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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-GertBernstein 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

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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 (Mohammad 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 intraperitonially, 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 the 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 for 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 H_2O_2 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 H_2O_2 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 millilitre 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 thiocholine 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.

Protein concentrations were determined according to Lowry et al. (1951) using purified bovine serum albumin as standard.

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).

Administration of scopolamine (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

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

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

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. $^{a}p < 0.001$ vs. corn oil, $^{b}p < 0.001$ vs. normal saline; $^{c}p < 0.001$ vs. group III.

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

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

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. $^{a}p < 0.001$ vs. corn oil, $^{b}p < 0.001$ vs. normal saline; $^{c}p < 0.001$ vs. group III.

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

Group (n = 6)	Drug treatment	Transfer latency (s)		Alteration (%)	Possible alteration (s)
		On day 1	On day 2		
I	Corn oil (1 ml/kg, i.p)	25.33 ± 2.32	11.21 ± 1.1	52.63 ± 5.34	29.5 ± 1.1
II	Normal saline (1 ml/kg, i.p)	22.42 ± 1.76	9.04 ± 0.39	48.87 ± 3.87	30.66 ± 2.3
III	SCOP (0.5 mg/kg, i.p)	55.37 ± 5.26^{ab}	46.73 ± 3.7^{ab}	27.47 ± 1.89^{ab}	11.37 ± 0.08^{ab}
IV	TQ (20 mg/kg, i.p)	28.48 ± 1.98	16.78 ± 2.13	62.49 ± 7.34^{b}	24.43 ± 2.1
V	TQ (20 mg/kg, i.p) + SCOP (0.5 mg/kg, i.p)	42.43 ± 5.78^{ab}	31.76 ± 4.15^{ab}	39.78 ± 5.78^{ab}	17.36 ± 1.19^{ab}

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. $^{a}p < 0.001$ vs. corn oil, $^{b}p < 0.001$ vs. normal saline.

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 guanylylcyclase 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 (Gilhotra 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 D_2 receptors 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 4. Effect of thymoquinone on TBARS, GSH and CAT levels in haloperidol induced catalepsy in mice.

Group (n = 6)	Drug treatment	TBARS (nm/mg protein)	GSH (µmoles/mg protein)	CAT (U/mg protein)
I	Corn oil (1 ml/kg, i.p)	4.86 ± 0.43	45.54 ± 4.84	12.46 ± 1.56
II	Normal saline (1 ml/kg, i.p)	5.16 ± 0.64	36.67 ± 3.96	10.67 ± 1.98
III	HAL (2 mg/kg, p.o)	8.14 ± 1.3$^{\#}$	25.76 ± 3.19$^{\#}$	6.15 ± 0.46*
IV	TQ (20 mg/kg, i.p)	1.48 ± 0.11*	71.75 ± 4.01**	18.9 ± 1.1*
V	TQ (20 mg/kg, i.p) + HAL (2 mg/kg, p.o)	3.28 ± 0.39	59.09 ± 4.69$^{\#}$	15.84 ± 2.4

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.

Group (n = 6)	Drug treatment	TBARS (nm/mg protein)	GSH (µmoles/mg protein)	CAT (U/mg protein)
I	Corn oil (1 ml/kg, i.p)	4.86 ± 0.11	45.54 ± 4.38	12.46 ± 2.46
II	Normal saline (1 ml/kg, i.p)	5.16 ± 0.37	36.67 ± 3.29	10.67 ± 1.98
III	APO (1 mg/kg, s.c)	5.68 ± 0.64	35.54 ± 2.34	9.08 ± 0.97
IV	TQ (20 mg/kg, i.p)	2.97 ± 0.23*	77.84 ± 6.43*$^{\#}$	17.68 ± 2.11*
V	TQ (20 mg/kg, i.p) + APO (1 mg/kg, s.c)	4.37 ± 0.32	72.45 ± 7.39**$^{\#\#\#}$	14.37 ± 1.87

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.

Group (n = 6)	Drug treatment	TBARS (nm/mg protein)	GSH (µmoles/mg protein)	CAT (U/mg protein)
I	Corn oil (1 ml/kg, i.p)	4.86 ± 0.98	45.54 ± 5.02	12.46 ± 1.04
II	Normal saline (1 ml/kg, i.p)	5.16 ± 0.13	36.67 ± 2.71	10.67 ± 1.29
III	SCOP (0.5mg/kg, i.p)	3.65 ± 0.08	22.18 ± 1.05	8.56 ± 0.89
IV	TQ (20 mg/kg, i.p)	2.01 ± 0.58*	69.78 ± 8.98*	18.59 ± 1.89*
V	TQ (20 mg/kg, i.p) + SCOP (0.5 mg/kg, i.p)	1.75 ± 0.89*$^{\#}$	32.83 ± 1.98	13.59 ± 0.29

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-nitroinidazole) 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.

Group (n = 6)	Drug treatment	Acetyl cholinesterase (mole/mg/protein)
I	Corn oil (1 ml/kg, i.p)	0.01 ± 0.003
II	Normal saline (1 ml/kg, i.p)	0.02 ± 0.002
III	SCOP (0.5 mg/kg, i.p)	0.50 ± 0.001$^{\#}$
IV	TQ (20 mg/kg, i.p)	0.03 ± 0.001
V	TQ (20 mg/kg, i.p) + SCOP (0.5 mg/kg, i.p)	0.24 ± 0.008*$^{\#}$

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 < 0.05 vs. corn oil; $^{\#}$p < 0.05 vs. normal saline.

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

Group (n = 6)	Drug treatment	Dopamine (ng/g weight of tissue)
I	Corn oil (1 ml/kg, i.p)	14.12 ± 1.1
II	Normal saline (1 ml/kg, i.p)	14.62 ± 0.86
III	HAL (2 mg/kg, p.o)	6.18 ± 0.57***, $^{\#\#\#}$
IV	TQ (20 mg/kg, i.p)	9.61 ± 0.43**
V	TQ (20 mg/kg, i.p) + HAL (2 mg/kg, p.o)	4.33 ± 0.21***$^{\#\#\#}$

All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison tests. 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 < 0.01 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.

Group (n = 6)	Drug treatment	Dopamine (ng/g weight of tissue)
I	Corn oil (1 ml/kg, i.p)	14.12 ± 1.1
II	Normal saline (1 ml/kg, i.p)	14.62 ± 0.86
III	APO (1 mg/kg, s.c)	21.29 ± 1.35***$^{\#\#\#}$
IV	TQ (20 mg/kg, i.p)	9.61 ± 0.92**
V	TQ (20 mg/kg, i.p) + APO (1 mg/kg, s.c)	16.64 ± 0.79***$^{\#\#\#}$

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 < 0.01 vs. corn oil, ***p < 0.001 vs. corn oil.,$^{\#\#\#}$p < 0.001 vs. normal saline.

al., 2006). 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.

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

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

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.

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 catalase levels as compared to corn oil treated control group. In so many experimental studies, it was reported that oxidative stress decreased GSH and catalase levels, while pre-treatment of different *Nigella sativa* extracts resulted in significant elevation of GSH levels and catalase 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.

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.

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Conflict of Interest

The authors declare no conflict of interest.

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