressed the expression of inducible nitric oxide synthase (iNOS) in rat macrophages.

The enzyme iNOS plays a central role in the inflammatory reactions that follow infection, disease and tissue damage (Hans-Gert Bernstein et al., 2005). Nitric oxide generated by iNOS plays a role in wide spectrum of diseases including septic shock, cerebral ischemia, multiple sclerosis and Alzheimer's disease (Fernandez-Vizarra et al., 2004). Since, schizophrenia is believed to have immune and inflammatory implications; the potential role of iNOS may be possible in schizophrenia (Hans-GertBerstein et al., 2005). TQ already reported the inhibition of iNOS earlier, may be an effective treatment for schizophrenia.

Reactive oxygen species (ROS) are generated as a result of various biochemical processes taking place in the living organisms. Toxic and harmful substances are removed via enzymatic and non-enzymatic anti-oxidative mechanisms. Conditions which alter this equilibrium may result an increase in oxidants and decrease antioxidants levels and the oxidative/anti-oxidative balance shifts towards oxidative stress status. Further, the role of oxidative stress has been implicated in the pathogenesis of schizophrenia (Lohr, 1991; Halliwell, 1994; Mahadick, 1996; Mahmood et al., 2012b) and some researchers already observed the reduction in anti-oxidant enzyme levels in the brains of schizophrenic patients (Halliwell, 1994; Benidicta, 2003). More recently different extracts of *Nigella sativa* reported anti-oxidant and free radical scavenger properties in animal model of stroke (Akhtar et al., 2012c, 2013d). With wide spectrum of pharmacological properties of TQ, the data on neuropsychiatric disorders is still scanty; so, the present study was planned to investigate the possible role of TQ in some animal models of schizophrenia.

**MATERIALS AND METHODS**

**Animals**

Albino mice 25 to 35 g were procured from the Central Animal House Facility of Hamdard University, New Delhi and acclimatized accordingly. The mice were maintained on a 12 h light/12 h dark cycle with free access to food. The mice were maintained on pellet feed and water ad libitum during the whole duration of study (28 days for each group). The study was approved by the Institutional Animal Ethics Committee (IAEC file No. 790.) of Jamia Hamdard (Hamdard University), New Delhi India.

**Drugs and chemicals**

Thymoquinone, haloperidol, apomorphine, and scopolamine were procured from Sigma Aldrich (USA) in pure form. Thymoquinone was dissolved in corn oil, whereas other drugs were dissolved in normal saline. All the chemicals and reagents used were of analytical grade.

**Methodology**

All the animals were divided into five groups of six animals each. Group I animals were corn oil treated; group II, normal control saline treated; group III, pathogenic control (haloperidol, apomorphine, or scopolamine); group IV, TQ per se; group V, co-administration of TQ with haloperidol, apomorphine, or scopolamine. TQ (20 mg/kg) was administered daily intraperitoneally (i.p) for 28 days, while scopolamine (0.5 mg/kg, i.p) was also administered intraperitonally, but as a single dose. Haloperidol was administered per orally (2 mg/kg, p.o) and apomorphine subcutaneously (1 mg/kg, s.c) as a single dose on the 28th day. Behavioural tests were performed on the 28th day, 1 h after the last dosing regimen. Animals were sacrificed after behavioural tests and the brains were removed for biochemical estimations.

**Haloperidol induced catalepsy in mice**

Haloperidol induced catalepsy was used to observe the negative symptoms in animals. It is the widely employed method for screening of neuroleptic drugs in rodents. Catalepsy was induced with haloperidol (2 mg/kg p.o) as described by Silva et al. (1995) and was determined every hour up to 4 h by means of a standard
bar test. The phenomenon was measured as the time when the mouse maintained an imposed position with both front limbs extended and resting on a 4 cm high bar (0.4 cm diameter). The total time during which animal stayed on the bar (even if it climbed back up) was recorded for a maximum period of 300 s.

**Apomorphine-induced climbing behaviour in mice**

The positive symptoms of schizophrenia were assessed using a dopamine agonist, that is, apomorphine. Apomorphine induced climbing behaviour was followed as previously described (Costall et al., 1978; Moore et al., 1992) with some modifications. Vehicle (corn oil) and TQ were administered 1 h and 2 h before the administration of apomorphine (1 mg/kg s.c.). Immediately after injection, the mice were then placed individually in cylindrical wire mesh cages (height 13 cm, diameter 14 cm, mesh size 3 mm). During the trial, the time when each mouse climbed on the inside of the wire cage was recorded over a 30 min period. Maximum time (that is, maximum time spent in a single climb throughout the duration of apomorphine effect) and percent climbing index (that is, the percent of time spent climbing during 30 min period following the first climb) were recorded.

