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Evaluation of Central Nervous System Activities of Dhanwantaram Kashayam in Experimental Animal Models

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Abstract

Ayurveda is a traditional Indian medicinal system that has been practiced for centuries and is known as the “Mother of All Healing.” According to the World Health Organization, 80% of the world’s population still relies primarily on traditional medicines for their healthcare. The purpose of the study was to evaluate and confirm the CNS activities of Dhanwantaram Kashayam, an ayurvedic formulation, in experimental animal models. It is a decoction of many herbs that is commonly used for labour pain relief and is given at random for several vata disorders. Because there is not much available literature on it, the following study is to confirm the hypothesis.The animal models selected were tests for anti-convulsant activity, anti-anxiety activity, anti-depressant activity, and muscle coordination activity. The study entails that Dhanwantaram Kashayam polyhedral formulation has highly significant (P<0.001) ant anxiety, antidepressant, analgesic, and muscle relaxant properties. In addition to that, it also has a sedative effect. It was concluded that the Dhanwantaram Kashayam at a high dose (0.5 ml/kg) shows anticonvulsant, antidepressant, anti-anxiety, analgesic, and muscle relaxant activity, whereas the low dose (0.25 ml/kg) shows a sedative effect.

Keywords: Dhanwantaram Kashayam, Anticonvulsant, Antidepressant, Analgesic, Muscle Relaxant.

Introduction

Central Nervous System (CNS) controls important aspects of body function and maintains homeostasis. CNS diseases can affect either the brain or the spinal cord, which leads to neurological or psychiatric disorders like Alzheimer’s disease, Parkinson’s disease, depressive disorders, etc (1,2). According to the World Health Organization (WHO), globally, more than 300 million people suffer from depression, 260 million with anxiety disorder; 50 million with epilepsy, and 5.5 million have Alzheimer (3,4).

In recent years, health planners and practitioners are recognizing the value of traditional medical systems like Ayurveda. The medicinal plants from indigenous pharmacopoeias like the compendia of Charaka and Suśrūta have shown significant healing power and have been postulated to be effective in a

wide variety of diseases, ranging from allergic rhinitis, cancer to neurodegenerative disorders, like memory impairment, senile dementia of the Alzheimer's type and Parkinson's disease (5). Ayurveda claims that several plants, called as 'medhya' possess beneficiary activities against neurodegenerative diseases (6). The use of Dhanwantaram Kashayam (DK) finds the way for curing many such ailments. Dhanwantaram kashayam polyherbal formulation (DKPHF) is the water decoction of various herbs having anticonvulsant, antidepressant, and cognitive properties. However, due to the lack of scientific data regarding the effect of Dhanwantaram Kashayam on CNS activities, the present study is focused on the evaluation of its possible CNS activities in experimental animal models.

Materials and Methods

Chemicals and apparatus used

The authenticated raw materials of the test formulation were procured from Yucca Enterprises, Wadale (E), Mumbai. Diazepam 5mg tab was purchased from Ranbaxy, India, ketamine hydrochloride 500mg/ml inj from Neon Pharmaceutical Ltd, India, isoniazid 100mg tab from Macleods, India, imipramine 10mg tab from Sun Pharmaceuticals, India, gabapentin 100mg tab from Intas Pharmaceuticals, India, pentazocine 30mg/ml inj from Ranbaxy, India, and phenytoin 25mg tab (Abbot, India).

The various equipment used in this study were electroconvulsiometer (Dolphine, India), Digital rotarod apparatus (Inco-Ambala, India), Actophotometer (Dolphine, India), Elevated plus maze (Elevated plus maze), Eddy's hotplate (Instruments and chemicals amber city, India), Analytical balance (Schimadzu, Japan), UV Spectrophotometer (Schimadzu, Japan), Centrifuge (Remi, India).

Composition of dhanwantaram kashayam

DKPHF is the water decoction of various herbs like Sida cordifolia, Hordeum vulgare, Ziziphus abyssinica, Macrotyloma uniflorum, Gmelina arborea, Aegle marmelos, Stereospermum colais, Oroxylum indicum, Premna corymbosa, Desmodium gangeticum, pseudarthria viscida, Solanum anguivi, Solanum surattense, Tribulus terrestris, Cedrus deodara, Rubia cordifolia, Santalum album, Hemidesmus indicus, Saussurea costus, Valeriana jatamansi, Trigonella foenum-graecum, Parmelia perlata, Acorus calamus, Boerhavia diffusa, Withania somnifera, Asparagus racemosus, Ipomoea mauritiana, Glycyrrhiza glabra, Terminalia chebula, Phyllanthus emblica, Terminalia bellirica, Commiphora myrrha, Anethum graveolens, Dysolobium pilosum, Vigna radiata var. sublobata, Elettaria cardamomum, Cinnamomum verum, Cinnamomum tamala. The Kashayam was manufactured by Arya Vaidya Sala, Kottakkal-676 503, Kerala, India.

