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ASSESSMENT OF POLYHERBAL FORMULATION FOR NOOTROPIC POTENTIAL

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Abstract

Polyherbal formulation consisting of plant ingredients of Brahmi (*Bacopa monniera),* Yastimadhu *(Glycyrrhiza glabra),* Tagar (*Valeriana wallechii)* and Ashwagandha *(Withania somnifera).*

Objective: The present study was undertaken to investigate the effects of polyherbal formulation on learning and memory in experimental animals.

Methods: Elevated plus-maze (EPM) and passive avoidance paradigm were employed to test learning and memory. Scopolamine (1mg/kg i.p.) and diazepam (1mg/kg i.p.) were used as interoceptive (stimulus inside the body) behaviour model. Three doses (5, 10 and 15 ml/kg p.o.) of polyherbal formulation were administered for 7-14 successive days in separate groups of animals.

Results: Elevated plus-maze (EPM) and passive avoidance paradigm model results show that dose of 15 ml/kg of polyherbal formulation significantly improved learning and memory of mice. Furthermore, this dose significantly reversed the amnesia induced by diazepam (1mg/kg i.p.) and scopolamine (1mg/kg i.p.).

Since scopolamine-induced amnesia was reversed by polyherbal formulation, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic-transmission in mouse brain, also diazepam which is a GABA mimetic agent induces memory impairment and the subsequent inhibition of diazepam induced amnesia by polyherbal formulation may be due to inhibition of GABA-B receptors has been found to facilitate learning and memory.

Conclusion: In the present investigation, polyherbal formulation (15ml/kg, p.o.) has shown promise as a memory enhancing agent in experimental animals in all the laboratory models employed.

Keywords: polyherbal formulation; Amnesia; Learning; Memory; Nootropic.

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1. Introduction

Memory is the ability of an individual to record sensory stimuli, events, information etc., retain them over short or long periods of time and recall the same at a later date when needed. Poor memory, lower retention and slow recall are common problems in today's stressful and competitive world[1]. Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities [2]. Age has main role in the prevalence of dementia[3]. Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capacity and memory[4]. Typically these are thought to work by increasing the brain's supply of neurochemicals (neurotransmitters, enzymes and hormones), improving brain's oxygen supply or by stimulating nerve growth. Nootropics agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil are being used for improving memory, mood and behaviour, but the resulting side-effects associated with these agents have made their applicability limited. The central cholinergic pathways play a vital role in learning and memory processes[5]. Centrally acting drugs (e.g. scopolamine, diazepam) impair learning and memory both in animals[6] and human beings[7, 8, 9]. Indian ayurvedic system of medicine emphasizes use of herbs, nutraceuticals of life style changes for controlling age related neurodegenerative disorders[4.]

In the Indian ayurvedic system of medicine, Brahmi (*Bacopa monniera)* used as a nerve tonic, antiepilepitic[10,11],diuretic[12], to reduce stress induced anxiety, nootropic[13,14], antipyretic, analgesic, sedative[12], anti-inflammatory[12,15,] antidepressant[16] and for adaptogenic activities[17], Yastimadhu *(Glycyrrhiza glabra)* used as a nerve tonic, demulcent, mild expectorant, peptic ulcer, rheumatoid arthritis, Tagar (*Valeriana wallechii)* used as a carminative, stimulant, antispasmodic and nervous disorder[18], and Ashwagandha *(Withania somnifera)* used as a anti-stress, nervine tonic, astringent, adaptogenic, febrifuge, sedative, hypnotic, anthelmintic and diuretic and as an immuno-modulatory agent[19].

In the present study, we have focused upon exploring the potential of an Indian ayurvedic poly-herbal formulation for its efficacy in reversing the memory deficits and for its improving acquisition and memory retention in experimental animals. Brahmi (*Bacopa monniera),* Yastimadhu *(Glycyrrhiza glabra),* Tagar (*Valeriana wallechii)* and Ashwagandha *(Withania somnifera)* are ingredients of polyherbal formulation.