**Elevated plus-maze test in mice**

The plus-maze test was used to study the drugs affecting learning and memory. The plus maze was constructed from acrylic resin, and consisted of two open arms (5 × 30 cm) and two enclosed arms (5 × 30 × 15 cm) facing each other. The entire apparatus was elevated to a height of 40 cm above the floor. The open arms and central platform were coloured white and covered with cellophane, and the enclosed arms were coloured black. On day 1, an acquisition trial was performed as follows: the mice were placed individually at the end of one open arm facing away from the central platform, and the time each mouse took to move from the open arm to either of the enclosed arms (transfer latency, TL) was recorded. The mice were allowed to explore the plus-maze for 150 s. On day 1, if the mice did not enter the enclosed arm within 90 s, they were pushed gently (on the back) into the enclosed arm and were permitted to explore the plus-maze for an additional 60 s. In such cases, TL was recorded as 90 s. Twenty-four hours later, a retention test was performed in the same manner as on day 1, and TL was recorded. If the mice did not enter the enclosed arm within 90 s on day 2, the test was stopped and TL was recorded as 90 s.

**Spontaneous alternation behaviour**

This method was also used to study the effect of drug on learning and memory. Spontaneous alternation behaviour was performed in a plus maze to assess effects of drugs on short-term memory with respect to spatial orientation and perception as described by Ragozzino et al. (1998). The animals were placed in a plus maze. The maze (85 cm height) was constructed of wood painted grey and contained a central platform (25 cm diameter), from which radiated four symmetrical arms (55 cm long × 10 cm wide), with 12 cm wall. After being placed in the central platform, rats were allowed to traverse the maze freely for 12 min. The number and sequence of entries were recorded. An alternation was defined as entry into four different arms on an overlapping quintuple set. Five consecutive arm choices within the total set of arm choices constitute a quintuple set. A quintuple consisting of arm choices A, B, A, C, D was considered as an alternation, while the set with A, B, A, C, B did not. Using this procedure, percentage alternation is equal to the ratio of actual alternation to possible alternation × 100. Possible alternation sequences are equal to the number of arm entries minus 4.

**Biochemical parameters**

**Dopamine estimation**

Animals were decapitated and brains were removed and kept on ice immediately, within 4 min striatum were removed and wrapped in aluminium foil and kept on deep freezer at -80°C, until dopamine analysis via liquid chromatography mass spectrometry (LC-MS-MS) was performed. 50 mg striatum was homogenized in 500 μl ice-cold methanol by vortex mixing for 10 min. One millilitre of acetonitrile was mixed with homogenate into 2.5 ml conical plastic centrifuge tube and centrifuged at 14,000 rpm for 20 min. Then the supernatant was evaporated to dryness by nitrogen evaporator at 14°C temperature. The dry residue was then reconstituted with 400 μl mobile phase 0.05% formic acid acetonitrile (92: 8, v/v) and vortex-mixed for 10 s, then the mixture was centrifuged at 14,000 rpm for another 20 min. The upper aqueous layer was injected into the LC-MS-MS. The sample volume injected was 10 μl for dopamine.

**Thiobarbituric acid reactive substances (TBARS)**

One millilitre of suspension medium was taken from the supernatants of 10% tissue homogenate and centrifuged at 10,000 rpm 0.5 ml of 30 % TCA, followed by adding 0.5 ml of 0.8% TBA. The tubes were covered with aluminium foil and kept in shaking water bath at 30 min at 80°C. After 30 min, tubes were taken out and kept in ice-cold water for 10 min. They were then centrifuged at 3000 rpm for 15 min. The absorbance of supernatants was read at 540 nm at room temperature against appropriate blank. Blank consists of 1.0 ml distilled water, 0.5 ml TBA, and 0.5 ml TCA.

**Glutathione (GSH)**

Brain tissue was homogenized in 0.02 M of EDTA (6%) and 5 ml of homogenate was mixed with 4 ml of distilled water and 1 ml of 50% TCA. The tubes were shaken for 10 to 15 min and then centrifuged at 300 rpm for 1 min. After that, 2 ml of supernatant was mixed with 4 ml of 0.4 M tris buffer (pH 8.9) and 0.1 ml of DTNB. The absorbance was read within 5 min of the addition of DTNB at 410 nm against a reagent blank with no homogenate (Ellman, 1959).