Experimental animals

Swiss albino mice of either sex weighing between (25-30g) were used in the present study. The experimental protocol was approved by the institutional animal ethics committee. The animals were maintained under standard conditions in the institutional animal house as per the guidelines of the Committee for the purpose of control and supervision on Experiments on Animals (CPCSEA). The animals were grouped and housed in polyacrylic cages (38x23x10cm) with not more than eight animals per cage and maintained under standard laboratory conditions (7).

Selection of dose

The doses which were administered to mice were calculated from human dose, which was converted to the mice dose by using human equivalent dose method (HED). The doses of high dose and low dose Dhanwantaram Kashayam were selected as 0.25ml/kg and 0.5ml/kg (8).

Human dose of kashayam =15ml/60kg/day

60kg(60000g) is equivalent to 15ml.

*Therefore, 1kg (1000g) is equivalent to 15*60000/1000 = 0.25ml/kg*

Low dose=0.25ml/kg High dose=0.5ml/kg

Experimental models

Anticonvulsant activity

Maximum electroshock induced seizures (MES)

Mice were divided into 4 groups of 6 animals each, where group 1 included normal mice, group 2 included mice treated with standard (phenytoin), group 3 included mice treated with low dose Dhanwantaram Kashayam, group 4 included mice treated with high dose Dhanwantaram Kashayam, the animals received a current of 45 mA for 0.2 seconds through an electroconvulsiometer using corneal electrodes, after 60 min of oral administration of Dhanwantaram Kashayam or phenytoin. The incidence and duration of the extensor tonus were noted. Complete abolition of hind limb tonic extension was considered 100% protection (9).

Isoniazid (INH) induced seizures

Wistar rats were divided into five groups with six animals in each group, similar to the above-mentioned test. All the treatments were administered intraperitoneally 30 min prior to the administration of INH (300 mg/kg). Animals that did not convulse within 30 min were considered protected. The number of rats protected in each group were expressed in terms of percentage. In the INH-treated group, the animals were monitored for 60 min, and the percent protection were determined. In unprotected animals, the latency to first convulsion and the durations of convulsions were recorded. The animals were observed for 24 h after the administration of INH for their mortality rate (10).

Antidepressant activity

Forced swim test (FST)

Mice were divided into four groups of six animals in each group, where group 1 included normal mice, group 2 included mice treated with standard (imipramine), group 3 included mice treated with low dose Dhanwantaram Kashayam, and group 4 included mice treated with high dose Dhanwantaram Kashayam. FST was performed in a glass jar. This test consisted of two parts, an initial training period of 15 min followed by the actual test for 5 min duration, 24 h later. Mice were individually forced to swim inside a vertical borosilicate glass cylinder (height: 40 cm; diameter: 15 cm; containing 15 cm height of water maintained at 25°C). The activity was recorded after 24 hours following the doses of Dhanwantaram Kashayam and imipramine respectively. The recordings were analyzed to find the duration of immobility, swimming behavior, and climbing behavior in the 5 min test period using a stopwatch. An animal was judged to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose just above the surface, with no additional activity other than that necessary to keep its head above water. Swimming is defined as active movement throughout the swim chamber, which includes crossing into another quadrant. Climbing activity (also termed thrashing) consisted of outward-directed movements of the forepaws along the side of the swim chamber.

Tail suspension test (TST)

The grouping of animals was similar to that of FST. The total duration of immobility induced by tail suspension was measured according to the described method as a facile means of evaluating potential anti-depressants. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. An animal was immobile when it did not show any movement of its body and hung passively (11).

Anti-anxiety activity by elevated plus maze

Mice were divided into four groups of six animals in each group, where group 1 included normal mice, group 2 included mice treated with standard (diazepam), group 3 included mice treated with low dose Dhanwantaram Kashayam, and group 4 included mice treated with high dose Dhanwantaram Kashayam. The wooden maze consisted of two open arms (length 50 cm x breadth 10 cm) and two closed arms of the same size (height 40 cm). The arms of the same type are opposite to each other, with a central square of 10 cm. The maze was elevated to a height of 50 cm above the floor. Each animal was tested initially in the plus maze. On the 10th day of drug or vehicle administration, 60 minutes after the last dose, each animal was placed in the centre square of the plus maze, facing one of the open arms. The number of entries into and the time spent in open and closed arms and the number of rears in each arm in a five-minute period were noted (12).