2. Materials and Methods

2.1. Animals

Swiss albino mice of either sex weighting 20-30 g used in the present study were procured from the Central Animal House. They had free access to food and water, and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h. The animals were fed with commercially available rat pelleted diet. The animals were acclimatized for at least 7 days before behavioural study. The experimental protocol was approved by the Institutional Animals Ethics Committee and animals were maintained as per CPCSEA guidelines.

2.2. Determination of acute toxicity (LD_{50})

The acute toxicity of polyherbal formulation was determined by using female albino mice (20-30g). The animals were fasted for 4 hrs prior to the experiment and up and down procedure (OECD guideline no. 425) method of CPCSEA was adopted for acute toxicity studies[20]. Animals were administered with twice daily (0.1ml) of formulation and observed for its mortality during 48 hours study

period (short term) toxicity. Based on the short term profile of drug, the dose for the next animals were determined as per as OECD guideline 425. All the animals were observed for long term toxicity (0.1ml, twice daily for 25 days). Polyherbal formulation did not produce any obvious toxicity or mortality when subjected to chronic toxicity studies for 25 days in mice according to OECD guidelines. Hence, the formulation was ensured to be devoid of any potential toxicity and obvious mortality. Further, the different doses of polyherbal formulation used in the study for evaluation of its nootropic activity was decided based on laboratory experience. Thus the doses 5ml/kg, 10ml/kg, 15ml/kg were used as low, medium and high doses respectively in the entire project[21].

2.3. Animal models for testing learning and memory

(i) Passive avoidance paradigm (Exteroceptive Behaviour Model)

(ii) Elevated plus-maze (Exteroceptive Behaviour Model)

(ii) Diazepam-induced amnesia (Interoceptive Behaviour Model)

(iii) Scopolamine-induced amnesia (Interoceptive Behaviour Model)

(i) Passive avoidance paradigm (Exteroceptive Behaviour Model) [22, 23]

Passive avoidance behaviour based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of an inverted petridish placed in the centre of the grid floor (Instruments and Chemicals Pvt. Ltd, Ambala) was used. The petridish served as the shock-free zone (SFZ). Each mouse was gently placed on the in SFZ set in the center of the grid floor. When the mouse stepped down and placed all its paws on the grid floor, shocks (20V) were delivered for 15 sec and the step-down latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from SFZ to grid floor. Animals were trained to remain on the SFZ for at least 60 sec and mice which did not meet these criteria in five trials were rejected. Observations were made for acquisition i.e. the number of trials required to reach the learning criteria and for retention of learning for 10 min at 2 h and 24 h post-training. The following retention parameters like step-down latency (SDL) in seconds, step-down error (SDE) as the number of times the animal stepped down from the SFZ and the time spent in the shock zone (TSZ) in seconds are noted**.**

Group of adult Swiss male albino mice 25-30g, each consisting of six animals (n=6) were divided into following groups and animals were fasted overnight prior to the test but water was supplied *ad libitum*. The memory- impairing dose of Phenytoin (25mg/kg) daily for 14 days.

Group I: Normal control group: distilled water (10 ml/kg) was administered p.o. for 14 days.

Group II: Negative control group: Phenytoin alone (25 mg/kg) was administered p.o. for 14 days.

Group III: Standard control group: Piracetam (standard 200 mg/kg) + Phenytoin (25 mg/kg) was administered p.o. for 14 days.

Group IV, V and VI: polyherbal formulation (5, 10 and 15 mg/kg, twice daily) + Phenytoin (25mg/kg, p.o.) was administered p.o. for 7 days i.e. 8^{th} to 14^{th} day.

(ii) Elevated plus-maze (Exteroceptive Behaviour Model) [22, 23, 24]

Elevated plus-maze served as the exteroceptive behaviour model to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory was followed as per the parameters described by the investigators working in the area of neuropsychopharmacology. The apparatus consisted of two open arms (16 cm × 5 cm) and two enclosed arms (16 cm×5 cm×12 cm). The arms extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor.

On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was the time taken by mouse with all its four legs to move into one of the enclosed arms. TL was recorded on the first day. If the animal did not enter into one of the enclosed arms within 90 s, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 s. Retention of this learned-task was re-examined 24 h after the first day trial.