**Catalase**

A 10% tissue homogenate was prepared in 2 ml of potassium phosphate buffer (pH 7.4). This homogenate was centrifuged at 3000 rpm for 15 min. Catalase activity was measured in the supernatant obtained after centrifugation. 2.95 ml of 19 mM H₂O₂ was put in cuvette. To it, 0.05 ml of cytosolic supernatant was added and a change in absorbance at 240 nm was recorded at 1 min interval for 3 min. The presence of catalase decomposes H₂O₂ leading to a decrease in absorbance (Claiborne, 1985).

**Estimation of brain acetyl cholinesterase (AChE) in mice**

Mouse brain was harvested by decapitation, weighed and kept at –
70°C until acetyl cholinesterase (AChE) assay. The whole brain AChE activity was measured according to the method of Ellman et al. (1961). A known weight of the brain tissue was homogenized in 0.32 M sucrose solution to get a 10% homogenate that was centrifuged at 3000 rpm for 15 min, followed by centrifugation at 10,000 rpm for 10 min at a constant temperature of 4°C. One milliliter of supernatant was mixed with 9 ml of sucrose solution to get a 1% post mitochondrial supernatant. Test samples were prepared by mixing 2.7 ml of phosphate buffer, 0.1 ml of DTNB and 0.1 ml of PMS. Reaction mixture was taken in a cuvette and pre-incubated for 5 min and 0.1 ml of acetyl thioccholine iodide was added to the mixture to initiate the reaction and immediately absorbance was taken at 412 nm for 3 min for every 1 min interval.

Statistical analysis

Data were expressed as the mean ± standard error of mean (SEM). For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests which can be used to identify differences between groups. P value < 0.05 was considered significant.

RESULTS

Effect of thymoquinone on haloperidol induced catalepsy

Administration of haloperidol (2 mg/kg, p.o), TQ (20 mg/kg, i.p) alone and in combination produced catalepsy. A highly significant prolongation of catalepsy times were observed in combination group post two hours drug administration (p < 0.001) (Table 1).

Effect of thymoquinone on apomorphine induced climbing behaviour.

Administration of apomorphine (1 mg/kg, s.c) resulted in significant increase in climbing index and climbing time (p < 0.001). Pre-treatment of TQ (20 mg/kg i.p) 2 h before the administration of apomorphine reduced maximum time of single climb and climbing index (p < 0.001) (Table 2).

Effect of thymoquinone on elevated plus maze test

Transfer latency was recorded on days 1 and 2. Administration of TQ decreased transfer latency (TL) on day 2 when compared with day 1. Scopolamine (0.5 mg/kg, i.p) exhibited prolongation of TL. Concurrent administration of scopolamine and TQ (20 mg/kg, i.p) reduced it (p<0.001).

There was no change in the percentage alternation of animals in TQ treated group as compared to their corn oil treated control group. However, a significant reduction in the percentage alteration in scopolamine treated group as compared to their saline treated control group was observed (p < 0.001). A significant possible alternation was observed in scopolamine and TQ per se and TQ + scopolamine treated group in comparison to their respective controls (p < 0.001) (Table 3).

Effect of thymoquinone on TBARS, GSH and Catalase levels in all behavioral models

Administration of TQ (20 mg/kg, i.p) alone and in combination with different treated groups such as haloperidol, apomorphine and scopolamine showed significant reduction in TBARS levels, increase in GSH and catalase levels in all models used in the present study (p < 0.01) (Tables 4, 5, and 6).

Effect of thymoquinone on acetyl cholinesterase levels in elevated plus maze

In elevated plus maze test, scopolamine per se (0.5 mg/kg, i.p) produced significant increase in acetyl cholinesterase level as compared to their control (p < 0.05). Administration of TQ showed decrease in acetyl cholinesterase level as compared to their control (Table 7).

Effect of thymoquinone on dopamine levels

In haloperidol induced catalepsy, haloperidol (2 mg/kg, p.o) treated group showed reduction in dopamine levels (p < 0.001). Administration of TQ (20 mg/kg, i.p) alone and in combination with haloperidol showed further reduction in dopamine levels as compared to their respective controls (p < 0.001) (Table 8).