Analgesic activity by hot plate method

Mice were divided into 4 groups of 6 animals each, where group 1 included normal mice, group 2 included mice treated with standard (pentazocine), group 3 included mice treated with low dose Dhanwantaram Kashayam, and group 4 included mice treated with high dose Dhanwantaram Kashayam. The parameter evaluated was the latency time for paw licking and jumping response after exposure on the surface of a hot plate. The standard used was pentazocine. The hot plate temperature was kept at (55°C), and the cut-off time will be 20 sec (13).

Acetic-acid induced writhing assay

Mice were divided into 4 groups of 6 animals each, where group 1 included normal mice, group 2 included mice treated with standard (diclofenac sodium), group 3 included mice treated with low dose Dhanwantaram Kashayam, group 4 included mice treated with high dose Dhanwantaram Kashayam. Analgesic activity of Dhanwantaram Kashayam was studied by the reduction of acetic acid-induced writhing in mice. Thirty minutes after the administration of Dhanwantaram Kashayam or standard diclofenac sodium (10 mg/kg, i.p.), the animals will receive acetic acid (0.6%, 10 ml/kg ip). The number of abdominal contractions (writhing) and stretching with a jerk of the hind limb were counted for 15 min after administering acetic acid, and percent inhibition was calculated as follows:

% inhibition = (1 - WT / WC) × 100

Where, WT is the writhings in drug-treated mice and WC is the writhing in control mice (14).

Motor in coordination activity by rotarod apparatus

Mice were divided into four groups of six animals in each group, where group 1 included normal mice, group 2 included mice treated with standard (diazepam), group 3 included mice treated with low dose Dhanwantaram Kashayam, and group 4 included mice treated with high dose Dhanwantaram Kashayam. The effect on motor coordination was assessed using the Rotarod apparatus. The animals were trained to remain for 3 min on the Rotar-rod rotating at a speed of 25 rpm. On the next day, either vehicle or Dhanwantaram Kashayam was administered orally, and their ability to remain on the rotating rod was assessed before and after the oral administration. The fall-off time from the rod was noted for each animal (15).

Motility test

The grouping of animals was similar to that of the motor in coordination test. 30 minutes after drug administration, the spontaneous locomotor activity was recorded using an activity cage, actophotometer with automatic counting of animal movements on the cage floor. The locomotor count for each animal was recorded for 5 minutes at 30-minute intervals for 2 h. The

data obtained were compared and studied (15).

Behavioral effect

Mice were divided into three groups of six animals in each group, where group 1 included normal mice, group 2 included mice treated with low dose Dhanwantaram Kashayam, and group 3 included mice treated with high dose Dhanwantaram Kashayam. The animals were observed for behavioral changes after treatment with Dhanwantaram Kashayam for up to 2 hours after 30 minutes of administration. The behavioral pattern of the vehicle-treated mice was studied prior to Varavisaladi Kashaya. Body position, locomotion, rearing, reparation, lighting reflex, and lacrimation were the observation parameters. The collected data were compared and studied (15).

Effect on sleep activity/locomotion/sedative/stimulatory by ketamine HCl-induced sleeping time

Mice were divided into 4 groups of 6 animals each, where group 1 included normal mice, group 2 included mice treated with standard ketamine HCl, group 3 included mice treated with low dose Dhanwantaram Kashayam, and group 4 included mice treated with high dose Dhanwantaram Kashayam. Ketamine HCl (100 mg/kg i.p) was injected 30 min before oral administration for all groups. The time elapsed between loss and recovery of the lighting reflex was noted and taken as sleeping time (16).

Effect on GABA in experimental animals

The grouping of animals was similar to that of the effect of sleep. According groups were administered gabapentin (20 mg/kg i.p.) alone on 1st day, and blood was collected through the caudal vein 30 min after administration. According groups were treated with Dhanwantaram Kashayam at different dose levels once daily for 7 days. On the 7th day, 60 min after administration of vehicle and extract blood sample were collected through the caudal vein of each animal. The serum was separated, transferred into a plastic tube, and stored at -XoC until analysis. All the glass and polypropylene apparatus were soaked in 10% (V/V) nitric acid for 12 h and rinsed with double distilled deionized water. The serum sample (0.1 ml) was added to 1.5 ml of absolute alcohol, centrifuged at 3000g for 15 min. The upper layer was aspirated, and 0.3 ml was put on Whatman's filter paper which was dipped in phenol for 24 h and subsequently dried in the air. Thereafter, ninhydrin salt solution was sprayed on chromatographic paper and heated at 65°C for 10 min. The spot developed due to the chromatographic mobility of GABA was cut and put in a 3ml solution of absolute alcohol for elution. The optical density was taken on a spectrophotometer at a wavelength of 509 nm and compared to the standard GABA solution (16).