Group of adult Swiss male albino mice 20-25g, each consisting of six animals (n=6) were divided into following groups and animals were fasted overnight prior to the test but water was supplied *ad libitum*.

Group I: Control group: distilled water (10 ml/ g) was administered p.o. for 7 days. After 90 min of administration on 7th day, transfer latency was recorded. Retention of learned task was examined after 24 h.

Group II: Standard group for elevated plus-maze (n = 6): piracetam (200 mg/kg) was administered p.o. for 7 days. After 90 min of administration on 7th day, transfer latency was recorded. Retention of learned task was examined after 24 h.

Groups III, IV and V (n = 6): Polyherbal formulation (5, 10 and 15 ml/kg, twice daily respectively) was administered orally for 7 days. TL was noted after 90 min of administration on 7th day and after 24 h. significant reduction in TL value of retention indicate improvement in memory.

The inflexion ratio was calculated by the formula as follows[25].

$$\text{Inflexion ratio (IR)} = \frac{(L_0 - L_1)}{L_0}$$

Where L_0 is the initial TL (s) on first day and L_1 is the TL (s) on the second day.

(iii) Diazepam-induced amnesia (Interoceptive Behaviour Model) [26]

In the present investigation the mice were divided into different groups (control, diazepam alone, piracetam + diazepam, polyherbal formulation +diazepam treated group) comprising six animals in each for investigation using various interoceptive (stimulus inside the body) memory models. Polyherbal formulation (5, 10, 15 ml/kg) was administered to mice of different groups for 7 days i.e. 8th to 14th day. These mice were exposed to the training session using elevated plus maze on 8th day after 90 min of the last dose. Retention (memory) of the learned task was recorded after 24hr i.e. on 9th day. Amnesia was induced in separate groups (interoceptive models) by diazepam (1mg/kg i.p.) on 8th day after 90 min of the last dose. Piracetam 200mg/kg i.p. an established nootropic agent was injected for 8th days to the positive control group of animals. All groups were treated respectively as mentioned above for a period of 14 days. Transfer latency and inflexion ration was calculated by using elevated plus maze model as described above.

(iv) Scopolamine-induced amnesia (Interoceptive Behaviour Model) [26, 27]

In the present investigation the mice were divided into different groups (control, scopolamine alone, piracetam + diazepam, polyherbal formulation + diazepam treated group) comprising six animals in each for investigation using various interoceptive (stimulus inside the body) memory models. Polyherbal formulation (5, 10, 15 ml/kg) was administered to mice of different groups for 7 days i.e. 8th to 14th day. These mice were exposed to the training session using elevated plus maze on 8th day after 90 min of the last dose. Retention (memory) of the learned task was recorded after 24hr i.e. on 9th day. Amnesia was induced in separate groups (interoceptive models) by scopolamine (1mg/kg i.p.) on 8th day after 90 min of the last dose. Piracetam 200mg/kg i.p. an established nootropic agent was injected for 8th days to the positive control group of animals. All groups were treated respectively as

mentioned above for a period of 14 days. Transfer latency and inflexion ration was calculated by using elevated plus maze model as described above.

2.5. Statistical analysis

All results were expressed as mean ± standard error of mean (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's't' test.

3. Results

(i) Effect of POLYHERBAL FORMULATION on passive avoidance learning and retention in mice

Piracetam (200mg/kg) and different dose levels of polyherbal formulation (5, 10 and 15ml/kg) was tested in different groups. Polyherbal formulation at a dose of (15ml/kg p.o.) shown statistically significant increased Step-down latency and decreased time spent in shock zone and number of errors as compared to standard (Table no 1, Figure no. 1,2 and 3).

(ii) Effect on transfer latency (using elevated plus-maze)

TL of first day reflected learning behaviour of animals whereas, TL of second day reflected retention of information or memory. In mice, Piracetam (200mg/kg, p.o.) and different dose levels of polyherbal formulation (5, 10 and 15ml/kg) was tested. Polyherbal formulation at a dose of (15ml/kg, p.o.) shown statistically significant increased in inflexation ratio as compared to standard (Table no 2, Figure no.4).

