Effect of Chronic Administration of Ursolic Acid on Haloperidol Induced Catalepsy in Albino Mice

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Neuroleptic drugs used in the treatment of schizophrenia and other affective disorders are known to produce extra pyramidal side effects. Catalepsy induced by these drugs in animals has been used as a model for the extra pyramidal side effects associated with antipsychotic agents in human beings. In the present study, we have attempted to evaluate the protective effect of the ursolic acid (UA) on haloperidol (1.0 mg/kg, intraperitoneal administration)-induced catalepsy (HIC) in mice by employing the standard bar test. Mice were allocated to five groups, each group containing six animals. The effects of the test drug UA (at 0.05, 0.1 & 0.2 mg/kg doses) and the standard drug, scopolamine (1.0 mg/kg) was assessed after repeated dose administration for seven days, 30 minutes prior to the haloperidol. The results suggest that UA has a protective effect against haloperidol-induced catalepsy, which is comparable to the standard drugs used for the same purpose. Our study indicates that UA could be used to prevent neuroleptic drug-induced extra pyramidal side effects.

Key words: Catalepsy, haloperidol, ursolic acid, scopolamine, mice

INTRODUCTION

Neuroleptic-induced catalepsy has long been used as an animal model for screening drugs for Parkinsonism[1]. There is considerable evidence that blockade of DA transmission produces catalepsy[2] in rats and extrapyramidal side effects in humans[3]. Catalepsy is defined as the failure to correct an externally imposed posture. This test is widely used to evaluate the effect of drugs on extrapyramidal system[1]. Haloperidol blocks dopamine D_2 receptors and produces a state of catalepsy in animals by reducing dopaminergic transmission in basal ganglion. Anticholinergic drugs are most effective in counteracting the catalepsy induced by haloperidol in experimental animals[4]. But these anti-cholinergic drugs produce various side effects like dryness of mouth, constipation and urinary retention. Hence the search for newer drugs with fewer side effects is continuing.

Ursolic acid is a triterpenoid compound which exists widely in natural plants in the form of free acid or aglycones for triterpenoid saponins[5]. Triterpenoids have many biological effects and interest in biological activity of triterpenoids is growing. Various plants having ursolic acid as an active ingredient have shown hepatoprotective activity[6]. Ursolic acid has also been implicated in inhibition of lipoxygenase and cyclooxygenase in HL60 leukemic cells[7], inhibition of mutagenesis in bacteria[8], antitumor-promotion[9], inhibition of histamine release[10], inhibition of lipid peroxidation and protection against adriamycin toxicity[11], antimicrobial activity[12], inhibition of mouse skin tumorigenesis[13], anti-inflammatory action[14],

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hypolipidemic and anti-atherosclerotic effects[15] , anti-ulcer activity[16] and cytotoxicity to leukemia cells[17].

Ursolic Acid has been identified as the active principle of *Ocimum sanctum*. From our laboratory we have reported ethanolic extract of leaves of Ocimum *sanctum* (OS) against haloperidol induced catalepsy in mice[18], antianxiety[19], antidepressant activity[20]. With this background, in the present study we have investigated the anti-cataleptic effect of ursolic acid on haloperidol induced catalepsy in mice.

Animals:

Adult male albino mice (weighing 25-30gm), bred in the central animal house of Kasturba medical college, Mangalore, were used for the study. The animals were housed under standard 12h: 12h light / dark cycle and supplied with food and water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for at least seven days prior to any experimentation. Each animal was used only once. The experiment procedures were performed between 10.00 and 16.00 hrs. The experimental protocol was approved by the Institutional Animal Ethics Committee and the study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs:

The test drug, ursolic acid(Sigma Aldrich Chemicals Pvt. Ltd, United Kingdom, HS No. 29181985900) was dissolved in 14% dimethyl sulfoxide (DMSO) and administered orally in a dose of 0.05, 0.1, 0.2 mg / kg). The standard drug scopolamine (German Remedies Ltd., Mumbai) was suspended in 1% gum acacia solution and administered orally ((1.0mg / kg). Haloperidol (RPG Life Sciences Ltd., Mumbai) was dissolved in distilled water and was given by the intraperitoneal route (1.0mg / kg). 14% DMSO (Sigma Aldrich Chemicals Pvt.Ltd, United Kingdom, HS No.29309085990) administered by oral route (10ml / kg) served as the vehicle.

Experimental design:

Haloperidol induced Catalepsy (HIC):

Thirty minutes after administration of vehicle / drugs, haloperidol (1mg / kg body weight) was administered by the intraperitoneal route to induce catalepsy. This dose of haloperidol was chosen to produce a moderate degree of catalepsy so that attenuation or potentiation of the phenomenon could be detected[21]. The degree of catalepsy was measured at 30, 60, 90, 120 and 240 minutes after haloperidol administration by using a method similar to the standard bar test[22].