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism v4 software (Graph Pad Inc., USA). Results were expressed as mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P < 0.05$ was considered significant.

Results and Discussion

Organoleptic evaluation of kashaya

Organoleptic evaluation of the formulation showed that it was a brown-coloured liquid with a characteristic odour and bitter taste.

Evaluation of anticonvulsant activity

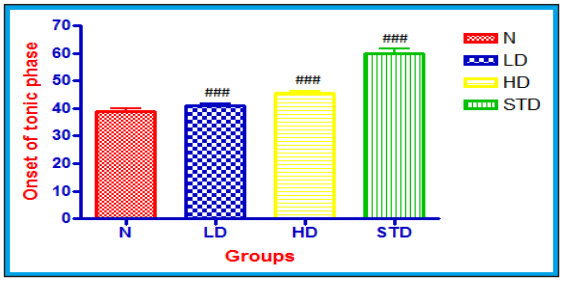
Isoniazid induced seizures

The animals were treated with a low dose of DKPHF (0.25 mg/kg) and exhibited no significant delay in the onset of clonic as well as tonic convulsions. However, those treated with a higher dose of DKPHF (0.5 ml/kg) exhibited a statistically significant ($P < 0.001$) delay in the

onset of clonic and tonic convulsions as compared to normal.

Table 1. Effect of DKPHF and Diazepam on INH-Induced Convulsion in Mice.

Groups	Duration of seizure (min)		Mortality
	On set of clonic phase (min)	On set of tonic phase (min)	
Normal	39±0.81	49±0.5	3
LD(0.25ml/g)	46.0±0.6###	56.0±1.2###	2
HD(0.5ml/kg)	48±0.8###	60.0±1.2###	2
STD (Diazepam)	60±1.5###	70.42±0.64###	1



Data was analyzed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests; values are expressed as mean ± SEM (n=6) *p<0.05; ** p<0.01; *** p<0.001.#p<0.05; ##p<0.01;###p<0.

Figure 1. Effect of DKPHF on INH-Induced Convulsion in Mice

Maximum electroshock induced seizures (MES)

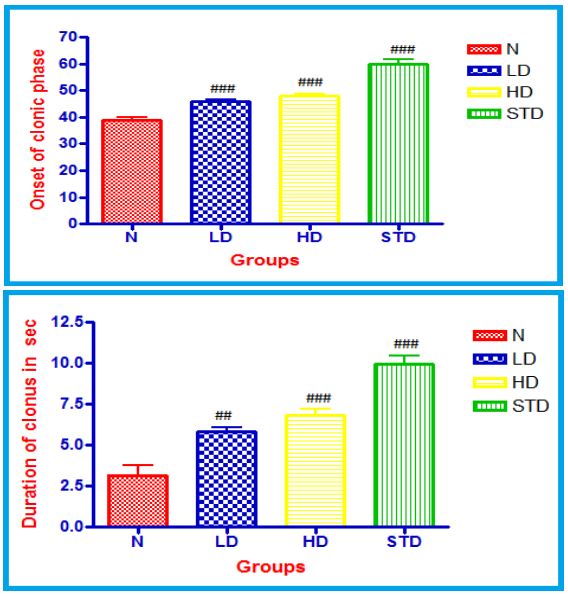


Figure 2. Effect of DKPHF on MES induced convulsion in mice

DKPHF showed a highly significant (P < 0.001) anticonvulsant activity. The duration of clonus in mice had decreased to 6.8 ± 0.4 (mean ± standard deviation) at a high dose of 0.5 ml/kg. The activity of DKPHF was comparable to that produced by phenytoin.

Table 2. Effect of DKPHF on MES Induced Convulsion

Groups	Flexion	Extension	Clonus	Stupor	Recovery/ Death
N	8.48 ± 0.18	16.03 ± 0.65	3.16 ± 0.62	10.40 ± 0.85	Recovery
LD	5.98 ± 0.43	5.49 ± 0.49	5.8 ± 0.3##	20.14 ± 0.88	Recovery
HD	5.34 ± 0.29	3.15 ± 0.30	6.8 ± 0.4###	20.79 ± 1.60	Recovery
STD (Phenytoin)	3.15 ± 0.35	-	9.93 ± 0.5###	3.21 ± 0.81	Recovery

Data was analysed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests,values are expressed asmean ± SEM (n=6)*p<0.05;** p<0.01;***p<0.001;#p<0.05##p<0.01;###p<0.001.

Antidepressant activity

Forced swim test

In this test, animals treated with a low dose of DKPHF (0.25 ml/kg) showed a slightly significant decrease in their immobility time, and those treated with a high dose of DKPHF (0.5 ml/kg) showed a highly significant (P < 0.001) decrease in immobility time compared to normal.

