Original Article

Evaluation of learning and memory activity of poly-herbal formulation by scopolamine and stress induced amnesia

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ABSTRACT

Normal ageing is not as much as responsible for causing memory disorder as such, but it is associated with a general decline in cognitive and neural systems, including memory. As people age, the likelihood of cholinergic dysfunction, beta-amyloid deposits, hippocampal neurofibrillary tangles or neurotic plaques in the cortex of the brain increases, so that memory connections can become blocked, memory functions decrease and the likelihood of memory disorders like dementia and Alzheimer's disease increases. Ageing is the single greatest risk factor for neurodegenerative diseases in general. Recent research reveals some major consequences between cognitive changes of normal ageing and other neurodegenerative diseases known as mild cognitive impairment were some memory loss occurs which doesn't interferes with normal daily functioning. More severe memory loss is defined as dementia; those patients who have mild cognitive impairment are at high risk of developing Alzheimer like diseases. Currently the commercial use of the synthetic cognitive enhancers shows some of its downs effects on the brain not only functionally but also have unexpected complications. That why the area of thirst is to explore such poly-herbal formulations with meets and fulfil the optimized level of therapeutic efficiency and efficacy without any side effects.

1. INTRODUCTION

Biodiesel has gained importance in the recent part for its according to Darwin's principle of "survival of the fittest" every person wants to become fittest in today's environment. To achieve this people do over struggle and comes under various types of stress along with that there are so many factors like biochemical, physical psychological which alters the perception of mind and leads to memory impairment. Stress and anxiety are very common factors in our life which reduces memory and power to concentrate. It's a quite well known mechanism that when we are in stress free radicals generation increases in our brain. Our body naturally produces free radicals as a by-product of cellular energy production and liver detoxification. This is similar to a car that produces toxic exhaust fumes as the waste product of the combustion of petrol. Whenever we use our body a lot, there will be increased free radical production. Emotional stress significantly increases free radical load on the body. As stress induces the release of hormones, the body mobilizes ready for action, known as the fight and flight response. This creates bio-chemical changes which increase free radical stress in the body [1-2]. There is number of memory boosters available in the markets that have been widely used by our young minds to achieve top position in academic era. The sales of these drugs are at high level near the board examination. It have been found that most of the allopathic cognitive enhancers have a wide range of side effect from mild to severe e.g. Piracetam acts by increasing cerebral blood flow and providing direct support to neuronal metabolism this drug have side effects like nervousness,

agitation, irritability, anxiety and sleep disturbances. Other effects are vertigo, headache etc [3]. Vinopcetine is another cognitive enhancer which inhibits platelet aggregation. There are so many examples but we mention only few here, that's why a need and importance of potent and safe poly herbal formulation that is in the reach of common people and having no side effects.

