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Evaluation of CNS effects of Dhatri Lauha: An Ayurvedic Preparation

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Abstract

A total of four experiments were carried out at different doses (100, 200 and 400 mg/kg, p.o.) of Dhatri Lauha in different animal model in an attempt to confirm the safety of the general patients or users of the society and country as a whole. These were Hole cross test, Hole board test, Open field test and Climbing out test. In Hole cross test highly significant ($p<0.005$) increase in motor activity was observed but only at a dose of 200mg/kg and only at min 30. Highly significant ($p<0.005$) increase in ambulation behavior was observed in Hole board test only at a dose of 100mg/kg after 240 min and a significant increase ($p<0.01$) was observed at the same dose after 180 min. No statistically significant changes were observed in case of head-dipping and emotional defecation in the Hole board test. Open field test showed no significant changes in ambulation, center ambulation, standing up behavior and emotional defecation. Climbing out test also did not produce any significant changes in activity. Overall the study reflects inconsistent and insignificant changes in behavior related to CNS activities which clearly demonstrates the safety of Dhatri Lauha.

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Introduction

Ayurveda is a major traditional medicinal system of Bangladesh and the Indian subcontinent originated several thousands year ago and it is still being successfully used in many countries. Ayurveda and variations of it have also been practiced for centuries

in Pakistan, Nepal, Bangladesh, Sri Lanka, and Tibet [1]. Ayurveda uses the concept of purification as a means to eradicate disease rather than to cure as perceived by modern medicine. Numerous Ayurvedic medicinal plants have shown strong chemotherapeutic and immunomodifying effect in experimentally induced infections.

Recapitulation and adaptation of the older science to modern drug discovery processes can bring renewed interest to the pharmaceutical world and offer unique therapeutic solutions for a wide range of human disorders. The global market for herbal and ayurvedic medicine is estimated to be more than $60 billion a year and many people in the West are showing growing interests [2].

Officially recognized by the government of Bangladesh shortly following independence, Unani and Ayurvedic drugs were brought under a drug control system in 1982 to provide oversight of manufacturing and marketing [3]. Though time-tested evidences show immense therapeutic benefits of these ayurvedic drugs but there is no pharmacologically established data to support these drugs for using against different diseases and for other benefits [4,5]. The present study aims to study the CNS effects of Dhatri Lauha, an Ayurvedic preparation with a view to evaluate the safety of the preparation.

Dhatri Lauha is a widely used formulation in folk and ayurvedic systems of medicine and it is commonly used for Sularoga (colic), pandu (anaemia), kamala (jaundice), and amlapitta (hyperacidity non ulcer dyspepsia). This ayurvedic drug is mainly formulated of Dhatri (amalaki) curna, Lauha curna (bhasma), Yastimadhuraja (curna), Amrta kvatha and these compounds are already well tested for their various types of therapeutic action and in certain diseases.

Materials and Methods

Collection of the Ayurvedic Formulation

For the pharmacological study DHATRI LAUHA (DTR) was collected from Sree Kundeswari Aushadhalaya Ltd, Chittagong, Bangladesh.

Dose

For the pharmacological experiment, the solution of powered tablets was administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. For all the experiments the drugs were administered per oral route. All of the four tests were performed using three different doses of 100 mg/kg, 200 mg/kg, 400 mg/kg of body weight.

Experimental Animals

Male and Female mice (Swiss-Webster strain, 20-40 gm body weight) bred in the Animal House of the Department of Pharmacy, Jahangirnagar University, were used for the pharmacological experiments. They were kept in cages having dimensions of 30 x 20 x 13 cm^3 and soft wood shavings were employed as bedding in the cages. The animals were provided with standard laboratory food and tap water '*ad libitum*' and maintained at natural day night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR). For both control and drug group female mice were used in all experiments.

Controls

A group of equal number of mice (as the drug treated group) was simultaneously employed in the experiment. They were administered distilled water as per the same volume as the drug treated group and this group served as the control. Six to ten mice were taken for each group for both the control and the experiment groups.

Pharmacological Study with Animal Models

Hole Cross Test

In this experiment, the method of Takagi et al [6] was employed. Spontaneous movement of the animals

through the hole from one chamber to the other was counted for a period of 2 minutes. The observation was conducted 30, 60, 120 and 240 minutes after oral administration of test drugs and was compared with control animal and normal saline was administered to the mice under this group. The weight range of female mice for this experiment was 20- 25 g.

Hole Board Test

This experiment was carried out by the following method of Nakama et al. [7] In this test, the number of ambulation (expressed as the number of holes passed), head dipping and number of fecal boluses excretion was recorded for a period of 2 minutes at pre 30 minutes and post 30, 60, 120 and 240 minutes intervals and were compared with the control animals administered with distilled water [7]. The weight range of mice for this experiment was 25 - 30 g.