Table 3. Effect of DKPHF on Forced Swim Testin Mice

Groups	Time of immobility(sec)
Normal	253.29 ± 1.78
LD(0.25ml/kg)	241.0 ± 0.93###
HD(0.5ml/kg)	240.10 ± 1.86###
STD(Imipramine)	127.73 ± 1.53###

Data was analyzed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests; values are expressed as mean ± SEM (n = 6) # p < 0.05; ## p < 0.01; ###

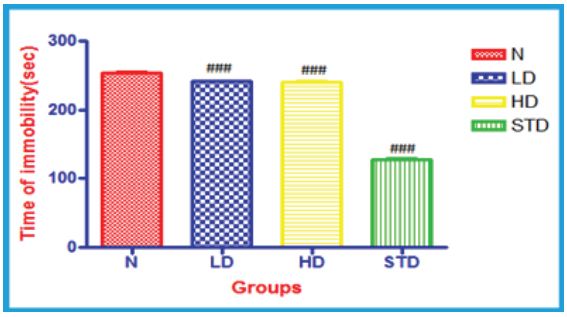


Figure 3. Effect of DKPHF on Forced Swim Test in mice

Tail suspension test

In this test, animals treated with a low dose of DKPHF (0.25 ml/kg) showed a slightly significant decrease in their immobility time, and those treated with a high dose of DKPHF (0.5 ml/kg) showed a highly significant (P < 0.001) decrease in immobility time compared to normal.

Table 4. Effect of DKPHF on Tail Suspension Test in Mice

Groups	Time of immobility(sec)
Normal	251.5 ± 0.76
LD(0.25ml/kg)	239 ± 0.63###
HD(0.5ml/kg)	238 ± 1.48###
STD(Imipramine)	129.83 ± 0.83###

Data was analysed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests; values are expressed as mean ± SEM (n = 6) # p < 0.05; ## p < 0.01; ### p < 0.001

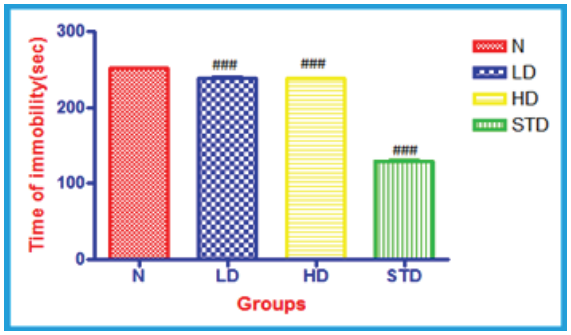


Figure 4. Effect of DKPHF on Tail Suspension Test in Mice

Anti-anxiety activity – elevated plus maze (epm)

The time spent in open arm was statistically increased to an extremely significant (P < 0.001) value after 0.5 mg/kg of DKPHF when compared with normal-treated group. Treatment with diazepam increased extremely significant (P < 0.001) time spent in the open arm.

Table 5. Effect of DKPHF Anti-anxiety Activity by Using EPM

Groups	Times pent (sec)	
	Openarm	Closed arm
Normal	98.28 ± 2.716	218.04 ± 2.62
LD (0.25ml/kg)	114.6 ± 2.37###	199.44 ± 2.2###

HD(0.5ml/kg)	118.00 ± 1.55$^{###}$	196.05 ± 1.64$^{###}$
STD(Diaze-pam)	173.18 ± 2.45$^{###}$	130.34 ± 1.67$^{###}$

Data was analysed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests, values are expressed as mean ± SEM (n = 6) # p < 0.05; ### p < 0.001.

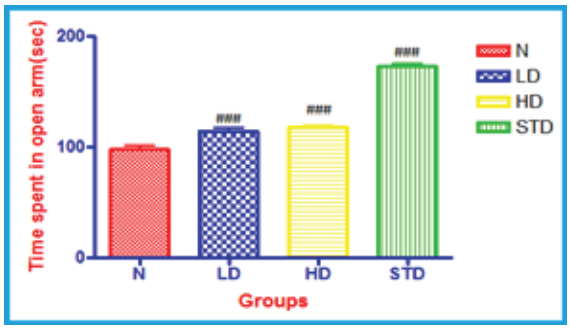


Figure 5. Anti-anxiety Activity of DKPHF by Using EPM

Behavioral studies

The behavioral assessment was performed by observing the mice for 2 hr after 1 hr oral administration of vehicle or DKPHF (0.25 and 0.5 ml/kg). The observations have been summarized in table 6.

