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Beneficial Interaction of Thymoquinone and Sodium Valproate in Experimental Models of Epilepsy: Reduction in Hepatotoxicity of Valproate

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Abstract

The antiepileptic potential of thymoquinone (TQ) was studied in mice by using pentylenetetrazole (PTZ) and maximal electroshock seizure (MES)-induced convulsion models. In this investigation, the combined treatment of TQ and sodium valproate (SVP) was also studied. The aim of this part was to minimize SVP-induced hepatotoxic implications, by reducing its antiepileptic dose. TQ in both PTZ- and MES- models increased SVP potency. The experiments dealing with the effects of TQ on the ED_{50} of SVP revealed that TQ reduced the ED_{50} of SVP in both the models. However, this potentiation of SVP antiepileptic response was relatively more significant in PTZ model at both 50 and 100 mg/kg doses of TQ. Although very well tolerated and effective, SVP is well known for its hapatotoxic implications. In the experiment dealing with subacute treatment, SVP (1300-1500mg/kg/day in drinking water) for 21 days, produced hepatotoxicity in mice, characterized by elevated serum ALT and AST, reduced levels of non protein sulfhydryls and increase in lipid peroxidation in hepatic cells. Hepato-protection was successfully achieved when TQ (5-5.5mg/kg/day) was combined with SVP in drinking water for the same duration. The protective activity of TQ was evident by averting any serum ALT rise seen in SVP treatment. Though there is no prevention to the rise in lipid peroxidation in hepatic tissue but the same time a significant recovery in glutathione was evident by TQ joint treatment.

However, a combination of TQ and low dose of SVP may be useful strategy to minimize adverse reactions of SVP. From the results of the present study that disclosed a protective role of TQ against SVP toxic damage to the liver, it could be mentioned safely that TQ behaves as an antioxidant and protected liver against its harmful effects. It also protected against the liver enzyme induction seen in the case of SVP alone.

Keywords

Thymoquinone, valproate, potentiation, maximal electroshock convulsions, hepatoprotection.

Abbreviations: maximal electroshock seizure = MES; pentylenetetrazole = PTZ; thymoquinone = TQ; sodium valproate = SVP; malondialdehyde = MDA; non-protein sulfhydryl groups = NPSH; glutathione = G-SH.

Introduction

Thymoquinone (TQ), 2-isopropyl-5- methyl- 1,4- benzoquinone is the major active constituent of volatile oil obtained from different *Nigella* species (family *Ranunculaceae*) [1]. Several years before the pharmacognostic identification and characterization, thymoquinone-containing seeds of these plants were frequently used as a folklore medicine in Arabian countries for the treatment of various ailments like headache, respiratory depression, asthma, calculus of bladder, kidney and as a diuretic [1,2]. Expressed oil of *Nigella* seeds that contains 18.4 to 24 % w/w TQ [3,4], is useful in asthma and cough [1], as a topical treatment for pain and stiffness of joints [5], rheumatism and related inflammatory disorders [6]. However, in the last few years thymoquinone in its pure form has been assessed in various pathological states. Abdel-Fattah *et al*. [7] claimed that pure TQ (1 - 6 mg/kg, i.p.; 2.5 - 10 mg/kg, p.o.; 1 - 4 µg/mouse, i.c.v.) suppressed the nociceptive response in the hot-plate, tail pinch test, acetic acid-induced writhing test and early phase of the formalin test and naloxone (1 mg/kg) blocked TQ-induced antinociception. El Tahir and Ageel [8] demonstrated that TQ decreased arterial blood pressure and the heart rate in the guinea pig *in vivo*. Nigellone (the carbonyl polymer of thymoquinone) has been used clinically in bronchial asthma. It inhibits histamine release from mast cells [9]. The crude total oil and TQ both inhibited the cyclooxygenase and 5-lipoxygenase pathways of arachidonic acid metabolism in rat peritoneal leukocytes and TQ was described to be very potent in this action [6]. The authors showed it to be due to an inhibition in the generation of thromboxane B_2 (TXB_2) and leukotriene B_4 (LTB_4). Thymoquinone produced anti-inflammatory effect using carrageenan-induced edema in rat hind paw and cotton-pellet granuloma in rat [10]. These anti-inflammatory effects are ascribed to the inhibition in generation of eicosanoids and lipid peroxidation. These properties support the traditional use of *Nigella sativa* for rheumatism and related inflammatory diseases. The crude fixed oil (total oil) from *Nigella* seeds and pure TQ inhibited non-enzymatic peroxidation in ox brain phospholipid liposomes [6]. Kruk *et al.* [11]