We have gone through number of literatures and journals, we found that most of the drug that are available in the market are either very costly or having number of side effects. That's why I have chosen a balanced combination of four plants along with honey. All these products are totally natural, safe and neuroprotective in nature. The bases of selection of these contents are pharmacologically and phonemically through their mechanism of action. **Amla:** Vitamin C is the chief constituent of Amla having potent antioxidant activity. It also protect brain cells from free radicals produced during stress and in normal metabolic process. It also contain gallic acid which protects our brain from oxidative stress. Ellagic acid plays very important role in attenuating neurophysiological and cognitive behavioural symptoms associated with infusion of amyloid-beta ($A\beta$) peptide fragment in adult rats [4-6]. **Tulsi:** Called as queen of herbs contains Eugenol and Carryophyllene possess anticholinesterase and anti-stress activity. It also Vitamin C and A having antioxidant activity. Tulsi leaves prevented changes in plasma levels of the stress hormone corticosterone induced by both acute and chronic noise stress [4]. Tulsi also contain gallic acid which acts as neuroprotective. Chlorogenic acid may exert anti-amnesic activity via inhibition of acetylcholinesterase and malondialdehyde in the hippocampus and frontal cortex (Kwon SHet al). Ursolic acid ameliorates cognition deficits and attenuates oxidative damage in the brain [2]. Administration of OS (100 and 200 mg/kg p.o.) and its saponin rich fraction (100 and 200 mg/kg p.o.) for 14 days significantly attenuated vincristine-induced neuropathic pain along with decrease in oxidative stress and calcium levels [10]. **Ginger:** It contains gingerol and shogaols are the principle constituents having anticholinesterase activity. Ginger also contains α-zingiberene and α- curcumin. It also posses' antioxidant activity.6-gingerol as an active ingredient is provided to suppress apoptosis and oxidative damage of nerve cells and use in pharmaceutical composition and health functional food for preventing or treating disorder relating to memory and cognitive ability. 6-shogaol protects neurons. Dietary lipids including lecithin are responsible for better memory [5]. *Zingiber Officinale* (Zingiberaccae) rhizomes posses potent memory enhance in scopolamine inducing memory impairment by significantly increased whole brain acetyl cholinesterase inhibition activity [9]. **Gurhal:** Contains Quercetin which enhances glutathione level which is an important antioxidant and neuroprotective substance. It also contain hentriacontane which is a chief constituent of brahmi also [8]. Honey is used as viscosity modifies as well as it improves memory by improving concentration of Ach. It is an excellent food supplement of glucose for brain cells.

2. EXPERIMENTAL

2.1 Collection and authentication of plant

The plants (fruit of *Emblica officinalis*, leaves of *Ocimum sanctum*, rhizome of *Zingiber officinale* and roots of *Hibiscus rosasinensis*) were collected in the month of February from the local area of Bairagarh, near Kailashnagar colony, Bhopal (M.P.). Herbarium file of plant part was prepared and authenticated by Dr. Zia Ul Hasan (Professor), Safia College, Bhopal and the specimen voucher no. assigned was450/Bot/Safia/13. After that Herbarium file was submitted in Truba Institute of Pharmacy, Bhopal.

2.2 Test material

All the plant materials were dried under shade. They was pulverized to coarse powder with the help of grinder. The coarse powder was passed through sieve No.20 to maintain uniformity and packed into airtight container and stored in cool and dry place. Ethanolic extracts were prepared by extracting each plant with ethanol using Soxhlet extractor.

2.3 Preparation of test PHE (poly herbal extract) suspension

The individual dose in mg/kg PO of Amla, Tulsi, Ginger and Gurhal was found to be 400, 200, 100 and 50 respectively. The dose was finally calculated for an avg. wt. of 30 gm. mice and on the basis of these doses a poly herbal formulation from these materials was prepared. 225 mg of total drug was dissolve in 10 ml of 30% (v/v) honey solution (3ml honey+7ml water). Finally the suspension was prepared in 0.5% CMC (carboxymethyl cellulose) and was sonicated.

Note: Honey used in this formulation had a dual benefit i.e. it have anticholinesterase activity as well as viscosity modifier for suspensions.

2.4 Animal care and handling

The animals were carried for experiments from the authorized animal house of Truba Institute of pharmacy, Bhopal. All the rats were healthy and 100- 250gm of body weight. The animals were kept in air conditioning environment and temperature was maintained at 22°C (± 3°C). The bedding of animals were changed every 3rd day.

2.5 Acute oral toxicity study

Oral Acute toxicity study was evaluated as per OECD guidelines (425) on Swiss albino mice. Animals were provided by Truba Institute of Pharmacy, Bhopal and experiment was done in the Institute. Three animals are selected which receives dose of 2000mg/kg (limit test), the volume should not normally exceed 1 ml/100g of body weight; however in the case of aqueous solutions, 2 ml/100g body weight can be considered.

Animals were observed individually for 48 hrs after dosing any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality. No significant signs were noticed in animals.