Table 6. Behavioral Assessment of DKPHF in Mice

Behavioral Parameters	Vehicle (10ml/kg)	DKPHF (0.25ml/kg)	DKPHF (0.5ml/kg)
Spontaneous Activity	—	↓	—
Lacrimation	—	—	—
Reactivityto touch stimuli	—	—	—
Righting Reflex	—	—	—
Alertness	—	—	↓
Pinnareflex	—	—	—
Body Position	—	—	—

—: Normal, ↑: Increased, ↓: Decreased.

Analgesic activity – hot plate method

The oral administration of the DKPHF at a dose of 0.5 ml/kg extremely significantly (P < 0.001) raised the pain threshold in 60 min and 90 min time of observation in comparison with normal. And in low dose, it only showed an extremely significant effect (P < 0.001) in 90 min.

Table 7. Effect of DKPHF on Analgesic Activity by Using Hot Plate Method in Mice

Group	Reaction time in sec			
	0 min	30 min	60 min	90 min
Normal	3.2± 0.12	4.6± 0.07	5.10± 0.17	6 . 6 0 ± 0.13
LD (0.25ml/kg)	3.19 ± 0.15$^{#}$	5.3 ±0.11$^{##}$	6.5 ±0.14$^{##}$	8.4 ± 0.16$^{###}$
HD (0.5ml/kg)	3.3 ± 0.9$^{#}$	5.60 ±0.12$^{###}$	6.8 ± 0.16$^{###}$	8.6 ± 0.17$^{###}$
STD(Pentazocine)	3.9 ± 0.28$^{#}$	7.33 ± 0.20$^{###}$	11.23 ± 0.39$^{###}$	11.9 ± 0.33$^{###}$

Data was analyzed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests, values are expressed as mean ±SEM (n=6) *p<0.05;** p<0.01;***p<0.001.$^{#}$p<0.05;$^{##}$p<0.01;$^{###}$p<0.001

0 min 30min-
60min 90min

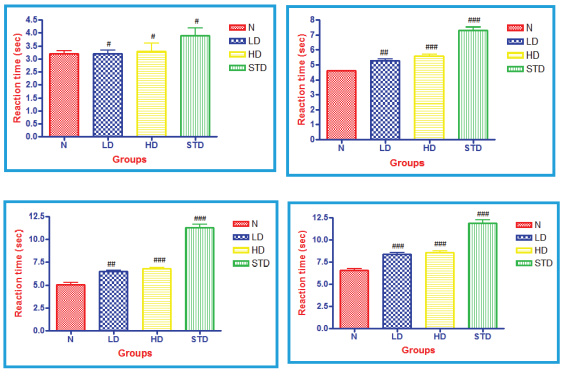


Figure 6. Analgesic Activity of DKPHF by Using Hot Plate Method in Mice

Motor in co-ordination activity – rota rod

In this test, DKPHF (0.25 and 0.5 ml/kg) significantly reduced the time spent by the animals on the revolving rod when compared to the normal treated group.

Table 8. Muscle Relaxant Activity of DKPHF by using Rota Rod

Groups	Times pent onrevolvin-grod (sec)
Normal	268 ± 4.90
LD(0.25ml/kg)	208 ± 3.5$^{###}$
HD(0.5ml/kg)	219 ± 5.46$^{###}$
STD(Diazepam)	105 ± 2.11$^{###}$

Data was analyzed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests; values are expressed as mean ± SEM (n=6) #p<0.05; ## p<0.0 1; ### p<0.001.

Figure 7. Muscle Relaxant Activity of DKPHF by Using Rota Rod in Mice

Effect on locomotion (Sedative/Stimulatory) - by Actophotometer A high dose of DKPHF (0.5 mg/kg, p.o) showed moderately significant, and a low dose of DKPHF (0.25 mg/kg) showed extremely significant locomotor activity when compared to the normal. Table 9. Effects of DKPHF on Locomotor Activity

Table 9. Effects of DKPHF on Locomotor Activity

Groups	Locomotoractivityfor5min. (Scores)
Normal	176.6 ± 2.3
LD(0.25ml/kg)	157.62 ± 3.64$^{###}$
HD(0.5ml/kg)	161.4 ±3.60$^{##}$
STD(Diazepam)	93.33 ± 0.98$^{###}$

Data was analyzed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests,values are expressed as mean ***±SEM(n=6)***#p<0.05;##p<0.01###p<0.001

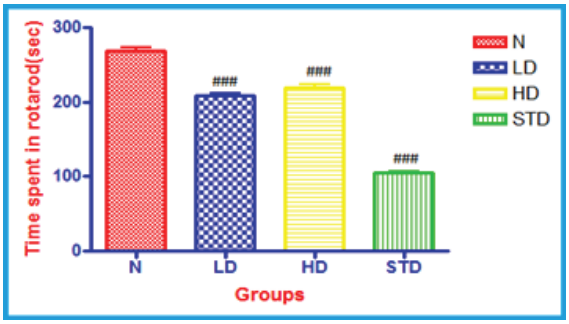


Figure 8. Effect of DKPHF on Locomotion in Mice

Ketamine induced sleeping time

Treatment with DKPHF at a dose of 0.25 ml/kg showed an extremely significant increase in sleep latency time when compared with the normal saline treated group, Whereas DKPHF at a high dose (0.5 ml/kg) increased the sleep latency time as compared to the normal (124.0 ± 1.45).