studied the effects of thymol, TQ and dithymoquinone on the reactions generating reactive oxygen species (ROS), such as superoxide anion radical ($O^{\cdot}_2$), hydroxyl radical ($HO^{\cdot}$) and singlet oxygen ($1O_2$), using the chemiluminescence and spectrophotometric methods. All test compounds acted as scavengers of various ROS. Co-administration of *Nigella sativa* seed extract with cisplatin (a well known free-radical generating chemotherapeutic agent) in rats prevented many toxic side effects of cisplatin, including reduction of serum GPT, GOT and LDH, and caused an increase in glutathione (G-SH) [12]. Thymoquinone pretreatment in drinking water for five days (8 mg/ kg /day) protected organs against oxidative damage induced by a variety of free radical generating agents including doxorubicin [13], cisplatin [14] and CCl_4 [15]. Badary *et al*., [16] administered TQ in drinking water to rats (5 mg/kg/day) for 5 days before and after ifosfamide (IFO), a cytotoxic agent. This treatment ameliorated the severity of IFO-induced renal damage. TQ also improved IFO antitumour toxicity against Ehrlich ascites carcinoma in mice. Nagi *et al*. [17] showed that oral administration of TQ as a single dose (100 mg/kg) immediately before CCl_4 administration offered protection to the hepatic cells from toxicity induced by CCl_4 in mice. TQ in drinking water (0.01 %) one week before, during and after benzo (α)-pyrene, an inducer of forestomach tumors, in mice, showed a powerful cytotoxic activity [18]. The authors suggest that TQ may act through its antioxidant and anti-inflammatory activity, coupled with enhancement of detoxification process. A recent study [18] has demonstrated that total oil from NS seeds is effective against PTZ-induced kindling in mice. Another study has indicated that treatment of mice with TQ reduced the duration of myoclonic seizure and effectively protected against mortality [19] and ascribed these effects through an opioid receptor mediated increase in GABAergic tone [20].

Despite the folkloric importance of thymoquinone containing seeds and the biological significance of thymoquinone there is dearth of literature on the pharmacological effects of TQ in epilepsy either alone or in combination with some potent drug like valproate. The present study is, therefore, designed to investigate the anticonvulsant potential of TQ alone and in combination with sodium valproate, a widely used antiepileptic drug (AED) against pentylenetetrazole (PTZ) and maximal electroshock seizure (MES)-induced convulsions. Review of the existing literature on biochemical aspects has indicated that TQ has a hepatoprotective activity against CCl_4. No work has been done to explore this activity against sodium valproate induced hepatotoxicity. Therefore, it was also aimed to explore this aspect precisely and to see if TQ can protect against SVP- induced hepatotoxicity.

Material and Methods

Chemicals and Drugs

Thymoquinone, pentylenetetrazole, and sodium valproate (Sigma Chemical Co., St. Louis, MO, USA), 5-5'-dithiobis-(2 nitrobenzoic acid) (BDH chemicals Ltd, Poole, UK), malondialdehyde bis (dimethyl acetal) tetra-ammonium (Fluka Biochemika-Buchs, Switzerland) and corn oil (Afia®, Savola Edible Oils, Saudi Arabia)were used. All the other chemicals and reagents used in this study were of analytical reagent grade procured from commercial sources.

Animals and treatments

Swiss albino (SWR) male mice, weighing 25-30 g, 8-10 weeks old, (bred at Experimental Animal Care and Breading Center, College of Pharmacy, King Saud University, Riyadh) were used. The animals were housed in groups to acclimatize to the laboratory conditions for three days before the start of the experiment as for diet, water, temperature (22±1°C), relative humidity (~50 %) and light cycle (7:0 am to 7:0 p.m.). Food and water were made freely accessible. The drug solutions were prepared freshly daily. Valproate and pentylenetetrazole were dissolved in distilled water and thymoquinone was dissolved in corn oil to be used in acute studies. Preliminary experiments revealed that i.p. injection of corn oil at 0.2ml dose had no significant effect on any of the parameters studied, when compared to the untreated control group.