2.6 Experimental design

In-vivo screening of Nootropic activity

Scopolamine induced amnesia in mice using Elevated plus maze (EPM)

Procedure: The Swiss albino mice of either sex were weighed and numbered. They were divided into 5 groups (n=6). All the animals were practiced on EPM (Elevated plus maze) for 1 hr. /day for 10 days. Group 1st –control, receives only vehicle, Group 2nd–Negative control receives only scopolamine (3mg/kg) i.p on 11th day, Group 3rd –standard, receives standard syrup Mentat (1ml) p.o. Group 4th –Test, receives test poly herbal formulation (11.5mg/ml)p.o, Group 5th –Test, receives test poly herbal formulation (22.5mg/ml)p.o. The animals from group 3rd to 5th receives the treatment for 10 days. On 11th day the animals of group 2nd to 5th receives scopolamine hydrobromide (Buscopan, Cadila Healthcare Ltd.) after 1 hr. of dosing of test and standard drug. All the animals were placed on elevated plus maze and transfer latency (TL) was evaluated.

2.7 Evaluation of transfer latency

TL is the time in which animal moves from the open arm to the enclosed arms putting all his 4 legs completely inside the enclosed arm [7].

2.8 Stress induced amnesia in mice using Elevated plus maze (EPM) and MWM (Morris water maze) test

Weighing and numbering of animals was done, they were divided into 5 groups (n=6). All the animals were practiced on MWM, 6-10 trials/day for 5 days and on EPM (Elevated plus maze) for 1 hr./day for 10 days. All the animals were given stress by restrain and cold (10mins at 4-8ºc) [8] followed by tail suspension (20mins) for 10 days. Group 1st –control, receives only vehicle, Group 2nd –Negative control, receives only by restrain and cold followed by tail suspension. Group 3rd–Standard, receives standard syrup Mentat (1ml) p.o and after 30 mins. animals were exposed to stress. Group 4th –Test, receives test poly herbal formulation (11.5mg/ml)p.o and after 30 mins. animals were exposed to stress and Group 5th –Test, receives test poly herbal formulation (22.5mg/ml) p.o. after 30 mins. animals were exposed to stress. The above procedure was performed for 10 days and finally on 11th day all the groups were evaluated on EPM for TL (Transfer latency) and on Morris water Maze for EL (Escape latency).

2.9 Evaluation of escape latency and transfer latency

EL is the time taken by the animal to find the hidden platform from the starting point. **TL** is the time in which animal moves from the open arm to the enclosed arms putting all his 4 legs completely inside the enclosed arm [7].

2.10 Statistical analysis

Data was expressed in mean ± S.E.M, One way ANOVA followed by Turkey-Kramer Multiple Comparisons Test using software Graph Pad Prism 6 and graph pad instat.

3. RESULTS AND DISCUSSION

We now are at the position to discuss the quite significant action of the poly herbal formulation from result of three observations. As the work was solely on the spatial memory so the selection of the evaluation parameter taken are Transfer latency and Escape latency.

In case of chemical induced amnesia the result of ANNOVA on the basis of TL reveals that the polyherbal formulation shows significant response as compared to standard formulation with respect to negative control group but the induction of amnesia is non-significant with respect to control group. In the second study which is of stress induced amnesia the observations from the TL is much better than that of chemically induced. The induction of amnesia by stress shows greater transfer latency than the control group which is very much significant. The most important thing that we observed that the behaviour of the animals were very much aggressive so the lag time of about 30 to 45mins are maintained before the animals are put on the EPM. The results of the second model are based on 2 observations i.e. TL and EL. The decrease in transfer latency due to standard treatment and test Polyherbal formulation are compared and shows the probable significance. The dose response effect of test Polyherbal formulation is also seen and is up to the expected level. In case of EL we got only two significant probabilities one shows the significant induction of amnesia and other shows the treatment effect of the standard drug. The comparison between negative control vs treatment is nonsignificant via ANNOVA.

4. CONCLUSION

Finally on the basis on the above result it has been concluded that the work that our polyherbal formulation shows dose dependent action and is effective in both scopolamine and stress induced amnesia. The rationale of my project may not be up to mark but it reveals some of its positive effects which in future may be tremendously explored as an ideal memory booster bullet.

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