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EXPERIMENTAL RESEARCH

The Effect Of Acute And Chronic Administration Of The Aqueous Extract Of Triphala On Haloperidol Induced Catalepsy In Mice.

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ABSTRACT

Neuroleptics that are commonly used in the treatment of schizophrenia and other affective disorders are often associated with distressing extrapyramidal side effects (catalepsy). Catalepsy induced by neuroleptics in animals has been used as a model for the extrapyramidal side effects associated with antipsychotic agents in human beings. In the present study, we have attempted to evaluate the protective effect of Triphala on haloperidol induced catalepsy in mice. Inbred albino mice were divided into five groups, each containing six animals. Both, the test drug, the aqueous extract of Triphala and the standard drug scopolamine were dissolved in 1% gum acacia solution. Catalepsy was induced with haloperidol (1mg/kg). The first group received the vehicle (10ml/kg), the second group received scopolamine (1mg/kg) and the remaining three groups of animals received the test compound, Triphala (2.5, 6.25 and 12.5 mg/kg respectively) orally. In the acute study, a single dose of vehicle and the test drug were administered, while in the chronic study, they were given once a day for seven days, 30 minutes prior to haloperidol administration. Catalepsy was determined by the standard bar test after 30 minutes of haloperidol administration and was scored as described by Ahtee and Benumbe. In the acute study, the aqueous extract of Triphala at all the doses tested, significantly ($P<0.01$) reduced the cataleptic score after the latency of 60 minutes. However, in the chronic study, the reduction in the cataleptic score was seen throughout the period of observations. These effects were comparable to that produced by the standard drug scopolamine. Pretreatment of Triphala decreased haloperidol induced catalepsy in mice, which is comparable to that produced by the standard drug scopolamine. Triphala seems to be more effective when it is repeatedly administered than with a single administration. It can be used as an alternative drug or with a combination of currently available drugs in treating drug induced extrapyramidal side effects.

Key Words: Triphala, catalepsy, haloperidol

Key Message: Antipsychotics are usually associated with extrapyramidal side effects. The mice were treated by standard drugs like scopolamine or by centrally acting anticholinergics; but these have many side effects like dryness of mouth, blurred vision etc. As Triphala is readily available in India and it can be used as an alternative to presently available drugs against drug induced extrapyramidal side effects.

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Introduction

Neuroleptics that are commonly used in the treatment of schizophrenia and other affective disorders [1], are often associated with distressing extrapyramidal side effects [2],[3]. The phenomenon of cataleptic immobility induced in rodents by typical neuroleptics (eg.haloperidol), is a robust behavioural model to study nigrostriatal function and its modulation by cholinergic, 5-hydroxytryptamine (5-HT, serotonergic), nitrergic and other neurotransmitter systems [4],[5]. Haloperidol induced catalepsy (HIC) occurs due to the blockade of dopamine (D2) receptors and reduced dopaminergic transmission [6]. Enhanced stimulation of the intrinsic central cholinergic system has also been implicated in haloperidol induced catalepsy, as it has been

reported to be enhanced and antagonised by a cholinergic agonist and the blocker, atropine respectively [7]. Hence, scopolamine (a known anticholinergic agent) was used as standard drug in this study to compare the anticataleptic effect of the test compound, Triphala.

Triphala, a cornerstone of traditional Ayurvedic medicine, is composed of equal parts of three dried fruits, namely, *Terminlia chebula, Terminalia belerica* and *Emblica officinalis*. Triphala is used in head ache, dyspepsia, constipation, ascitis and leucorrhoea, and also as a blood purifier. It is also reported to have antiinflammatory, analgesic, antiarthritic, hypoglycaemic, antioxidant and anti-aging properties [8],[9],[10],[11],[12]. Gallic acid has been found to be a major ingredient of Triphala[13]. The standardization of the aqueous extract of Triphala and the estimation of the active principle was done by the Quality Control Laboratory, M/s. Natural Remedies, Bangalore, lab reference no.0505211, dt.31-05-2005.

Experimental evidence indicates that Triphala has very good antioxidant and nitric oxide scavenging activities [14],[15].

From our laboratory, we have reported the anticataleptic activity of *Emblica officinalis* and NR-ANX-C (a polyherbal product) on haloperidol induced catalepsy in mice [16],[17]. *Emblica officinalis* is one of the components of Triphala and Triphala is one of the components of NR-ANX-C. As evidence indicates that the involvement of the reactive oxygen species involved in haloperidol induced catalepsy and Triphala has very good antioxidant activity, this study was undertaken to evaluate the anticataleptic activity of Triphala in mice.