Open Field Test

In this experiment, the method of Gupta [8] was employed. The number of squares, traveled by the animal, was recorded for a period of two minutes. The weight range of female mice for this experiment was 25- 30 g.

Climbing Out Test

This experiment was carried out by the method of Sandberg [9]. The animals were put in a cage with dimension of 60 X 50 X 30 cm and having dark walls. Animals were supplied with a ladder and the time taken to climbs out of the cage was recorded for a maximum period of 10 minutes. In this experiment the decrease in the number of animals climbed out of the cage or an increase in time taken to come out of the cage is directly proportionate to the CNS depressant property. For this experiment female mice weight 25-30 g were used.

Results and Discussion

Hole Cross Test

At dose of 100 mg/kg, response of the mice at 30 and 60 min were decreased but it was increased in 120, 180 and 240 min. But no results were statistically significant (Table 1). At dose of 200 mg/kg and response of the mice at min 30, the result was found to be statistically highly significant ($p<0.005$). Although there was overall increase in the response, none of the other results were significantly different from the corresponding control animals (Table 1). At dose of 400mg/kg the overall response of the mice decreased compared to the control animals (Table 1).

Table 1: The effect of DTR in the Hole Cross Test

Group (no. of mice)	Dose (mg/kg)	Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=6)		2.333 ± 1.054	3.500 ± 1.408	3.167 ± 0.910	2.667 ± 0.715	2.833 ± 0.703	1.500 ± 0.563
DTR (n=6)	100	0.000 ± 0.000	0.167 ± 0.167	0.333 ± 0.211	0.333 ± 0.211	1.000 ± 0.683	1.333 ± 0.843
	200	2.000 ± 0.856	2.333 ± 0.494[a]	2.333 ± 1.054	1.667 ± 0.919	1.500 ± 0.671	1.667 ± 0.615
	400	1.833 ± 0.980	2.833 ± 0.872	3.000 ± 0.683	1.833 ± 0.401	1.667 ± 0.558	1.000 ± 0.258

[a]$p<0.005$

Hole Board Test

The experiment was carried out to get a clear picture of the effect of the drugs under consideration on the pattern of behavior characterized by spontaneous ambulatory activity, exploratory activity and emotional defecation of the animals. This experiment presents with a different and more complex environment to explore.

Ambulation

At dose of 100 mg/kg the overall response of the mice increased, whereas at min 180 the result of increase was found to be statistically significant ($p<0.01$) and in 240 min, it was highly significant ($p<0.005$) (Table 2). At dose of 200 mg/kg the overall response of the mice increased in 60, 120 and 240 min. The exceptions were in 30 and 180 min when the responses were decreased compared to the respective control animals. But no results were found as statically significant (Table 2). At dose of 400 mg/kg the overall response of the mice were increased except in 30 min the response were found decreased (Table 2).

Head dipping

At dose of 100 mg/kg the response of the mice overall increased but none of the result found as statistically significant (Table 2). At dose of 200mg/kg the response of the mice were found to be increased after 30, 120, and 240 min. The exception was found in 60 and 180 min when it was decreased (Table 2). At dose of 400 mg/kg the response of the mice overall increased but none of the results were found statistically significant (Table 2).

Emotional defecation

At dose of 100 mg/kg the response of the mice found to be increased in 60 and 240 min and then in 30 and 120 min the response was decreased whereas in 180 min the response was found as same (Table 2). At dose of 200 mg/kg the response of the mice overall increased but none of the results were found statistically significant (Table 2). At dose of 400 mg/kg the overall response of the mice increased in 30, 60 and 120 min but none of the results were found statistically significant and the defecation were decreased in 180 and 240 min (Table 2).