(iii) Effect of polyherbal formulation on inflexion ratio in mice (Diazepam-induced amnesic model)

Diazepam (1 mg/kg) injected before training impaired learning significantly. Diazepam has induced dose dependent amnesia and in this amnesic model, a decrease in inflexation ratio was observed when compared to normal control group. Piracetam and all doses of polyherbal formulation (5, 10 and 15ml/kg, p.o.) treated groups had exhibited a highly significant nootropic activity (memory enhancing) with increase an inflexation ratio and reduction in transfer latency observed with EPM and reversed the diazepam-induced amnesia. Statistically significant reduction in transfer latency was observed in piracetam and polyherbal formulation (15ml/kg, p.o.) treated groups (Table no. 3, Figure no.5).

(iv)Effect of polyherbal formulation on inflexion ratio in mice (scopolamine-induced amnesic model)

Scopolamine treated group of mice exhibits impairment of memory and decrease in inflexion ratio as compared to normal control group, which indicates the induction of amnesia. Piracetam and 15ml/kg, p.o. of polyherbal formulation treated groups had shown significant increased inflexion ratio, significant reduction in TL and reversed the scopolamine- induced amnesia (Table no. 4, Figure no.6)

4. Discussion

Memory enhancing drugs are thought to work by increasing the brain's supply of neurochemicals (neurotransmitters, enzymes and hormones), improving brain's oxygen supply or by stimulating nerve growth. Nootropics agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil are being used for improving memory, mood and behaviour but not used generally because of more side effects associated with these agents have made their applicability limited [4]. In the present study, we have focused upon exploring the potential of ayurvedic poly-herbal formulation, polyherbal formulation for its efficacy in reversing the memory deficits, improving acquisition and memory retention in experimental animals using passive avoidance and EPM model.

In the present study, polyherbal formulation (5, 10, 15 ml/kg) administered orally improved learning and memory of mice significantly in both the exteroceptive (stimulus lie outside) and interoceptive

(stimulus lies within the body) behavioural models. Furthermore, pre-treatment with polyherbal formulation (5, 10, 15 ml/kg) protected the animals from learning and memory impairment produced by interoceptive stimuli (diazepam and scopolamine). These findings suggested the possible neuroprotective role for polyherbal formulation [28].

The polyherbal formulation (5, 10 and 15 ml/kg) and piracetam (200mg/kg) when given along with Phenytoin, significantly reversed Phenytoin-induced impairment, protective effect was observed with parameters tested i.e. increased in step-down latency (SDL) and decreased in time spent in shock zone (TSZ) and step-down error (SDE) at a dose of 15ml/kg, p.o. of polyherbal formulation. In EPM acquisition (learning) can be considered as transfer latency on first day trials and the retention/ consolidation (memory) is examined 24 h later[29]. The animal shows significant decrease in transfer latency as compared with the control group at a dose of 15ml/kg, p.o. of polyherbal formulation.

Diazepam (1mg/kg) has prolonged TL from the open arm to the closed arm i.e., decreased IR. The polyherbal formulation (5, 10 and 15 ml/kg) and piracetam (200mg/kg) have decreased TL from the open arm to the closed arm i.e., increased inflexation ratio thus confirms their nootropic activity. Statistically significant results were observed at a dose polyherbal formulation (15ml/kg, p.o.) as compare to standard. The protective effect offered by polyherbal formulation (5, 10 and 15 ml/kg) and Piracetam (200mg/kg) against diazepam- induced amnesic model may be due to indirect release of Ach in the brain[30-56].

The impairment of learning and memory induced by scopolamine (1.0mg/kg), an anticholinergic agent was reflected by prolonged TL from the open arm to the closed arm i.e., decreased IR was observed with EPM. The polyherbal formulation (5,10 and 15 ml/kg) and piracetam (200mg/kg) have reversed the amnesia induced by scopolamine, i.e. decreased TL from the open arm to the closed arm i.e., increased IR, indicates that they are acting on Ach receptors because they had shown nootropic activity in presence of scopolamine which is a muscarinic receptor antagonist. Statistically significant results were observed at a dose of POLYHERBAL FORMULATION (15ml/kg, p.o.) as compare to standard[30].