Materials and Methods

Animals

Adult male albino mice (weighing 25-30gm), which were bred in the central animal house of kasturba medical college, Mangalore, were used for the study. The animals were housed under standard12h: 12h light/dark cycle and *ad libitum* food and water. They were allowed to acclimatize to laboratory conditions for at least seven days prior to any experimentation. Each animal was used once. The experimental procedures were performed between 10.00 and 16.00hrs. 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 and Dosage

The Test Drug: Triphala (dry powder supplied by Natural Remedies Pvt. Ltd., Bangalore). It is a brown to very dark brown powder with characteristic odour and taste, stored in air tight containers and protected from light. The standard drug, scopolamine (German Remedies Ltd., Mumbai) and Triphala were suspended/ dissolved in 1% gum acacia solution[18], while haloperidol (RPG Life Sciences Ltd.,Mumbai) was dissolved in distilled water and the doses of Triphala (2.5, 6.25 and 12.5 mg / kg) were selected on the basis of earlier studies (25% of Triphala was present in NR-ANX-C). The treatments were received by each group (each group consisted of six animals, n=6) are shown in [Table1/Fig 1],[Table/Fig 2]. The control group was the receiving vehicle; 1% gum acacia (10ml/kg), Scopolamine (1.0mg/kg) and Triphala (2.5, 6.25 and 12.5 mg / kg) were given orally, whereas haloperidol was given intraperitoneally.

Experimental Design

Haloperidol Induced Catalepsy (HIC): Catalepsy induced with haloperidol (1.0mg/kg i.p.) and was assessed at 30 minutes intervals until 120 minutes and at the end of 240 minutes, by means of a standard bar test [19]. Haloperidol 1mg/kg i.p. was chosen so that it could elicit and thus enable the detection of either attenuation or potentiation of the phenomenon [6]. 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 cms high wooden bar (1.0cm 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 between determinations [18]. The animals were returned to their individual home cages. 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 was given one point. For every additional 20 seconds that the cataleptic posture was maintained, one extra point was given. The animals were tested twice

at 30 minute time intervals and only the greater duration of immobility was considered [20].

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

Statistical Analysis

For each group, mean± SEM was calculated and the data was analyzed by one way ANOVA, followed by Dunnet's multiple comparison test. P<0.05 was considered to be statistically significant.

Results

Acute study [Table/Fig 1]

In the acute phase of the study, after the administration of 2.5, 6.25 and 12.5 mg/kg of Triphala and Scopolamine,a significant (P<0.01) decrease in the cataleptic score was observed at all time intervals, except at the 30 min interval.

Chronic study [Table/Fig 2]

In the chronic phase of the study, after the administration of 2.5, 6.25 and 12.5 mg/kg of Triphala and Scopolamine,a significant (P<0.01) decrease in the cataleptic score was observed throughout the period of observation.

(Table/Fig 1) Effect of acute administration of Triphala on haloperidol induced catalepsy in mice.

Groups (n=6)	Dose/kg.b.w.	Cataleptic scores at different time intervals after haloperidol administration				
		30 min	60 min	90 min	120min	240 min
I-Control-1%gumacacia	10.0ml	20.66 ± 0.80	29.66 ± 2.67	31.44±1.44	31.00 ±3.16	39.16 ±2.91
II-Scopolamine	1.0mg	13.16 ±1.10	17.16 ± 0.94**	22.55 ±1.45**	18.14 ±2.44**	17.66 ±1.43**
III-Triphala	2.5mg	16.66 ±2.73	22.63 ±1.22	19.33 ±2.62**	18.66 ±2.21*	12.66 ±1.02**
IV-Triphala	6.25mg	15.00 ±1.12	13.00 ±1.88**	12.66 ± 0.95**	9.33 ± 0.80**	11.33 ± 0.66**
V- Triphala	12.5mg	16.66 ±3.92	17.64 ±2.91**	23.33 ±2.30*	18.00 ±2.44**	15.16 ±2.88**
F value(df)		1.17 (4,25)	9.44 (4,25)	13.63 (4,25)	10.86(4,25)	32.09(4,25)

(Values are mean±SEM, statistical analysis by one way ANOVA followed by Dunnet's multiple comparison test; *P<0.05,P<0.01 compared with control, df,degrees of freedom, b.w., body weight)**