Catalepsy was assessed in terms of the time for which the mouse maintained an imposed position with both front limbs extended and resting on a four cm high wooden bar (1.0 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. A cut-off time of 1100 seconds was applied during the recording of observations[23]. The animals were returned to their individual home cages in between determinations. All observations were made between 10.00 and 16.00 hrs in a quiet room at 23-25º C.

Scoring method:

If the animal maintained the imposed posture for at least 20 seconds, it was considered to be cataleptic and given one point. One extra point was given for every additional period of 20 seconds that the cataleptic posture was maintained. The animals were tested twice at 30 minute time intervals and only the greater duration of immobility was considered[21].

In the chronic study, these drugs were administered once daily 30 min prior to the haloperidol administration for seven days. Catalepsy was determined 30 min after haloperidol administration on the first and on the seventh day of treatment.

Time (mn)	Control (14% dmso) 10ml/kg	Scopolamine 10mg/kg	Ursolic acid mg/kg		
			0.05	0.1	0.2
30	16.3±0.5	12.0±0.6*	10.0±1.5*	12.0±0.7	14.0±0.9
60	23.0±1.3	14.0±0.7***	11.0±1.6***	14.0±0.9***	15.0±1.2**
90	24.6±1.4	16.0±0.7**	13.0±0***	14.0±1.0***	17.0±1.0**
120	28.5±0.6	17.0±1.3***	16.0±0.7***	18.0±0.7***	20.0±1.5**
240	33.0±0.6	17.1±1.3***	19.0±0.6***	19.3±1.2***	20.0±1.2***

Table 1: Chronic administration of ursolic acid on haloperidol induced catalepsy in mice.
Time after haloperidol administration, number of animals in each group, number of animals; n=6, values are mean ± SEM.
*P<0.05; **P<0.01; ***P<0.001

Statistical analysis:

For each group, mean ± SEM was calculated and the data was analyzed by one way ANOVA followed by Dunnet's multiple comparison tests. P<0.05 was considered to be statistically significant. The statistical package used for the analysis was SPSS version 11.0.

RESULTS

In the chronic phase of the study (Table 1), the standard drug, scopolamine and the test drug, ursolic acid at 0.05mg/kg dose showed a significant reduction in the cataleptic scores when observed at the end of 30 min after the last dose of haloperidol administration. In the subsequent observations, ursolic acid showed significant reductions in the cataleptic scores at all the doses used. Moreover, the reduction in cataleptic score was dose dependent and comparable to the reduction shown by the standard drug, scopolamine.

DISCUSSION

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. Neuroleptic-induced catalepsy has been linked to a blockade of postsynaptic striatal dopamine D1 and D2 receptors. Despite this evidence, theories implicating central cholinergic dysfunction[4], g-amino butyric acid (GABA) deficiency[24], oxidative stress[25], and 5-hydroxy tryptamine (5-HT) dysfunction[26] have also been proposed. In addition to various neurotransmitters, many preclinical and clinical studies have also proposed reactive oxygen species as causes of haloperidol-induced toxicity[27]. Evidence indicates that drugs which potentiate or attenuate neuroleptic catalepsy in rodents might also aggravate or reduce the extrapyramidal signs respectively in human beings[28].

The present study revealed the anti cataleptic effect of ursolic acid on chronic administration in a murine model of haloperidol induced catalepsy. Pre-treatment of ursolic acid protected the mice from catalepsy induced by haloperidol as effectively as the standard drug scopolamine. The anticataleptic effect observed was dose dependent manner.

The protective effect of UA against HIC is consistent with our earlier report on anticataleptic effect of an herbal product, ethanolic leaf extract of *Ocimum sanctum*[18] containing UA as one of its active principles. Earlier behavioral studies in rodents have suggested that OS facilitates activation of dopaminergic neurons and increases

dopamine levels in the corpus striatum[29]. Thus, the anticataleptic effect of UA might be due to both its dopamine facilitatory and antioxidant properties. Previous studies have reported the antioxidant properties of ursolic acid and it has been claimed to give remarkable protection against lipid peroxidation[30]. Since reactive oxygen species have been implicated in haloperidol induced toxicity it can be safely assumed that the antioxidant property of ursolic acid may contribute towards its anticataleptic activity also. However, further studies are needed to elucidate its exact mechanism of action.

To conclude, the results of the present study indicates that ursolic acid can be further screened for its potential as an alternative/adjuvant drug in preventing and treating the extrapyramidal side effects of antipsychotic agents in clinical practice.

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