In apomorphine (1 mg/kg, s.c) induced climbing behaviour, a significant increase in dopamine levels was observed after administration of apomorphine alone. However, combination of TQ and apomorphine produced a significant decrease in dopamine level as compared to control (p < 0.001) (Table 9).

In elevated plus maze test, scopolamine per se (0.5 mg/kg, i.p) in elevated plus maze test, showed significant increase in dopamine levels as compared to controls (p < 0.001). However, when TQ and scopolamine were given in combination, a reduction in dopamine level as compared to control was observed (p < 0.001) (Table 10).

DISCUSSION

In this study, the development of catalepsy in haloperidol induced catalepsy model after administration of TQ in
mice was observed. It has been shown previously that nitric oxide synthase (NOS) inhibition contributes to the development of catalepsy. Del Bel et al. (2004) showed in their study that systemic administration of NOS inhibitors induced catalepsy in a dose dependant manner in male albino Swiss mice. In another study it was observed that combined treatment of ascorbic acid with dopamine receptor antagonist or NOS inhibitor potentiated cataleptic effect in mice (Lazzarini et al., 2005). Echeverry et al. (2007) observed that intra cerebroventricular administration of nitric oxide sensitive guanylylcy clase inhibitors induced catalepsy in mice. It was already reported that TQ significantly suppresses the expression of inducible nitric oxide synthase (iNOS) (El-Mahmoudy et al., 2002) and decreases nitrate level (Gilihotra et al., 2011). Catalepsy induced by NOS inhibitors involves striatal DA mediated neurotransmission (Lazzarini et al., 2005). In the present study, TQ potentiated haloperidol induced catalepsy throughout the experiment (p < 0.001), suggesting its role in blocking dopamine D2 receptor and exhibiting its antipsychotic like effect and its potential of reducing negative symptoms of schizophrenia.

Apomorphine, a dopamine receptor agonist, exhibited climbing behaviour in mice. The climbing behaviour was

Table 1. Effect of thymoquinone on haloperidol induced catalepsy in mice.

<table>
<thead>
<tr>
<th>Group (n = 6)</th>
<th>Drug treatment</th>
<th>Catalepsy duration (s)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>After 1 h</td>
<td>After 2 h</td>
<td>After 3 h</td>
<td>After 4 h</td>
</tr>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>34.32 ± 4.5</td>
<td>43.56 ± 3.7</td>
<td>39.67 ± 9.8</td>
<td>30.75 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>10.45 ± 0.18</td>
<td>13.78 ± 5.8</td>
<td>11.54 ± 3.6</td>
<td>8.29 ± 6.9</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>HAL (2 mg/kg, p.o)</td>
<td>162.13 ± 7.42</td>
<td>246.33 ± 7.07</td>
<td>266 ± 7.16</td>
<td>272 ± 3.78</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>135.17 ± 2.92</td>
<td>236 ± 6.19</td>
<td>259 ± 7.09</td>
<td>264 ± 5.68</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + HAL (2 mg/kg, p.o)</td>
<td>283 ± 5.68</td>
<td>292 ± 1.57</td>
<td>293 ± 3.62</td>
<td>297 ± 1.94</td>
<td></td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. p value < 0.05 was considered significant and p value < 0.001 was considered highly significant. n = 6, number of animals in each group. *p < 0.001 vs. corn oil, †p < 0.001 vs. normal saline; ‡p < 0.001 vs. group III.

Table 2. Effect of thymoquinone on apomorphine induced climbing behaviour in mice.

<table>
<thead>
<tr>
<th>Group (n = 6)</th>
<th>Drug treatment</th>
<th>Maximum time (s)</th>
<th>Climbing index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>15.86 ± 1.24</td>
<td>8.05 ± 0.97</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>20.45 ± 2.23</td>
<td>5.62 ± 0.56</td>
</tr>
<tr>
<td>III</td>
<td>APO (1 mg/kg, s.c)</td>
<td>329.38 ± 20.64a</td>
<td>55.01 ± 4.56a</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>6.78 ± 0.056ab</td>
<td>2.33 ± 0.001ab</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + APO (1 mg/kg, s.c)</td>
<td>162.57 ± 10.45abc</td>
<td>34.38 ± 2.45ab</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparisons. p value < 0.05 was considered significant and p value < 0.001 was considered highly significant. n = 6, number of animals in each group. *p < 0.001 vs. corn oil, †p < 0.001 vs. normal saline; ‡p < 0.001 vs. group III.