Table 10: Effect of DKPHF on Latency to Loss of Lighting Reflex

Groups	**Latency of sleep(sec)**
Normal	124.0 ± 1.45
LD(0.25ml/kg)	92.6 ± 2.66$^{###}$
HD(0.5ml/kg)	97.56 ±12.3$^{##}$
STD(KetamineH-Cl)	56.95 ± 1.77$^{###}$

Data was analyzed by one-way ANOVA followed by Tukey-Karmer multiple comparison tests, values are expressed as mean ***±SEM(n=6)***#p<0.05;##p<0.01;### p<0.001; *p<0.05;**p<0.01; ***p<0.001

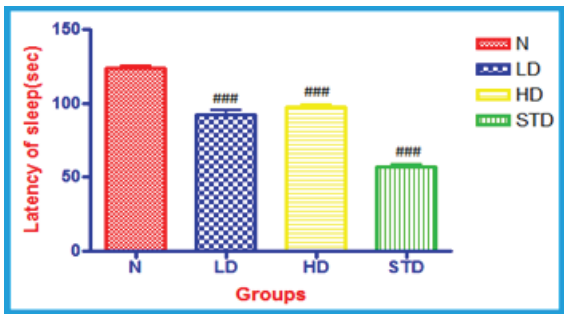


Figure 9. Effect of Ketamine Induced Sleep in Mice

Estimation of GABA by Spectrophotometric Method

Groups treated with low doses (0.25 ml/kg) of DKPHF showed that there was not any significant increase in GABA levels in mice serum with reference to the normal group, whereas high doses (0.5 ml/kg) of DKPHF increased the GABA level to an extremely significant level ($P < 0.001$). A standard Gabapentin extremely significantly ($P < 0.001$) increased GABA levels in the serum when compared to normal.

Table 11. Effect of DKPHF in Serum GABA Levels of Mice

Groups	Optical density(509nm) Mean ±SEM	GABA levels(p-mol/ml) (O.D of Test/O.D of Std =pmol/ml)
Normal (saline10ml/kg)	0.030±0.0007	217.39
EPHF(100mg/kg)	0.033±0.001	239.13###
EPHF(500mg/kg)	0.034±0.0009	275.36###
STD(Gabapentin)	0.06 ±0.0007	500.60###
Standard GABA solution (0.1ug/ml)	0.138±0.005	1000

Data was analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison tests, Values are expressed as mean ± SEM. *(n=6)#p<0.05; ## p<0.01;###p<0.001.*

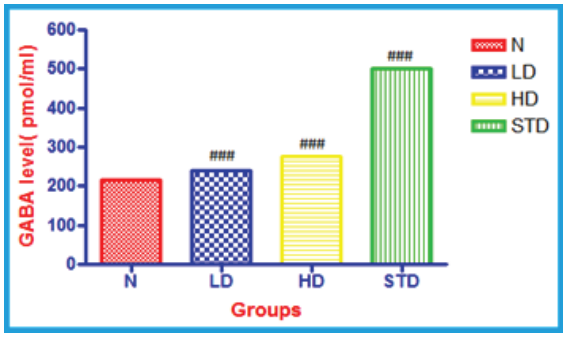


Figure 10. Effect of DKPHF on Serum GABAL evelin Mice

Discussion

Ayurveda is a traditional medicinal system that has been practiced for centuries. Furthermore, this ancient vedic knowledge is considered to be one of the oldest healing sciences and has survived many centuries of tradition (17, 18, 19). Psychoactive drugs interact at target sites or receptors found in the nervous system to induce widespread changes in physiological or psychological functions. Any substance that crosses the blood-brain barrier, and thus influences behavior, mood, or cognition is of interest to the researchers. The present study was carried out to elucidate the CNS activity of polyherbal formulation Dhanwantaram Kashayam by using various experimental animal models.

Dhanwantaram Kashayam demonstrated effective anticonvulsant, antidepressant, analgesic, anti-anxiety properties in animal models. It significantly ($P < 0.001$) increased the total sleep time induced by ketamine; a potent anesthetic, implying that it may possess sedative properties. The two aspects of its effect, lengthening total sleep time and facilitating sleep induction, would account for at least some of the decrease in spontaneous motor activity.