Groups of mice were given test drugs intraperitoneally and tested for anticonvulsant activity 1 hour post-treatment using PTZ and MES models. Minimum of 8 animals per group were used. In the experiments on the interaction of TQ with SVP anticonvulsant activities of SVP (against PTZ and MES) were first carried out 15 min after i.p. administration and repeatedly after every 10 min for the next three time points. The effect of TQ (50 and 100 mg/kg, i.p.) on the anticonvulsant potency (against both PTZ and MES) of two SVP doses (200- or 400-mg/kg) was studied. SVP was injected 45 min after TQ administration and this combined treatment was subjected to PTZ or MES seizures 15 min later.

Methods for the determination of anticonvulsant activity

Anticonvulsant activity was evaluated by pentylenetetrazole-induced seizure threshold test and Maximal electroshock seizure test.

Pentylenetetrazole-induced seizure threshold test

Pentylenetetrazole (PTZ) seizure threshold test is one of well-known chemical tests used to evaluate anticonvulsant activity [21] that was utilized as a model for petit-mal epilepsy [22]. The dose of PTZ (83 mg/kg, s.c.) used in this study was determined in our preliminary studies and was found to be the approximate minimal dose (CD_{100}) that induced convulsion in 100 % male SWR mice (i.e. control group, in this study). The mice that received PTZ (s.c.) were observed for 30 min.

The mice after PTZ treatment was kept on the force platform of convuls-1-meter (Columbus Instruments International Corp., USA) designed to record the convulsive activity of mice. The force-platform is connected to the convuls-1 control box. Any force exerted on the platform was measured by the force platform. The convuls-1-meter responds only to those changes in the force exerted on the force-platform and the static force exerted by the weight of the cage or animal was not recorded. The instrument was run without animals and the noise counts/second figures were used to correct the experimental measurements. In controlled experiments, the noise (counts/sec) was 4-5 times less than the reading obtained with animal under the same conditions [23,24].

Maximal electroshock seizure test

The maximal electroshock seizure (MES) test was employed as a model for grand-mal epilepsy [25]. This test is one of the reliable electrical tests used to evaluate anticonvulsant activity [21]. Mice were restrained by hand and subjected to electric shock through their ears using ECT UNIT (Model, 7801; UGO Basile, Varese, Italy), and released immediately following electrical stimulation to permit observation of the maximal seizure. The maximal seizure typically consists of a short period of initial tonic flexion and a prolonged period of tonic extension (in particular the hind limb). Various frequencies, current intensities and duration of stimulation were tried on male SWR mice (control group). The minimal electroshock that induced 100% maximal seizures was found to be 50mA alternating current of 100 Hz frequency for 0.2 seconds duration. Protection was defined as complete absence of hind limb tonic extension. The test was repeated by 10 min intervals for 30 min after the treatment.

Determination of ED_{50} for SVP in MES- and PTZ- models

From dose/response curve analysis of anticonvulsant activities, median anticonvulsant doses (ED_{50}) were calculated by Litchfield and Wilcoxon test. A computer program (Pharmacological Calculation System, version 3.2, medical college of Georgia, Augusta, Georgia, USA) was used to calculate this parameter.

Assessment of the thymoquinone (TQ)-hepatoprotective activity against sodium valproate (SVP)-induced hepatotoxicity

Sodium valproate (SVP) was dissolved in drinking water and made freely available to male SWR mice for 21 days. The concentration of SVP from day 1 to day 5 was 0.26 %, 0.33 %, 0.43 %, 0.55 % and 0.71 % w/v, respectively (an increase in concentration in logarithmic ratio of 0.11). Thereafter, the concentration of the drug remained constant throughout the study period. The daily intake of animals was recorded and SVP dose was calculated [24,26,27]. The dose was kept in the range of 1300-1500mg/kg/day that has already been described in the literature and proven to be hepatotoxic [27,28]. In the hepatoprotection studies, finely powdered TQ was suspended in water along with valproate or alone in a concentration of 0.025% w/v. The daily dose intake was calculated to be in the range of 5-5.5 mg/kg/day that has already been reported in the literature [16] and the treatment continued for the same duration as for valproate.