Table 3. Effect of thymoquinone on elevated plus maze test in mice.

<table>
<thead>
<tr>
<th>Group (n = 6)</th>
<th>Drug treatment</th>
<th>Transfer latency (s)</th>
<th>Alteration (%)</th>
<th>Possible alteration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>On day 1</td>
<td>On day 2</td>
<td>On day 1</td>
</tr>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>25.33 ± 2.32</td>
<td>11.21 ± 1.1</td>
<td>52.63 ± 5.34</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>22.42 ± 1.76</td>
<td>9.04 ± 0.39</td>
<td>48.87 ± 3.87</td>
</tr>
<tr>
<td>III</td>
<td>SCOP (0.5 mg/kg, i.p)</td>
<td>55.37 ± 5.26ab</td>
<td>46.73 ± 3.7ab</td>
<td>27.47 ± 1.89</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>28.48 ± 1.98</td>
<td>16.78 ± 2.13</td>
<td>62.49 ± 7.34b</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + SCOP (0.5 mg/kg, i.p)</td>
<td>42.43 ± 5.78ab</td>
<td>31.76 ± 4.15ab</td>
<td>39.78 ± 5.78ab</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparisons. p value < 0.05 was considered significant and p value < 0.001 was considered highly significant. n = 6, number of animals in each group. *p < 0.001 vs. corn oil, †p < 0.001 vs. normal saline.
Table 4. Effect of thymoquinone on TBARS, GSH and CAT levels in haloperidol induced catalepsy in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug treatment</th>
<th>TBARS (nm/mg protein)</th>
<th>GSH (μmoles/mg protein)</th>
<th>CAT (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>4.86 ± 0.43</td>
<td>45.54 ± 4.84</td>
<td>12.46 ± 1.56</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>5.16 ± 0.64</td>
<td>36.67 ± 3.96</td>
<td>10.67 ± 1.98</td>
</tr>
<tr>
<td>III</td>
<td>HAL (2 mg/kg, p.o)</td>
<td>8.14 ± 1.33</td>
<td>25.76 ± 3.19</td>
<td>6.15 ± 0.46*</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>1.48 ± 0.11*</td>
<td>71.75 ± 4.01**</td>
<td>18.9 ± 1.1*</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + HAL (2 mg/kg, p.o)</td>
<td>3.28 ± 0.39</td>
<td>59.09 ± 4.69*</td>
<td>15.84 ± 2.4</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparisons. p value < 0.05 was considered significant and p value < 0.001 was considered highly significant. n = 6, number of animals in each group. *p < 0.05 vs. corn oil, ** p < 0.01; *p < 0.05 vs. normal saline.

Table 5. Effect of thymoquinone on TBARS, GSH and CAT levels in apomorphine induced climbing behaviour in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug treatment</th>
<th>TBARS (nm/mg protein)</th>
<th>GSH (μmoles/mg protein)</th>
<th>CAT (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>4.86 ± 0.11</td>
<td>45.54 ± 4.38</td>
<td>12.46 ± 2.46</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>5.16 ± 0.37</td>
<td>36.67 ± 3.29</td>
<td>10.67 ± 1.98</td>
</tr>
<tr>
<td>III</td>
<td>APO (1 mg/kg, s.c)</td>
<td>5.68 ± 0.64</td>
<td>35.54 ± 2.34</td>
<td>9.08 ± 0.97</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>2.97 ± 0.23*</td>
<td>77.84 ± 6.43*</td>
<td>17.68 ± 2.11*</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + APO (1 mg/kg, s.c)</td>
<td>4.37 ± 0.32</td>
<td>72.45 ± 7.39****</td>
<td>14.37 ± 1.87</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparisons. p value < 0.05 was considered significant and p value < 0.001 was considered highly significant. n = 6, number of animals in each group. *p < 0.05 vs. corn oil, ** p < 0.01; *p < 0.05 vs. normal saline, ##p < 0.01 vs. normal saline, ###p < 0.001 vs. normal saline.