Studies on locomotor activity by acto-

photometer proved that the Dhanwantaram Kashayam at high dose didn't produce any significant reduction in locomotor activity whereas low dose did, establishing that it has sedative properties at low doses. Furthermore, at a dose of 0.25 ml/kg, it demonstrated a highly significant ($P < 0.001$) anticonvulsant activity by suppressing extension, generalized tonic–clonic on MES and INH-induced convulsion by increasing onset time for both clonic and tonic phases. GABA is known to be an important inhibitory neurotransmitter in the brain, whereas glutamate is the excitatory neurotransmitter. GABA activates GABA receptors whereas glutamate activates N-methyl-D-aspartate (NMDA) and non-NMDA receptors. When these receptors are activated, they modify voltage-gated Na+, K+, Ca++, and Cl- ion channels, which either excites or inhibits the neuron. Abnormalities in the GABA system have been found in neurological and psychiatric diseases. INH is regarded as a GABA-synthesis inhibitor. Clonic tonic seizures are induced in mice which are inhibited by AED (Anti-Epileptic Drugs) that either inhibit voltage-gated channels or block glutaminergic excitation mediated by the N-methyl-D-aspartame receptor (20). Dhanwantaram Kashayam treated groups were protected from seizures either by increased GABA synthesis via L-glutamate stimulation or prevention of GABA degradation via GABA transaminase. Furthermore, spectrophotometric methods revealed a statistically significant ($P < 0.001$) increase in the amount of GABA in rat serum following a high dose of Dhanwantaram Kashayam. Gabapentin, the standard drug, is GABA analogue that improves GABAergic transmission. Seizures caused by MES and INH can be prevented with standard phenytoin and diazepam.

The forced swimming model of depression provides a rapid and reliable behavior screening test for anti-depressants (21). Immobility has been expected to reflect a state of behavioral despair and failure to adapt to the stress. The results of the forced swimming and tail suspension tests showed a highly significant ($P < 0.001$) decrease in the immobility time upon treatment with low dose and high dose of Dhanwantaram Kashayam. Imipramine acts by inhibiting NE (Nor Epinephrine) reuptake. The beneficial effect of imipramine in the FST model seems to be due to the increased availability of these neurotransmitters nor epinephrine (NE) and serotonin (5-HT) at the post synaptic site following reuptake inhibition. It has been established that the shortening of immobility time in forced swimming and tail suspension tests depends mainly on the enhancement of central 5-HT and catecholamine neurotransmission (22). Thus, the overall results seem to be predictive for the antidepressant action of DKPHF.

The EPM test is considered one of the most widely validated tests for assaying new benzodiazepine-like anxiolytic agents. Anxiolytic compounds increase the number of entries in the open arm. The time spent in the open arm was statistically increased to an extremely significant value ($P < 0.001$) after 0.5/kg of Dhanwantaram Kashayam. The anxiolytic agents are thought to act via GABA-A receptor complex. Diazepam enhances the frequency of Cl- channel opening and thus Cl- flux through the GABA-A receptor, thus potentiating the inhibitory effect of GABA (23).

In addition to this, administration of Dhanwantaram Kashayam had a profound effect on motor coordination, which indicated some muscle-relaxant activity. Hot plate test and tail immersion test were used to investigate both peripheral and central activity. Nociceptive reaction towards thermal stimuli in hot plate test and tail immersion hot water test using mice is a well-validated model for the detection of opiate analgesic as well as several types of analgesic drugs from spinal origin (23).

Thus, the above observations indicate that Dhanwantaram Kashayam can overcome various CNS disorders like epilepsy, depression, anxiety, etc., and it also has analgesic

properties. The potential effect of its polyherbal formulation may be attributed to one or more bioactive principles present in these drugs such as steroidal saponins, flavonoids, alkaloids (mainly trigonelline), zingiberol, and free amino acids. There may be synergistic herb-herb interactions enhancing the total efficacy of the formulation. The exact mechanism of action of the drug needs to be evaluated by further extensive studies.

Conclusion

In conclusion, preliminary pharmacological testing in mice demonstrated that DKPHF was found to have significant anticonvulsant, antidepressant, anti-anxiety, and analgesic activity in experimental animals. Thus, this formulation can be used in the prevention and treatment of epilepsy, depression, anxiety at a marked level. Apart from this, when used in high doses, it provides promising results for analgesic activity. The present study opens new windows for further research on this classical combination at different dose levels, as an adjuvant with and in comparison with various anticonvulsant, antidepressant, and anxiolytic drugs. This may lead to better integrative management of these psychiatric disorders in the future.

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