5. Conclusion

In the present investigation, polyherbal formulation has shown promise as a memory enhancing agent at a dose of 15ml/kg, p.o. in Elevated plus-maze (EPM) and passive avoidance paradigm model.

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Table no .1. Effect of polyherbal formulation (PHF) on passive avoidance learning and retention in mice (Mean ± SEM)

Group	Treatment	Dose (per Kg)	No. of trials for acquisition	STEP-DOWN LATENCY (SDL)		TIME SPENT IN SHOCK ZONE (TSZ)		STEP-DOWN ERROR (SDE)	
				Learning	Retention	Learning	Retention	Learning	Retention
I	Control	10 ml p.o.	2.5	13.66±0.61	85.50±2.82	44.83±0.30	15.16±0.16	4.16±0.16	3.2±0.25
II	Phenytion	25mg p.o.	2.6	12.33±1.02**	84.16±2.27**	45.16±0.30**	16.00±0.25	4.33±0.21	3.44±0.22
III	Piracetam	200 mg p.o	3.0	91.16±1.19**	274.83±0.94**	13.33±0.33**	3.36±0.16**	1.23±0.21*	0.63±0.21*
III	PHF	5ml p.o.	2.7	47.33±0.42*	193.33±0.61*	24.66±0.21*	6.26±0.16*	3.26±0.16*	1.44±0.22*
IV	PHF	10ml p.o.	2.7	71.66±0.49*	255.16±0.30*	14.33±0.33*	4.36±0.16*	1.93±0.30*	1.18±0.16*
V	PHF	15 ml p.o.	2.9	81.66±0.66**	266.50±0.42**	10.00±0.36**	3.40±0.22**	1.76±0.21*	0.80±0.16*

n=6 in each group. Data is expressed as mean ±SEM. Statistical analysis by one-way ANOVA followed by Dunnett's't' test Significance at $p<0.05$*, p <0.01** .

Table no. 2. Effect of PHF on inflexion ratio in mice (EPM model)

Group	Treatment	Dose (per Kg)	Inflexion ratio (Mean±SEM)
I	Control(vehicle)	10 ml p.o.	0.2567± 0.011
II	Piracetam	200 mg p.o.	0.71±0.007**
III	PHF	5ml p.o.	0.44±0.0156*
IV	PHF	10ml p.o.	0.62±0.00902**
V	PHF	15ml p.o.	0.64±0.0299**

n=6 in each group. Data is expressed as mean ±SEM. Statistical analysis by one-way ANOVA followed by Dunnett's't' test Significance at p<0.05*, p <0.01**

Table no 3. Effect of PHF on inflexion ratio in mice (Diazepam-induced amnesic model)

Group No.	Treatment	Dose (per kg)	Inflexion ratio (mean ± SEM)
I	Normal control	10 ml p.o.	0.34^{ns}± 0.05
II	Diazepam alone	1.0 mg i.p.	0.21± 0.04
III	Piracetam	200 mg p.o.	0.64**± 0.02
IV	PHF	5ml p.o.	0.48* ± 0.02
V	PHF	10ml p.o.	0.53**± 0.02
VI	PHF	15ml p.o.	0.65**± 0.02

n=6 in each group. Data is expressed as mean ±SEM. Statistical analysis by one-way ANOVA followed by Dunnett's't' test Significance at $p<0.05$*, $p<0.01$** and ns-not significant vs. control group.

Table no. 4. Effect of PHFon inflexion ratio (Scopolamine-induced amnesic model)

.Group	Treatment	Dose (per Kg)	Inflexion ratio (Mean±SEM)
I	Vehicle	10 ml p.o.	$0.29^{ns} \pm 0.02$
II	Scopolamine	1.0 mg i.p.	0.13 ±0.04
III	Piracetam	200 mg p.o.	0.65** ± 0.08
IV	PHF	5ml p.o.	0.38** ± 0.03
V	PHF	10ml p.o.	0.65** ± 0.04
VI	PHF	15ml p.o.	0.63** ± 0.03