Isolation of serum and liver specimen

Treated and control groups were sacrificed after 21 days. Blood samples were drawn by direct cardiac puncture under light ether anaesthesia into non-heparinized capillary tubes. Serum was separated by centrifugation for 5 min at 4000 rpm and stored at -20°C until biochemical assessment of serum ALT and AST was done. The liver tissues of treated groups were isolated, washed with saline, immediately frozen by using Histofreez® (Fisher Scientific Company, Pittsburgh, PA, USA) and kept refrigerated at -20°C until used for analysis.

Plasma biochemical analysis

Activities of enzymes glutamic-oxaloacetic transaminase (AST, EC 2.6.1.1), glutamic-pyruvic transaminase (ALT, EC 2.6.1.2) were determined in the plasma by using commercially available kits from Boehringer Mannheim GmbH (Germany).

Estimation of lipid peroxides

The method described by Ohkawa *et al*., [29] was used with some modifications. Malondialdehyde (MDA) was measured as an indicator of lipid peroxidation. Liver tissues were thawed and homogenized in aqueous KCl solution supplemented with butylated hydroxyl toluene to avoid further formation of MDA, by using Ultra-Turrax® (Janke and Kunkel GmbH & Co. KG IKA-WERK Staufen) homogenizer at 20×1000 rpm for few seconds. The aliquots of tissue homogenate were immediately treated with 20% acetic acid (pH 3.5) and sodium dodecyl sulphate was added. The whole mixture was incubated with thiobarbituric acid (0.8% aqueous) for one hour at 95°C using ball condensers and cooled to room temperature. After centrifugation the pink clear layer was extracted with methanol-pyridine mixture (9:1) and read on a spectrophotometer against reagent blank. Malondialdehyde bis (dimethyl acetal) tetra ammonium salt was used as an external standard.

Estimation of non-protein sulfhydryls (NPSH)

Reduced glutathione (GSH) contents in liver tissues were estimated as non-protein sulfhydryls by using the method described elsewhere [30]. The liver tissues were homogenized in ice cold 0.02M ethylenediaminetetraacetic acid disodium (EDTA). Aliquots of tissue homogenate were treated with 50% w/v trichloroacetic acid and centrifuged. Supernatant fractions were mixed with Tris buffer; 5-5'-dithiobis-(2 nitrobenzoic acid) (DTNB) was added. After shaking the contents, its absorbance was measured at 412 nm within 5 min of the addition of DTNB against reagent blank with no homogenate.

Statistical analysis

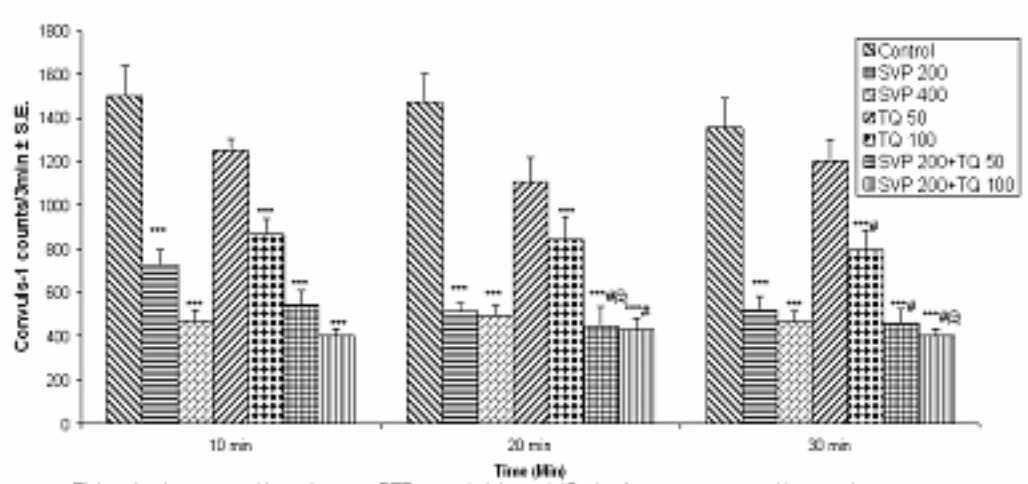
All the data are expressed as mean ± SEM. Analysis of variance (ANOVA one way) was used and critical values for $P < 0.05$ were considered significant. In post-hoc analysis Student's t-test for comparison of two means or Tukey-Kramer multiple comparison test was employed and is stated at appropriate places.

