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Design Development and Evaluation of Memory Enhancing Potential of Poly-Herbal Formulation

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Abstract-

Ayurvedic arrangement of medication is as old as human progress. Present study includes the development of a Polyherbal formulation by utilizing four different herbs, Pumpkin seed (*Cucurbita maxima*), Sunflower seed (*Helianthus annus*), Spinach leaves (*Spinach oleracea*), and Beet root (*Beta vulgaris*). Ethanol (90%) was utilized for preparation of polyherbal tincture. Freshly collected and authenticated herbs were characterized by pharmacognostical character and physicochemical assessment. Phytochemical screening showed the presence of alkaloids, glycosides, carbohydrates, amino acid, tannin, steroids and flavonoid in the tincture. Physical parameters like solubility, pH, ash values, LOD, extractive value etc. has been studied. The antioxidant activity of the tincture was determined by using DPPH free radical scavenging method. The results showed that the tincture has best antioxidant effect at a dose of 100µg/ml when it was compared with ascorbic acid as reference standard. From the acute toxicity studies safe dose and therapeutic experimental dose was found to be 300mg/kg. *In-Vivo* memory enhancing activity of Polyherbal formulation was evaluated by radial arm maze model in rats using Piracetam as a standard. Polyherbal formulation at the dose of 300mg/kg conc showed significant to highly significant memory enhancing potential. The result suggested that Polyherbal formulation possess *in vivo* memory enhancing activity and this might be due to Flavonoid, Phenolic compounds, Steroids or other constituents present in formulation.

Keywords: Polyherbal formulation, Physicochemical, Antioxidant, Memory enhancing activity.

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Introduction

Components like mental pressure, pollutants, hereditary preference increments or energizesthe danger of neurocognitive or neurodegenerative issues like Alzheimer's disease (AD), despondency, multi dementia, senile dementia which prompts disability of learning and memory (S. Shajahanet al 2014).Despite the fact that AD is basic in aged people however the side effect of Alzheimer's ailment is dynamic cognitive decline followed by a general intellectual decrease because of cholinergic lack in cortical areas. In India, it is time of immemorial practice of learning and memory upgrading by specific activities like Yoga and utilizing few any dietary parts, medicinal plants and herbs (Acharya J, et al 2015). It is accepted for a few centuries that, memory improvement has been a subject for science fiction, however now we know the idea of the neurobiological principle of memory. In light of the clinical information on memory work, numerous scientists are toward building up a medication, or finding a wellspring of characteristic intensifies that may improve our ability to recall. The nature gives another chance to recapture one's full intellectual ability. Various herbs generally utilized in the Indian System of Medicine Ayurveda have yielded positive outcomes. Present study includes the development of a polyherbal

formulation by utilizing four distinct herbs for example Pumpkin seed (*Cucurbita maxima*), Sunflower seed (*Helianthus annus*), Spinach leaves (*Spinach oleracea*), and Beet root (*Beta vulgaris*) (Sahoo R., 2011).

The effect of Polyherbal formulation on memory in Wistar rats with Radial arm maze (RAM) model and also to compare effects with standard drug Piracetam, this was presently used as memory enhancer in cognitive impairment (Ahuja D., Bapna J., 2016).

Material and Method

Plant material

The vegetative portion of plants for example seeds and leaves of Pumpkin, Sunflower, Spinach, and Beetroot were obtained from nearby market of Nanded, Maharashtra, India. The plant was recognized and validated by Dr. S. S. Bodke, Head Department of botany & Horticulture, Yeshwant Mahavidyalaya, Nanded, India.

The voucher specimen of the plant was deposited at the college for further reference. The vegetative portion of plants were dried under shade, powdered and stored in an air tight container.

Animals

Wistar rodents of either sex weighing (150-200 gm) were utilized in the present study. The experimental animals were maintained under standard laboratory conditions in an animal

house of Nanded Pharmacy College, which is approved by the committee for the purpose of control and supervision on experiments on animals Protocol (Registration No. 1613/PO/Re/S/12/CPCSEA). Animals were kept under 12 h light/dark cycles and controlled temperature ($24 \pm 2^\circ\text{C}$) and fed with commercial pellet diet and water *ad libitum*.

