Effect of *Tribulus terrestris* on Haloperidol-induced Catalepsy in Mice

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Haloperidol, an antipsychotic drug, leads to the development of a behavioural state called catalepsy, in which the animal is not able to correct an externally imposed posture. In the present study we have attempted to evaluate the anticataleptic effect of *Tribulus terrestris* on haloperidol-induced catalepsy in albino mice. Mice were allocated to four groups, each group containing six animals. Both, the test drug, *Tribulus terrestris* and the standard drug trihexyphenidyl were uniformly suspended in 1% gum acacia solution. Catalepsy was induced in mice with haloperidol (1.0 mg/kg, intraperitoneally). The first group received the vehicle (10 ml/kg, orally), the second group received trihexyphenidyl (10 mg/kg, orally) and the remaining two groups received *Tribulus terrestris* (100, 200 mg/kg, orally). The animals were assessed after single and repeated dose administration for ten days, 30 min prior to haloperidol, using standard bar test. The result of the present study demonstrates *Tribulus terrestris* has a protective effect against haloperidol-induced catalepsy, which is comparable to the standard drug used for the same purpose. Our study indicates *Tribulus terrestris* can be used to prevent haloperidol-induced extrapyramidal side effects.

Key words: *Tribulus terrestris,* catalepsy, haloperidol, trihexyphenidyl

Haloperidol is an antipsychotic drug, which is used in the treatment of schizophrenia and other psychotic disorders. Antipsychotics are often associated with distressing extrapyramidal side effects[1,2]. Haloperidol-induced catalepsy occurs due to the blockade of dopamine (D2) receptors and reduced dopaminergic transmission[3]. Enhanced stimulation of the intrinsic central cholinergic system has also been implicated in haloperidol-induced catalepsy as it has been reported to be intensified and antagonized by pilocarpine and atropine, respectively[4]. Evidence

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also suggests that the central serotonergic system modulates nigrostriatal dopaminergic transmission with 5-HT_3 antagonists reported to alleviate neuroleptic-induced catalepsy[5]. The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics (e.g. haloperidol) is a robust behavioral model to study nigrostriatal function and its modulation by cholinergic, serotonergic, nitrergic and other neurotransmitter systems[5,6]. Trihexyphenidyl, a central anticholinergic has been used as standard drug in this study to compare the anticataleptic effect of the test compound, *Tribulus terrestris*. *Tribulus terrestris* (TT, puncture vine, caltrop, *Gokshura, Gokhru*), is an indigenous medicinal plant of the *Zygophyllaceae* family, native to warm temperature and tropical regions of the old world in Southern Europe, Southern Asia, Africa and Northern Australia. It can thrive even in desert climates and poor soil[7]. *Tribulus terrestris* is a rich source of biologically active compounds such as saponins, flavonoids, amides, alkaloids and the therapeutic properties of the plant have been known for many years[8]. *Tribulus terrestris* is used in the Indian and Chinese system of medicine for treating various male reproductive disorders, and is also used as a tonic, analgesic, astringent, antihypertensive, diuretic, aphrodisiac[9], urinary antiinfective[10] and antidepressant[11]. Its aqueous extract has shown diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization *in vitro*[12]. Tribulosin, a methanol extract of *Tribulus terrestris* protects rat heart from ischemia/perfusion injury[13]. The whole plant extract of *Tribulus terrestris* showed antioxidant activity and has exerted protective effect on streptozotocin-induced diabetic rats by inhibiting oxidative stress[14]. Protodioscin, a steroidal glycoside found in *Tribulus terrestris*[8] increased the levels of testosterone, dihydrotestosterone, and dihydroepiandrosterone, and there by improved libido, erectile dysfunction[15] and low seminological indices[16]. The effect of this plant on nigrostriatal function has not been studied yet. Hence the present study was undertaken to evaluate the anticataleptic activity of *Tribulus terrestris* in Swiss albino mice.

Healthy adult male Swiss albino mice of either sex weighing 20-30 g inbred in the institutional Central Animal House were used for the study. Mice were housed in clean transparent polypropylene cages, with dust free rice husk as a bedding material; six mice in each cage, under controlled laboratory conditions. Controlled temperature (23±2°), humidity (60±10%), and free access to standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth laboratory animal feed manufactured by Pranav Agro Industries Ltd., Sangli) and water *ad libitum* were provided. The mice were allowed to acclimatize to the laboratory conditions for one week prior to the commencement of the study. Experiments were performed between 10:00 and 16:00 h during the light phase of the cycle. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the study was conducted according to CPCSEA guidelines for the use and care of experimental animals.

The animals were divided into four groups (n=6). Group I received the vehicle 1% gum acacia solution (10 ml/kg)[17] and served as the control, group II received the standard drug trihexyphenidyl (German Remedies Ltd, Mumbai) at the dose of 10 mg/kg[17], groups III and IV received the test drug *Tribulus terrestris* fruit extract powder (Provided by M/s. Himalaya Health Care, Bangalore) suspended in doses of 100 and 200 mg/kg, respectively[11]. All drug solutions were freshly prepared and suspended in 1% gum acacia solution and administered orally using a feeding tube. Thirty minutes after administration of drugs/vehicle haloperidol (RPG Life Sciences Ltd., Mumbai) at a dose of 1 mg/kg body weight[17] was administered intraperitoneally. In the acute study, vehicle/drugs were administered, 30 min prior to haloperidol administration. While in the chronic study, standard and test drugs were administered once daily for 10 days, and the last dose was given on the 10th day, 30 min prior to haloperidol administration.