Results

Comparison of SVP treatment with the TQ and SVP combined treatment

SVP at both the doses was very effective in preventing PTZ induced seizures at all the time points. On the other hand TQ 50 mg/kg alone was found ineffective. The high dose of TQ (100 mg/kg) was significantly effective in preventing PTZ induced seizures. In the combined treatment both the doses of TQ potentiated the low dose of valproate and the protection by the low dose of TQ with low dose of SVP was comparable to the high dose of SVP at all the three observation time points (Figure 1).

Similarly, subjected to MES-induced seizure model TQ at both the doses (50- and 100 mg/kg) had no intrinsic protective effects. High dose of TQ tended to protect but this was statistically insignificant ($p>0.05$). However, TQ (100 mg/kg) combined with SVP (200 mg/kg) significantly ($p<0.05$) protected against MES-induced seizures at all the three time points (Table 1).



Eight animals were used in each group. PTZ was administered 15 min after pretreatments with protective agents. Treatment groups at each time point were compared by using one way ANOVA. The level of significance was accepted at P<0.01. In post hoc analysis Tukey Kramer Multiple comparison was used at each time point independently. * P<0.05; ** P<0.01; ***P<0.001 compared to control. Compared to group 3. # P<0.001; @ Compared to group 5. P<0.05

Fig. 1. Effect of sodium valproate and thymoquinone on the PTZ-induced convulsions in mice using Convuls-1 meter

Group N°.	Treatment/dose mg/kg	Convulsion counts (Mean ± SEM)		
		10 min	20 min	30 min
1	Control (Saline, 10ml/kg)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
2	Sod. Valproate (200 mg/kg)	0.625 ± 0.183	0.625 ± 0.183**	0.125 ± 0.125***
3	Sod. Valproate (400 mg/kg)	0.125 ± 0.125***	0.0 ± 0.0***	0.0 ± 0.0***
4	Thymoquinone (50 mg/kg)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
5	Thymoquinone (100 mg/kg)	1.0 ± 0.0	1.0 ± 0.0	0.625 ± 0.183*
6	SVP 200 mg/kg +TQ 50mg/kg	0.125 ± 0.125***#@	0.0 ± 0.0***#@	0.0 ± 0.0***@
7	SVP 200 mg/kg +TQ 100 mg/kg	0.0 ± 0.0***#@	0.0 ± 0.0***#@	0.0 ± 0.0***@

Eight animals were used in each group. Animals were subjected to MES 15 min after pretreatments with protective agents. In each column the treatment groups were compared by using one way ANOVA. The level of significance was accepted at P<0.01. In post hoc analysis Tukey Kramer Multiple comparison was used in each column independently. * P<0.05; ** P<0.01; ***P<0.001 compared to control. # P<0.05, Compared to group 2; @P<0.001, Compared to group 4.

Table 1: Effect of valproate and thymoquinone on the MES-induced convulsions using ECT UNIT.

TQ at doses of 50 and 100 mg/kg, significantly ($p< 0.05$) reduced the ED_{50} value of SVP from 162.5 to 119 and 41.9 mg/kg, respectively in PTZ model. On the other side, TQ at a dose of 50 mg/kg, had no significant additive protective effect ($p>0.05$) on the ED_{50} value of SVP in MES-induced seizure model. But TQ at a higher dose (100 mg/kg), significantly ($p < 0.05$) reduced the ED_{50} of SVP from 173.55 to 125.3 mg/kg in MES model (Figure 2).