Preparation of Polyherbal Formulation (PHF)

300gm of freshly chopped herbs were taken in clean glass container and were mixed in with 90% alcohol. The mixture was shaken well for 1-2 minutes and afterward put away in cool dark place for 10-14 days. Intermittent shaking of the container each 1-2 days was done. This mixture was separated by utilizing muslin cloth by application of pressure and the filtrate was gathered in a container. Pour the tincture into clean, dark glass bottles by using a funnel. At the point when full, plug with a stopper or screw top and label the container (Kokate CK., 1994).

Phytochemical analysis

The preliminary phytochemical studies of PHF revealed the presence of Flavonoids, Phenolic compounds, Steroids and other phyto-constituents (Alamgir H., Mahbub S., 2016).

Pharmacological Screening

Acute oral toxicity study

Determination of LD₅₀ of PHF-The PHF was administered in a single dose by gavage using a stomach tube. Rats overnight fasted prior to dosing. The starting dose level was 300 mg/kg. Before dose administration, the body weight of each animal was determined, and the dose was calculated according to the body weight. Three female rats were used for each step, sequentially dosed at intervals of 48 h. Animals were observed individually after dosing during first 30 min and periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 d. An acute oral toxicity study was conducted in accordance with OECD Guideline 423 (Belhekar S., 2016).

Radial Arm Maze Model

Rats were divided into five groups of 6 animals such as follows: Group-I animals served as control group and received (vehicle p.o.) Group-II standard group received Piracetam (200mg/kgp.o.) Group-III received PHF (100 mg/kg p.o.) Group-IV received PHF (200 mg/kg p.o.) Group-V received PHF (300 mg/kg p.o.). The apparatus was fabric elevated eight arm radial arm maze with the arms extending from a central platform. 26cm in diameter. Each arm is 56cm long, 5cm wide with 2cm high rails along the of the arm. The maze was well illuminated and numerous cues were present. Food pellets were placed at the end of

the arm. To motivate the rat to run the maze. Animals were trained on a daily basis in the maze (Vogel G, Sandow J., 2002).

Each rat maintains at 85% of its total diet weight, was exposed to the maze daily with the food pellet in a fix arm. The apparatus was cleaned with damp cloth after each trial. The evaluation was carried out on 8th day, 30 minutes after the respective drug treatment. Food pellet was placed in a variable arm for evaluation of working memory.

Results

Latency to find food was recorded as a measure of working memory evaluation. Table 1 shows the consolidated data of number of entries as well as time spent in C and P zone respectively. When the PHF treated groups were compared with control and standard groups, it was observed that all the three PHF treated groups (i.e. PHF-100, PHF-200, PHF-300) showed increased in number of entries of the animals at P zone.

Table 1: Comparative Study of Number of Entries and Time Spent in C And P Zone of Rats for Memory Enhancing Activity of Polyherbal Formulation (PHF).

Group name	Number of Entries in				Time Spent in			
	C-zone		P-zone		C-zone		P-zone	
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
Ctrl	25.2 ±1.66	23.8 ±2.8	24.4 ±0.87	25.4 ±0.98	51.8 ±3.21	55.6 ±2.31	156.2 ±5.54	157.2 ±4.51
Std	24.0 ±4.41	12.4 ±1.72*	23.4 ±0.55	38 ±0.57**	62.2 ±5.25	31.0 ±1.39**	145.8 ±16.4	220.2 ±6.02**
PHF-100	24.4 ±3.71	14.2 ±1.4*#	27.8 ±2.52	39 ±0.51**#	60.2 ±4.01	37.0 ±2.58**#	133.0 ±4.76	221.4 ±4.59**#
PHF-200	26.8 ±2.6	16.2 ±1.82#	28.4 ±1.89	40.6 ±0.61**#	54.0 ±4.03	34.6 ±1.47**#	144.8 ±2.65	225.8 ±2.65**#
PHF-300	28.0 ±1.52	17.2 ±0.47#	30.8 ±1.49	42.8 ±0.6*Δ	51.8 ±3.09	33.4 ±1.64**#	149.4 ±2.73	231.6 ±2.73**#

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ-Significant difference when compared to Standard but more activity.