Table 6. Effect of thymoquinone on TBARS, GSH and CAT levels in Elevated plus maze test in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug treatment</th>
<th>TBARS (nm/mg protein)</th>
<th>GSH (μmoles/mg protein)</th>
<th>CAT (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>4.86 ± 0.98</td>
<td>45.54 ± 5.02</td>
<td>12.46 ± 1.04</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>5.16 ± 0.13</td>
<td>36.67 ± 2.71</td>
<td>10.67 ± 1.29</td>
</tr>
<tr>
<td>III</td>
<td>SCOP (0.5mg/kg, i.p)</td>
<td>3.65 ± 0.08</td>
<td>22.18 ± 1.05</td>
<td>8.56 ± 0.89</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>2.01 ± 0.58*</td>
<td>69.78 ± 8.98*</td>
<td>18.59 ± 1.89*</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + SCOP (0.5 mg/kg, i.p)</td>
<td>1.75 ± 0.89**</td>
<td>32.83 ± 1.98</td>
<td>13.59 ± 0.29</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison. p value < 0.05 was considered significant. n = 6, number of animals in each group. *p < 0.05) vs. corn oil; #p < 0.05 vs. normal saline group.

elicited by stimulation of dopamine receptors in the striatum. In one of the studies, it was shown that NOS inhibitors (7-nitroindazole) block the apomorphine induced stereotypy and climbing. Myricitine, a NOS and protein kinase C inhibitor exerts antipsychotic like effects in this model (Pereira et al., 2011). Some researcher reported that nitric oxide (NO) donor restores the apomorphine induced climbing behavior which was inhibited by NMDA receptor antagonist. NOS inhibitor inhibited apomorphine induced climbing behavior which was enhanced by NMDA receptor agonist (Hong et al., 2005). In apomorphine induced climbing behaviour, TQ was seen to decrease maximum time of single climb and climbing index as compared to corn oil treated control group. Pre-treatment of TQ (20 mg/kg i.p) 2 h before the experiment with apomorphine was also seen to reduce maximum time of single climb and climbing index (p < 0.001), this may suggest that TQ has dopamine receptors blocking effects in striatum and can also be useful in reducing positive symptoms of schizophrenia. In one study, it was observed that impairment of memory formation and facilitation of retrieval induced by morphine involves decreased synthesis/release of NO and can be counteracted by NOS substrate (Rezayof et
Table 7. Effect of thymoquinone on acetyl cholinesterase activity in elevated plus maze test in mice.

<table>
<thead>
<tr>
<th>Group (n = 6)</th>
<th>Drug treatment</th>
<th>Acetyl cholinesterase (mole/mg/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>0.01 ± 0.003</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>0.02 ± 0.002</td>
</tr>
<tr>
<td>III</td>
<td>SCOP (0.5 mg/kg, i.p)</td>
<td>0.50 ± 0.001*</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>0.03 ± 0.001</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + SCOP (0.5 mg/kg, i.p)</td>
<td>0.24 ± 0.008**</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. *p < 0.05 vs. corn oil; **p < 0.05 vs. normal saline.

Table 8. Effect of thymoquinone on dopamine levels in haloperidol induced catalepsy in mice.

<table>
<thead>
<tr>
<th>Group (n = 6)</th>
<th>Drug treatment</th>
<th>Dopamine (ng/g weight of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>14.12 ± 1.1</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>14.62 ± 0.86</td>
</tr>
<tr>
<td>III</td>
<td>HAL (2 mg/kg, p.o)</td>
<td>6.18 ± 0.57***, ###</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>9.61 ± 0.43**</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + HAL (2 mg/kg, p.o)</td>
<td>4.33 ± 0.21***##</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison tests. **p < 0.01 vs. corn oil, ***p < 0.001 vs. corn oil., ###p < 0.001 vs. normal saline.

Table 9. Effect of thymoquinone on dopamine levels in apomorphine induced climbing behaviour in mice.

<table>
<thead>
<tr>
<th>Group (n = 6)</th>
<th>Drug treatment</th>
<th>Dopamine (ng/g weight of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>14.12 ± 1.1</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>14.62 ± 0.86</td>
</tr>
<tr>
<td>III</td>
<td>APO (1 mg/kg, s.c)</td>
<td>21.29 ± 1.35***###</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>9.61 ± 0.92**</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + APO (1 mg/kg, s.c)</td>
<td>16.64 ± 0.79**###</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. **p < 0.01 vs. corn oil, ***p < 0.001 vs. corn oil, **p < 0.001 vs. normal saline.