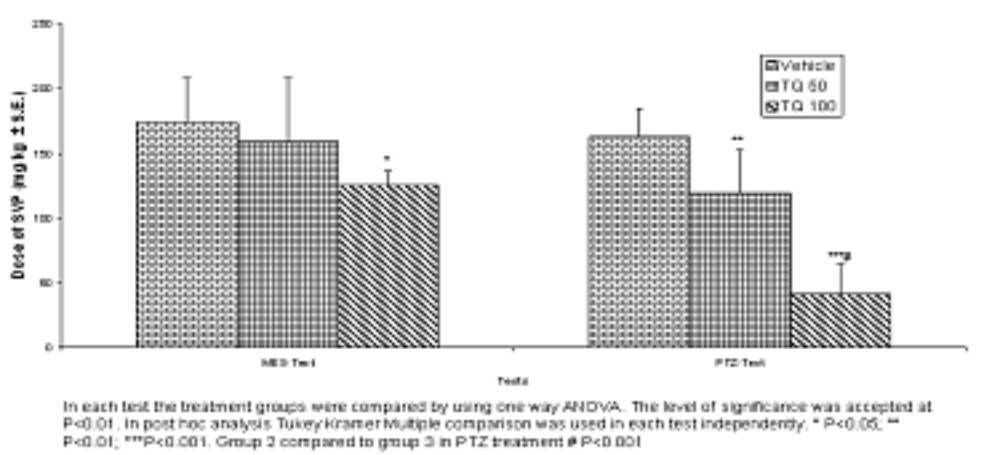


Fig. 2. Synergistic effect of TQ on the ED_{50} of SVP in MES- and PTZ-induced convulsions tests in mice

Effect of TQ on SVP induced aminotransferases, lipid peroxidation and glutathione

SVP caused an increase in the ALT activity with the values 26.50±1.76 U/l. This increase was statistically significant (P<0.01) when compared with the control values 18.33±0.84 U/l. Co-administration of SVP with TQ to the 4th group lowered the ALT activity slightly significantly (P<0.05) when compared to SVP alone (Table 2). SVP significantly (P<0.05) increased the AST activity from 10.30±1.06 (control group) to 13.73±0.67 U/l (group 3). Co-treatment with TQ altered the effect of SVP in group 4 and lowered the AST activity when compared to TQ alone (Table 2).

Group N°.	Treatment group	ALT, U/l Mean ± SEM	AST, U/l Mean ± SEM
1	Control (saline, i.p.)	18.33 ± 0.84	10.30 ± 1.06
2	Thymoquinone (5mg/kg)	22.83 ± 1.49*	9.93 ± 0.84
3	Sodium Valproate	26.50 ± 1.76**	13.73 ± 0.67*
4	Sodium Valproate + Thymoquinone	19.66 ± 0.66[a]	12.18 ± 0.78

Compared to group 1 (*P<0.05; **P<0.01); Compared to group 3, [a]P<0.05 (Students t-test).

Table 2: Effect of thymoquinone and valproate on ALT and AST levels in mice blood plasma.

SVP induced a significant (P<0.05) increase in the mice liver lipid peroxides from 257.72±7.23 (Control) to 278.05±6.17 nmol/g wet liver tissue. Co-treatment of mice with TQ did not significantly lower the effect of SVP and values remained significantly (P< 0.01) high (289.60±6.92) as compared to control group (Table 3).

Group N°.	Treatment Group	MDA nmole /g wet tissue Mean ± SEM	G-SH nmole/100mg tissue Mean ± SEM
1	Control (Vehicle)	257.72 ± 7.23	66.87 ± 1.66
2	Thymoquinone (5mg/kg)	246.03 ± 6.15	68.79 ± 2.80
3	Sodium Valproate	278.05 ± 6.17*	40.77 ± 2.63***
4	Sodium Valproate + Thymoquinone	289.60 ± 6.92**	61.36 ± 3.80[b]

Treatment groups were statistically compared to group 1. (Students t-test, *P<0.05; **P<0.01; ***P<001). Compared to group 3, [b]P<0.01

Table 3: Effect of thymoquinone on valproate induced lipidperoxidation and G-SH levels in mice liver after 21 days treatment.

SVP also caused a significant ($P<0.001$) lowering of glutathione in mice liver tissue to 40.77±2.63 as compared to 66.87±1.66 nmol/100mg wet tissue of control group. Co-treatment with TQ significantly ($P<0.01$) averted any decline in G-SH contents by SVP and the values remained essentially closer to the control group (Table 3).

Discussion

In the first part anticonvulsant activities of TQ 50- and 100-mg/kg acute doses were investigated and high dose was found to be minimally effective in preventing MES- and PTZ-induced convulsions. However, 50 mg/kg was selected as a sub-therapeutic dose. Ilhan et al., [18] has recently demonstrated NS total oil effectively prevented PTZ-induced kindling in mice by attenuating PTZ-induced injury to the brain tissue and ascribed this effect to its antioxidant properties. Hosseinzadeh and Parvardeh [19] reported a reduction in the duration of myoclonic seizures and protection against mortality by TQ. In another study [20] the authors reported that TQ was effective in petit mal epilepsy in mice most probably through an increase in opioid recptor mediated GABAergic tone.