Table 2: The effect of DTR on Ambulation in Hole Board Test

	Group	Dose (mg/kg)	Min0	Min30	Min60	Min120	Min180	Min240
Ambulation	Ctrl (n=6)		26.333 ± 15.136	33.333 ± 12.808	32.000 ± 12.332	25.000 ± 9.623	14.833 ± 3.646	23.167 ± 6.462
	DTR (n=6)	100	28.833 ± 4.771	27.500 ± 6.908	29.000 ± 3.256	24.833 ± 4.665	28.000 ± 2.000[a]	25.000 ± 4.830[b]
		200	11.667 ± 3.818	14.833 ± 4.199	16.333 ± 3.703	17.500 ± 1.522	12.833 ± 1.701	16.167 ± 3.478
		400	38.667 ± 3.947	30.333 ± 4.432	34.833 ± 5.412	25.500 ± 3.510	23.333 ± 2.654	30.667 ± 4.924
Head Dipping	Ctrl (n=6)		5.667 ± 2.836	4.500 ± 1.765	6.000 ± 2.129	2.500 ± 0.764	4.333 ± 1.520	3.667 ± 0.919
	DTR (n=6)	100	2.833 ± 1.515	1.833 ± 1.222	1.333 ± 1.333	1.167 ± 1.167	2.500 ± 1.628	1.833 ± 1.327
		200	4.333 ± 2.076	5.167 ± 2.548	5.000 ± 1.713	4.000 ± 2.251	1.833 ± 0.872	2.000 ± 0.966
		400	1.833 ± 1.447	3.167 ± 2.197	3.000 ± 1.770	3.833 ± 1.990	7.000 ± 1.571	6.333 ± 2.404
Emotional Defecation	Ctrl (n=6)		1.333 ± 0.803	1.500 ± 0.563	0.500 ± 0.342	0.500 ± 0.342	0.833 ± 0.401	1.000 ± 0.632
	DTR (n=6)	100	1.000 ± 0.365	0.667 ± 0.333	0.500 ± 0.224	0.167 ± 0.167	0.333 ± 0.333	0.667 ± 0.333
		200	2.000 ± 0.447	1.000 ± 0.516	1.167 ± 0.401	1.167 ± 0.543	0.167 ± 0.167	0.167 ± 0.167
		400	2.333 ± 0.615	1.833 ± 0.654	1.167 ± 0.401	0.833 ± 0.307	0.667 ± 0.422	0.333 ± 0.333

Open Field Test

The experiment was carried out to get a clear picture of the effect of the drugs under consideration on the pattern of behavior. This experiment presents with a different and more complex environment to explore.

Ambulation

DTR treated mice at dose 100 mg/kg levels exerted overall increase in ambulation compared to the control animals except in 30 min when it was found to be decreased (Table 3). At dose 200 mg/kg, the mice exerted overall increase in ambulation as compared with the control group but none of them were found as significant (Table 3). At dose 400 mg/kg the drug-treated group exerted an overall decrease in ambulation compared to the control animals except in 240 min when it was found as almost similar to the control animals (Table 3).

Center ambulation

DTR treated mice at dose levels 100 mg/kg exerted overall increase in total movement in the center region except in 120 and 240 min and at 30 min it was found as similar as compared to the control animals (Table 3). At dose level 200 mg/kg except in 0 and 30 min the total ambulation in the center region had decreased but none of them were found statistically significant (Table 3). At dose 400 mg/kg there was an overall decrease in total movement in the center region except in 30 and 120 min compared to the control animals and none of them were found as statistically significant (Table 3).

Standing up behavior

At dose 100 mg/kg except at 120 and 240 min the number of standing was decreased as compared with the control group (Table 3). Similarly at dose 200 mg/kg except at 180 and 240 min the number of standing was increased in comparison with the control group (Table 3). The exceptions were at the highest dose of 400 mg/kg where DTR treated mice exerted an insignificant decrease (p=0.036) after 30 min in the standing up behavior in comparison to that of control group (Table 3).

Emotional defecation

At dose 100 mg/kg, increased number of stool counted compared to the control group (Table 3). At dose 200 mg/kg the number of stool was found more than control group except in 60 and 240 min when it was found as similar (Table 3). At dose 400 mg/kg in 60 and 240 min, the number of stool counted were more whereas in 30 and 120 min the number of stool count decreased. In 180 min, it was found as similar to the control group (Table3).