In another study it was shown that L-NAME impairs memory consolidation (Khavandgar et al., 2003). Scopolamine, a muscarinic cholinergic antagonist, has been widely used to disrupt learning and memory in several types of animal experiments (Miyazaki et al., 1995). In the present study, administration of scopolamine (0.5 mg/kg s.c), prolonged transfer latency (TL) on day 2 compared with the saline-treated group in the elevated plus-maze test. TQ reduced the prolongation of TL induced by scopolamine in the elevated plus-maze test suggesting its role in learning and memory. In the present investigation, TQ showed anti-amnestic activity against scopolamine induced amnesia in elevated plus maze model. These behavioural results were correlated with decrease in acetylcholine esterase (AChE) activity in mice brain by TQ as evident by our observations. Spontaneous alteration behaviour (SAB) has been adopted as a measure of memory (Ragozzino et al., 1998). Scopolamine treated animals showed reduction of percentage alteration as compared to control group; thus confirming its amnesic effect. TQ was seen to slightly increase percentage alteration as compared to corn oil treated control group showing its anti-amnesic effect and thus, improves memory. It seems likely that administration of TQ may ameliorate the learning and memory deficit shown by schizophrenic patients.
Evidence for increased oxidative stress in chronic schizophrenic patients is primarily based on the altered levels of antioxidant enzymes as previously reported (Lohr, 1991; Halliwell, 1994; Mahadick, 1996; Mahmood et al., 2012b). In haloperidol induced catalepsy, apomorphine induced climbing behaviour and in elevated plus maze test, administration of TQ produced a significant reduction in thiobarbituric acid reactive substance (TBARS) levels, increase in glutathione levels and elevation in catalepsy levels as compared to haloperidol treated control group. In so many experimental studies, it was reported that oxidative stress decreased GSH and catalepsy levels, while pre-treatment of different Nigella sativa extracts resulted in significant elevation of GSH levels and catalepsy levels as compared to MCA occluded rats, thus confirming its antioxidant and free radical scavenging properties (Abdulhakeem et al., 2006; Akhtar et al., 2006a, 2008b, 2012c, 2013d; Pourghassem-Gargari et al., 2009).

It was also observed that TQ significantly decreased dopamine (DA) levels in haloperidol (HAL) induced catalepsy, apomorphine induced climbing behaviour and elevated plus maze model. In HAL induced catalepsy, administration of TQ (20 mg/kg, i.p) alone and in combination with HAL showed reduction in DA levels as compared to their respective controls (p < 0.001). In apomorphine induced climbing behaviour, administration of TQ alone and in combination with apomorphine reduced DA levels as compared to their respective controls. In elevated plus maze test, similar observations were recorded. Thus, in the present study, TQ decreased the DA levels which suggest its antipsychotic like actions.

**Table 10. Effect of thymoquinone on dopamine levels in elevated plus maze test in mice.**

<table>
<thead>
<tr>
<th>Group (n = 6)</th>
<th>Drug treatment</th>
<th>Dopamine (ng/g weight of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Corn oil (1 ml/kg, i.p)</td>
<td>14.12 ± 1.1</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline (1 ml/kg, i.p)</td>
<td>14.62 ± 0.86</td>
</tr>
<tr>
<td>III</td>
<td>SCOP (0.5 mg/kg, i.p)</td>
<td>16.76 ± 1.08</td>
</tr>
<tr>
<td>IV</td>
<td>TQ (20 mg/kg, i.p)</td>
<td>9.61 ± 0.42*</td>
</tr>
<tr>
<td>V</td>
<td>TQ (20 mg/kg, i.p) + SCOP (0.5 mg/kg, i.p)</td>
<td>11.40 ± 0.59</td>
</tr>
</tbody>
</table>

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett’s multiple comparison test. p value < 0.05 was considered significant. n= 6 is the number of animals in each group. *p < 0.05 vs. corn oil; †p < 0.05 vs. normal saline group.

Conclusions

The present study observed the role of TQ in different models of schizophrenia, that is, positive, negative and learning and memory deficit models in mice. TQ exhibited antipsychotic like activities in the used animal models of schizophrenia (improved memory, improved positive and negative symptoms). Our results are preliminary, further studies are required for determining the mechanism and the exact role of TQ in schizophrenia. Although, TQ has no role in schizophrenia at this stage, but establishing the role of decreasing the dopamine levels in brain may be a good approach in schizophrenia treatment.

**ACKNOWLEDGEMENT**

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**Conflict of Interest**

The authors declare no conflict of interest.

**REFERENCES**


Hosseinzadeh H, Parvardeh S (2004). Anticonvulsant effects of thymoquinone, the major constituent of Nigella sativa seeds in mice. Phytomedicine 11:56-64


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