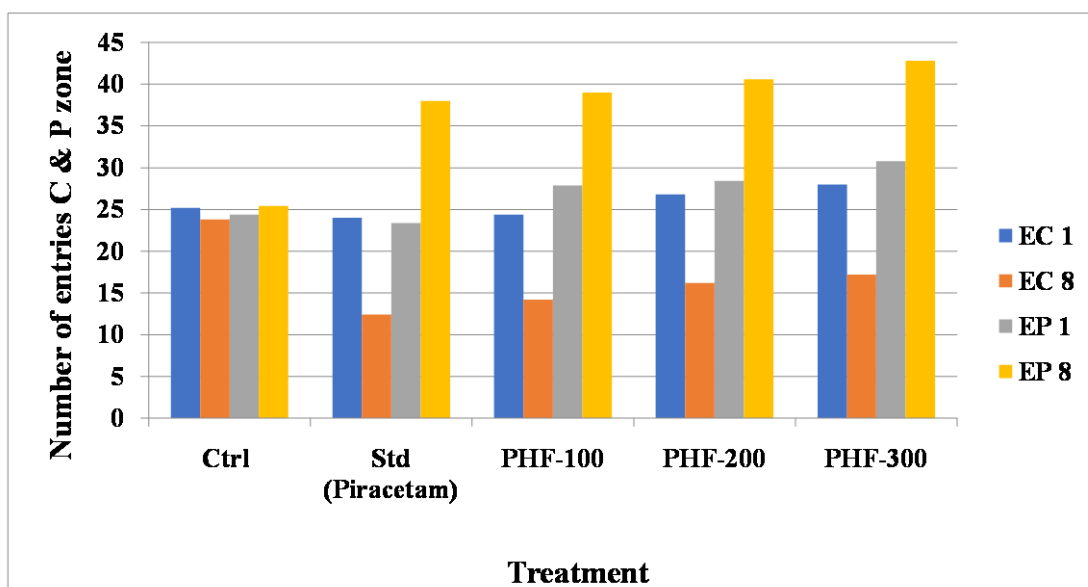


Fig. 1: Number of Entries in C and P Zone of Rats during Experimental Period

(EC1- Number of entries in C-zone on day 1,

EC8- Number of entries in C-zone on day 8;

EP1- Number of entries in P-zone on day 1,

EP8- Number of entries in P-zone on day 8.)

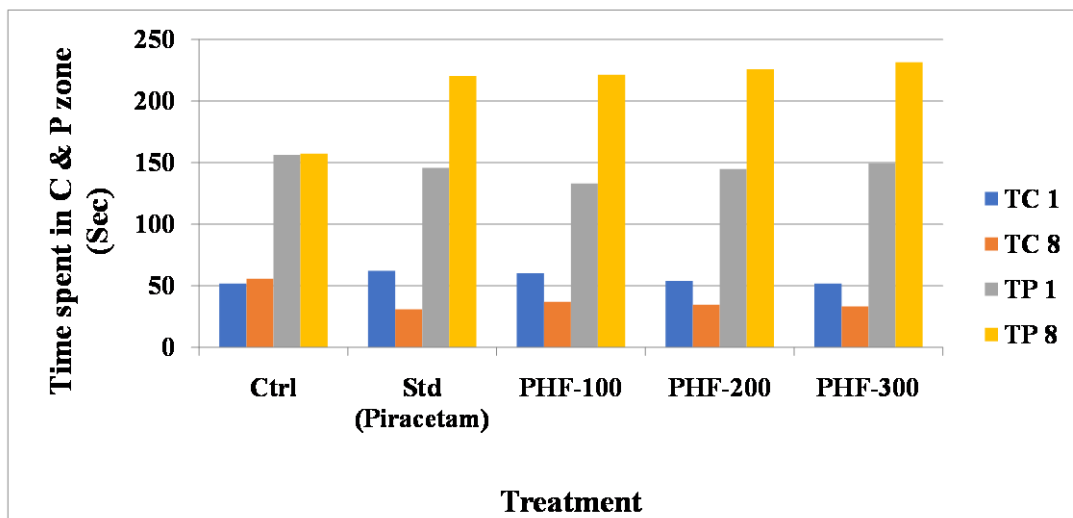


Fig 2: Time Spent in C and P Zone of Rats during Experimental Period

(TC1- Time spent in C-zone on day 1,

TC8- Time spent in C-zone on day 8;