Table 3: The effect of DTR on in the Open Field Test

	Group	Dose (mg/kg)	Min0	Min30	Min60	Min120	Min180	Min240
Ambulation	Ctrl (n=6)		155.167± 6.274	89.167 ± 16.993	64.833 ± 8.738	55.167 ± 8.845	72.333 ± 18.645	31.167 ± 8.163
	DTR (n=6)	100	112.333 ± 10.382	82.667± 20.381	73.500 ± 9.062	31.833 ± 14.791	35.833 ± 5.724	32.167 ± 9.432
		200	134.333 ± 21.773	74.833± 28.669	38.333 ± 13.994	40.500 ± 13.488	40.833 ± 21.706	16.333 ± 8.728
		400	90.667 ± 21.263	70.333 ± 9.570	33.667 ± 11.687	40.833 ± 11.473	39.500 ± 12.622	32.000 ± 6.938
Center Ambulation	Ctrl (n=6)		0.333 ± 0.211	0.167 ± 0.167	0.333 ± 0.211	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
	DTR (n=6)	100	0.833 ± 0.401	0.167 ± 0.167	0.833 ± 0.307	0.000 ± 0.000	0.000 ± 0.000	0.333 ± 0.333
		200	0.667 ± 0.333	0.333 ± 0.333	0.167 ± 0.167	0.167 ± 0.167	0.000 ± 0.000	0.000 ± 0.000
		400	1.000 ± 0.365	0.167 ± 0.167	0.000 ± 0.000	0.333 ± 0.333	0.167 ± 0.167	0.000 ± 0.000
Standing Up Behavior	Ctrl (n=6)		16.500 ± 1.522	14.000 ± 3.183	12.667 ± 3.393	7.833 ± 2.358	10.833 ± 3.177	2.833 ± 1.195
	DTR (n=6)	100	7.000 ± 0.966	8.167 ± 2.104	6.833 ± 1.833	4.500 ± 1.821	2.500 ± 0.847	2.333 ± 1.229
		200	4.833 ± 1.447	3.833 ± 0.980	3.500 ± 1.432	2.000 ± 0.683	2.500 ± 1.176	0.333 ± 0.211
		400	23.833 ± 13.651	5.333 ± 1.647	5.000 ± 2.733	3.000 ± 1.461	3.833 ± 1.515	1.833 ± 0.792
Emotional Defecation	Ctrl (n=6)		0.500 ± 0.342	1.167 ± 0.477	0.833 ± 0.477	0.500 ± 0.224	1.167 ± 0.477	1.833 ± 0.703
	DTR (n=6)	100	0.833 ± 0.654	1.167 ± 0.601	1.000 ± 0.447	0.833 ± 0.307	0.667 ± 0.333	0.833 ± 0.167
		200	1.500 ± 0.224[a]	1.500 ± 0.619	0.833± 0.543	1.8333 ± 0.654	1.333 ± 0.422	1.833 ± 0.601
		400	1.167 ± 0.167	0.333 ± 0.211	0.833± 0.401	0.333 ± 0.211	0.833 ± 0.167	0.667 ± 0.211

Climbing Out Test

DTR treated mice (at dose levels of 100 mg/kg) exerted increase in time taken to come out of the cage in 120, and 240 min. The exceptions were observed in 30 min when the time required for the drug treated mice to come out of the cage was decreased than the control group. But no results were found statically significant (Table 4). DTR treated mice at the dose of 200 mg/kg exerted decrease in time taken to come out of the cage in 60, 180 and 240 min. The exceptions were in 120 min when the required time was increased than the control group (Table 4). DTR treated mice at 400 mg/kg dose exerted decrease in time taken to come out of the cage in 60, 180 and 240 min. The exceptions were noticed in 30 and 120 min when the time required for the drug treated mice to come out of the cage was increased than the control group. But no results were found statically significant (Table 4).

Table 4: The effect of DTR in the Climbing out Test

Group	Dose (mg/kg)	Min0	Min30	Min60	Min120	Min180	Min240
Ctrl (n=10)		108.100 ± 71.286	54.500 ± 43.923	12.200 ± 8.826	40.500 ± 27.542	65.200 ± 43.107	.000 ± .000
DTR (n=10)	100	134.600 ± 69.997	22.200 ± 11.522	12.500 ± 9.064	94.500 ± 44.226	138.700± 48.842	39.400 ± 28.570
Ctrl (n=10)		80.100 ± 38.548	198.200± 72.650	74.000 ± 44.683	14.400 ± 9.951	157.600± 81.134	122.500± 56.373
DTR (n=10)	200	50.800 ± 24.747	110.700± 51.494	47.300 ± 24.424	127.000 ± 59.499	125.200± 59.152	99.500 ± 61.173
Ctrl (n=10)		122.2 ±60.169	34.1 ±22.657	53.2 ±27.177	.000 ± .000	133.1 ±70.527	100.9 ±67.686
DTR (n=10)	400	185.4 ±65.153	126.1 ±72.982	44.1 ±29.727	83.4 ±61.671	19.7 ±19.7	.000 ± .000

The Hole Cross Test produced mixed results which showed that the motor activity both increased and decreased at different doses but in 200 mg/kg dose at 30 min ($p<0.005$) showed highly significant increase of motor activity. The Open Field Test did not produce significant results throughout the experiment in all the 3 doses. According to the results for both Hole cross test and Open field test we can assume that the drug may have a little stimulant property and no depressant property. In Hole Board Test, at dose of 100 mg/kg DTR showed significant ($p<0.01$) increase and highly significant ($p<0.005$) decrease of ambulatory activity whereas other results were insignificant which indicates that DTR does not have any significant effect on emotional defecation and the ambulatory activity was changed in a time dependant manner. Climbing out test also indicates that DTR does not have any profound depressant effect on the experiment model.

Conclusion

All these experiment were executed in an attempt to confirm the safety of the Dhatri Lauha (DTR) for the general patients or users of the society and the country as a whole. After completion of this research work we can suggest that DTR can be prescribed for treating different diseases without or with a minimal central nervous system side-effect. Further extensive tests including clinical trial is warranted before any definitive conclusion is made in order to clinically delineate the safety profile of this preparation.

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