Haloperidol-induced catalepsy was induced and assessed at 30 min intervals until 120 min and at the end of 240 min on a standard bar test[18]. Haloperidol 1 mg/kg i.p. was chosen to induce so that it could elicit a moderate degree of catalepsy and thus enable the detection of either attenuation or potentiation of the phenomenon. Catalepsy was assessed in terms of the duration for which the mouse maintained an imposed position with both front limbs extended and resting on a 4 cm high wooden bar (1 cm diameter). The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. If the animal maintained the imposed posture

for at least 20 s, it was said to be cataleptic and given one point. For every further 20 s that the animal continued to maintain the cataleptic posture one extra point was given[18]. The animals were tested twice at 30 min intervals and only the greater duration of immobility was considered.

The results were expressed as the mean±standard error of mean (SEM) and analyzed by using one-way analysis of variance (ANOVA), followed by Dunnett's *Post hoc* test. A $P<0.05$ was considered statistically significant.

The cataleptic score of the acute and chronic study of the test drug *Tribulus terrestris* on haloperidol-induced catalepsy are given in Tables 1 and 2, respectively. A significant reduction ($P<0.001$) in cataleptic score was observed throughout the period of observations, compared to control (Vehicle+haloperidol) with both the standard drug trihexyphenidyl 10 mg/kg and the test drug *Tribulus terrestris* at all doses tested (100 and 200 mg/kg). The anticataleptic activity of *Tribulus terrestris* is comparable to that of the standard drug trihexyphenidyl.

Typical neuroleptic agents such as chlorpromazine, haloperidol and reserpine induce a cataleptic state in rodents, which is widely used as a model to test the extrapyramidal side effects of antipsychotic agents. Haloperidol is a well-known neuroleptic, primarily acting as a D2 receptor antagonist in the mesolimbic-mesocortical pathway. Due to its non-selective action, it also produces blockade of post-synaptic D2 receptors in the nigrostriatal pathway leading to the development of extrapyramidal side effects in humans[19] and catalepsy in animals[20]. Neuroleptic-induced catalepsy is a robust behavioural method for studying nigrostriatal function and its modulation by cholinergic[21], GABAergic[22], serotonergic[23] and nitrergic[24] systems. Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine or opioids have also been implicated in the catalepsy induced by neuroleptic agents[25].

In addition to the implications of various neurotransmitters in catalepsy, reactive oxygen species have also been proposed to play a role in haloperidol-induced toxicity[25]. Several earlier behavioural studies have demonstrated dopamine facilitator activity and have reported the antioxidant properties of *Tribulus terrestris*[12,13] and it has been claimed to give remarkable protection against lipid peroxidation[14]. Since reactive oxygen species have been implicated in haloperidol-induced toxicity it can be safely assumed that the antioxidant property of *Tribulus terrestris* may contribute towards its anticataleptic activity too. Hence further studies are required using more experimental paradigms and neurochemical analysis to elucidate targets of action and the possible mechanism of action of *Tribulus terrestris*.

The present study demonstrates that *Tribulus terrestris* has a protective effect against haloperidol-induced

TABLE 1: EFFECT OF ACUTE ADMINISTRATION OF *TRIBULUS TERRESTRIS* ON HALOPERIDOL-INDUCED CATALEPSY

Groups	Dose (/kg)	Cataleptic score at different time points (min)				
		30	60	90	120	240
I. Vehicle+haloperidol	10 ml+1 mg	13.50±0.95	15.00±0.00	13.50±0.71	11.67±0.91	8.33±0.55
II. Trihexyphenidyl+haloperidol	10 mg+1 mg	11.67±0.55	9.33±0.42*	8.17±0.91*	6.33±0.71*	3.33±0.55*
III. *Tribulus terrestris*+haloperidol	100 mg+1 mg	13.00±0.93	11.50±0.92*	10.67±0.88*	8.00±0.68*	3.17±0.54*
IV. *Tribulus terrestris*+haloperidol	200 mg+1 mg	11.50±0.76	9.33±0.49*	9.17±0.30*	6.00±0.57*	3.00±0.68**

Values are expressed as mean±standard error of mean (SEM), each group comprises of 6 animals. Results were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's Post hoc test. *$P<0.05$, was used to indicate statistical significance when compared with control (vehicle+haloperidol)

TABLE 2: EFFECT OF CHRONIC ADMINISTRATION OF *TRIBULUS TERRESTRIS* ON HALOPERIDOL-INDUCED CATALEPSY

Groups	Dose (/kg)	Cataleptic score at different time points (min)				
		30	60	90	120	240
I. Vehicle+haloperidol	10 ml+1 mg	14.83±0.16	14.67±0.33*	14.33±0.33*	11.67±0.71*	12.00±0.81*
II. Trihexyphenidyl+haloperidol	10 mg+1 mg	12.17±0.30	9.33±0.21*	7.83±0.87*	6.00±0.57*	3.17±0.30*
III. *Tribulus terrestris*+haloperidol	100 mg+1 mg	13.17±0.79	10.33±0.21*	8.83±1.16*	5.83±1.01*	1.67±0.33*
IV. *Tribulus terrestris*+haloperidol	200 mg+1 mg	14.67±0.33	12.00±0.36*	10.00±0.73*	6.33±0.49*	2.00±0.258*

Values are expressed as mean±standard error of mean (SEM), each group comprises of 6 animals. Results were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's Post hoc test. *$P<0.05$, was used to indicate statistical significance when compared with control (vehicle+haloperidol)

catalepsy, which is comparable to the standard drug trihexyphenidyl. Our study indicates that *Tribulus terrestris* could be used as an alternative/adjuvant drug in preventing and treating the extrapyramidal side effects of antipsychotic agents in clinical practice. However, it requires further preclinical and clinical studies to prove it.

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