(Table/Fig 2) Effect of chronic administration of Triphala on haloperidol induced catalepsy in mice

Groups (n=6)	Dose/kg.b.w	Cataleptic scores at different time intervals after haloperidol administration				
		30 min	60 min	90 min	120min	240 min
I-Control-1%gumacacia	10.0ml	25.00 ± 2.49	29.66 ± 2.67	39.00± 4.18	41.83 ± 2.66	45.55 ± 2.91
II-Scopolamine	1.0mg	13.66 ±0.49**	15.83 ± 0.47**	18.33 ± 0.40**	16.66 ± 0.49**	14.50 ± 0.56**
III-Triphala	2.5mg	12.00 ±1.09**	9.16 ± 0.60**	8.66 ± 1.05**	11.16 ± 0.60*	6.16 ± 0.47**
IV-Triphala	6.25mg	9.16 ± .1.13**	9.83 ± 0.87**	6.83 ± 0.70**	8.66 ± 0.33**	7.83 ± 0.40**
V- Triphala	12.5mg	9.50 ± 0.99**	9.66 ± 1.20**	13.83 ± 1.83*	13.66 ± 1.43**	8.16 ± 0.54**
F value(df)		(4,25) 21.24	(4,25) 38.21	(4,25) 38.62	(4,25) 91.52	(4,25) 73.09

(Values are mean±SEM, statistical analysis by one way ANOVA followed by Dunnet's multiple comparison test; *P<0.05,P<0.01 compared with control, df, degrees of freedom, b.w., body weight)**

Discussion

Typical neuroleptic agents like chlorpromazine, haloperidol and reserpine induce a cataleptic state in rodents and it is being used as a model to test the extrapyramidal side effects. Neuroleptic induced catalepsy has been linked to a blockade of the postsynaptic striatal dopamine D1 and D2 receptors [20]. Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine, or opioids have also been implicated [21]. In addition to the implications of various neurotransmitters in catalepsy, many preclinical and clinical studies have proposed reactive oxygen species in haloperidol induced toxicity [22]. Evidence indicates that drugs which potentiate or attenuate neuroleptic induced catalepsy in rodents might aggravate or reduce extrapyramidal signs, respectively in human beings [23].

In the present study, the aqueous extract of Triphala decreased the haloperidol induced catalepsy, which is comparable to that of the standard drug, scopolamine. The anticataleptic effect is more pronounced when Triphala was administered repeatedly (chronic administration), than with a single dose administration. The protective effect of Triphala against HIC was consistent with our earlier reports on the anticataleptic effect of a polyherbal product, NR-ANX-C [18], in which Triphala is one of the components. The reports on the individual components of Triphala; *Terminalia chebula* belongs to the family Combretaceae, commonly known as 'haritaki' in Ayurveda. The dried fruits are rich in tannins and also the presence of a variety of carbohydrates, glucose, sorbitol, saponins, anthrones and anthranols has also been documented [24]. *Terminalia bellerica* belongs to the family Combretaceae, commonly known as 'vibhitaki' in Ayurveda. The dried fruit contains about 20% of both condensed and hydrolysable tannins, lipids, b-sitosterol, saponins, gallic and ellagic acid and their

derivatives, glycosides and various carbohydrates. Fruits of *Terminalia chebula* and *Terminalia bellerica* were reported to be stronger antioxidants than alpha-tocopherol[25]. *Emblica officinalis* (EO) belongs to the family Euphorbiaceae, commonly known as 'amalaki' in Ayurveda. It contains tannins and other phenolic compounds. These include hydrolysable tannins (10-12%) such as emblicanins A and B, punigluconin, pedunculagin and an ellagitannin, putranjivain A. Recent investigations have shown that the tannoid principles of *Emblica officinalis* have significant antioxidant effects in rat brain frontal cortex and striatum [26], and also, EO was found to be effectively reduce haloperidol induced catalepsy in mice [16]. Triphala has been found to attenuate cold-stress induced elevation in lipid peroxidation (LPO) and corticosterone levels [27].

Based on the present study results and earlier antioxidant reports on Triphala, it is clear that further human studies are required to confirm the effectiveness of Triphala against the extrapyramidal side effects of antipsychotic drugs. At this juncture, it is difficult to point out which constituent of the preparation is /are responsible for the anticataleptic activity of this test compound.The anticataleptic effect of Triphala could be due to its antioxidant and free radicals scavenging action [14],[15]. Further investigations on their mode of action are needed to unravel the molecular mechanisms involved in the observed effects.

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