TP1- Time spent in P-zone on day 1,

TP8- Time spent in P-zone on day 8.)

The maximum entries were showed by PHF-300 treated group. This group showed significant increase in number of entries i.e. $42.8 \pm 0.6^* \Delta$ as compared to standard group. Similarly when these groups screened for Time spent in P zone with Standard and control, here also all tested groups showed moderate to significant increase in time spent at P zone. Here also group no.5 i.e. PHF-300 showed highly significant memory enhancing effect when compared with control group.

The result confirms memory enhancing potential of all three tested groups when compared with control and standard groups. The effect showed by PHF-300 was found to be highly significant as compare to other groups. Same results were represented in the form of bar diagram in Fig. 1 and Fig. 2.

Conclusion

Poly-herbal formulation (PHF) of crude drugs contain several chemical constituents which are pharmacologically important as they have been proved to be beneficial in many specific diseases like cancer, hypertension, diabetes, hepatotoxicity where its memory enhancing potential is claimed to be useful. The crude drugs already tested individually for different CNS related activities. No methodical reports on memory enhancing activity of combine form of crude drugs were available. Therefore, it was thought worthwhile to explore this

mixture of crude drugs for its memory enhancing activity in the form of poly-herbal formulation

The PHF was prepared in the form of tincture in ethanol. Preliminary phytochemical evaluation of PHF was carried out for the determination of presence of phytoconstituents which showed presence of Flavonoids, Tannins, Phenolic compounds, Steroids, Proteins, Alkaloids, Carbohydrate and Glycosides etc. These phytochemicals were further confirmed by thin layer chromatography (TLC).

The results of acute oral toxicity studies of PHF as per standard references revealed that in single dose; the PHF had no adverse effect, indicating that the medium lethal dose (LD_{50}) could be greater than 3000mg/kg body weight in rat. Accordingly, safe experimental dose was calculated as ≤ 300 mg/kg and was used accordingly for further screening of PHF.

Memory enhancing activity was carried out on rats by using radial arm maze model. Equal numbers of animals group were treated with (100mg/kg, 200mg/kg and 300mg/kg) of PHF and Piracetam as standard. Observations were made for the decrease in number of errors in rats by screening time spent and number of entries of animals in different arms.

The result showed that all the four selected crude drugs proved to have promising and

highly significant memory enhancing effect ($P < 0.001$) when given together in the form of a Poly-herbal Formulation. Furthermore, study is requiring finding out the exact mechanism of action.

Statistical Analysis

The data were expressed as mean \pm Standard error of mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) test.

Conflict of Interest

The authors declare no conflicts of interest.

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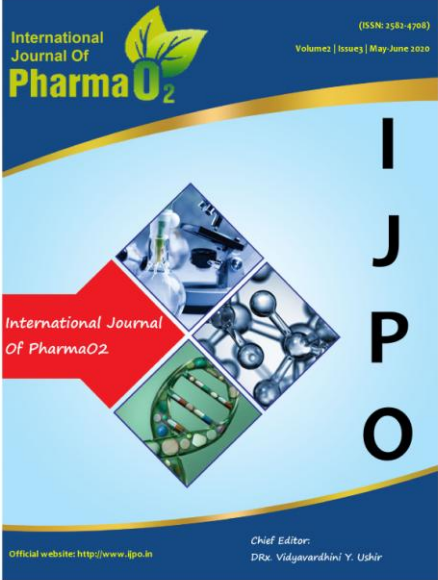
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