In these experiments, using PTZ and MES models the effects of two doses (50 and 100 mg/kg) of TQ on the dose-response curve of SVP were investigated. TQ at 50 and 100 mg/kg doses significantly reduced the ED_{50} value of SVP in PTZ model from 161 mg/kg to 112 and 35 mg/kg, respectively. Against MES-induced seizures, 50 mg/kg of TQ had no significant additive effect on the ED_{50} value of SVP. However, the high dose of TQ (100 mg/kg) significantly reduced ED_{50} of SVP from 170.1 mg/kg to 124.8 mg/kg.

It can be mentioned that TQ (in both PTZ and MES models) increased SVP potency, and apparently reduced its dose. However, the reduction in SVP dose (required for anticonvulsant activity) may be valuable to reduce or eliminate its unwanted effects like hepatotoxicity and teratogenic implications.

On the other hand, subacute administration of SVP (i.e. for 21 days) produced hepatotoxicity characterized by elevated serum ALT. This result is consistent with previous reports [31,32]. Addition of TQ with SVP in drinking water for the same period protected liver against SVP induced toxic effect indicated by inhibition in serum ALT activity as compared to SVP alone.

There is sufficient information in the literature that SVP is metabolized to unsaturated toxic products in the body [33,34,35]. These studies have shown that metabolites formed by omega oxidation pathway, 2-n-propyl-4-pentenoic acid and other delta dehydrogenation products may cause hepatotoxicity. The 4-ene

metabolic moieties interfere with both cytochrome P-450 and β-oxidation and lead to ultra structural changes. Raza *et al*., [27] have demonstrated that these ultra structural changes to be the results of free radicals damage that are produced during the metabolism of SVP. They used the same protocols and doses and described the generation of free radicals being the bases for the liver and kidney damage and confirmed it by the histopathological evidence [28]. It is also well known that the TQ could protect against toxic damage induced by free radicals generation [15,36] and a further support comes from the reports on its antioxidant properties in a certain dose range in vivo [37].

The findings of the present study indicating that TQ possesses hepatoprotective activity are in agreement with literature. Daba and Abdel-Rahman [38] tested TQ in isolated rat hepatocytes as a protective agent against tert-butylhydroperoxide (TBHP) toxicity. Preincubation of hepatocytes with 1 mM of TQ resulted in the protection against TBHP.

It has been reported that TQ pretreatment in drinking water for 5 days (8 mg/kg/day p.o.) protected mice against hepatotoxicity induced by CCl_4 [15]. The hepatoprotective effect of TQ against CCl_4-induced hepatotoxicity was demonstrated by the significant prevention to any increase in serum ALT, AST and LDH associated with a parallel significant inhibition in production of hepatic lipid peroxide content. Our results on SVP toxicity are compatible with studies demonstrating amelioration by pretreatment with free radical traps [36] or antioxidants [37]. Reports in the literature are suggestive that the ameliorative effect of TQ versus CCl_4-induced hepatotoxicity is mediated through decreased hepatic lipid peroxidation [15,39]. Nagi and colleagues [17] evaluated TQ as an inhibitor of lipid peroxidation using liver homogenate as the source of lipid, and ferric ascorbate to induce lipid peroxidation. In their findings TQ inhibited the *in vitro* peroxidation with median inhibitory concentration (IC_{50}) of 0.87 μM that lead Nagi and colleagues to suggest that the inhibition of lipid peroxidation might be the initial event in the mechanism by which TQ ameliorates hepatotoxicity. In the same report [17] dihydrothymoquinone (DHTQ) was found to be a powerful antioxidant compared to TQ. They strongly suggested that *in vivo* protection by TQ might be due to combined action of TQ and DHTQ, as the former is likely to be biotransformed into DHTQ by DT-diaphorase in the liver.

From the results of the present study that reveal a protective role of TQ against SVP-induced toxic damage, it could be mentioned safely that TQ behaves as an antioxidant and protects liver against detrimental effects of SVP. It also,

protect against the liver enzyme induction seen in the case of SVP alone. TQ significantly reduced the ED_{50} values of SVP in both MES- and PTZ- models; and a reduction in the dose of SVP might prove a useful strategy in reducing its hepatic and teratogenic implications.

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