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COGNITIVE EFFECT OF MAHAPAISHACHIK GHRITA IN DIAZEPAM INDUCED AMNESIA IN ADULT MICE.

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ABSTRACT **BACKGROUND:** Learning is an acquisition of information and skills whereas retention of the acquired information is called memory. Diazepam (DZP), widely prescribed agent for anxiety, depression and insomnia of old age is associated adverse effect of amnesia which further worsens the natural neurodegenerative amnesia of old age. Hence present study was undertaken to explore effect of Mahapaishachik Ghrita (MG), a fat based Ayurvedic formulation in learning, memory and Diazepam induced amnesia in adult mice.

AIM: To assess nootropic and anti- amnesic effect of Mahapaishachik Ghrita (MG) in adult Swiss albino mice.

METHOD: The experiment was conducted on adult mice of age 52-53 wks in five groups as Normal control, Disease control and standard control with known memory enhancer drug Rivastigmine (RV) (dose=2.4mg/kg/p.o.) and two groups of MG in two doses (MG1=5.2 gm /kg/p.o., MG2= 10.4 gm / kg/p.o.). Effect of MG on learning was assessed on day 15 & on memory was assessed on day 16, whereas diazepam induced amnesia (dose=1mg /kg/i.p.) was noted on 26 and 27 day. The transverse latency of the animals in extroceptive behavioral animal models - EPM & MWM were employed to assess learning and memory.

RESULTS: The higher dose of MG showed less TL, compared to standard (Rivastigmin)and diseases (Diazepam) control group. MG also demonstrated significant ameliorative effect in Diazepam induction amnesia. The obtained results may be contributed to the nootropic ingredients of the MG.

CONCLUSION: MG demonstrates nootropic effect in adult mice and also prevents Diazepam induction amnesia

KEYWORDS : Learning, Memory, Ageing, Mahapaishachik Ghrita.

INTRODUCTION:-

Cognition is the mental action or process of acquiring knowledge and understanding through, experience, and the senses. Learning and memory are component of human cognition. Learning is defined as the acquisition of information and skills and retention of the acquired information is called memory. Learning and memory can be conceived as both a psychological process, as well as change in synaptic neural connectivity.

Ageing is a normal phenomenon in human life cycle. Normal ageing is known to deteriorate memory due to free oxygen radical causing organic damage in human being. A survey conducted on 1,637 people above 64 years of age revealed that 524 persons (32.4%) were suffering from subjective memory complaints (SMC). The survey also reported that the memory complaints gradually rise with age and reaches to 57% in people up to 90 years or above group. SMC are also reported to be higher (52.8%) in people with anxiety and depression. Thus age related memory deficit is one of the psychosomatic problem and characteristic symptom of various neuro-generative disorders including Alzheimer's disease. Another associated chief complaints arising with ageing is anxiety, depression and insomnia. Diazepam, derivative of Benzodiazepines is one of the most widely prescribed pharmacologic agents to resolve anxiety, depression and insomnia complaints . The pharmacological agent used for treatment of ailment of old age (insomnia) further worsens the memory impairment due to ageing. Nootropic agents such as Piracetam, Aniracetam and choline esterase inhibitors like Donepezil are being used to improve memory, mood and behaviour, but the associated side effects such as headache, nausea, insomnia, anxiety, increase heart rate, agitation, dizziness have made their applicability limited.

Mahapaishachik Ghrita (MG) is a fat based herbal formulation prescribed in Ayurvedic classics with indication of learning and memory enhancement. The ingredients of MG are *Medhya* (cognitive enhancer) and *Rasayan* (Drugs capable to rejunevate and promotes healthy life) and widely used by the Ayurved physicians for psychosomatic ailments.

Considering the long duration required evaluate effect of pharmacological agent on cognitive functions the animal model is better choice. It has been reported that one human year is equivalent to nine mice days. Hence present study has undertaken to evaluate cognitive potential of *Mahapaishachik Ghrita* (MG) on learning-memory and diazepam induced amnesia employing exteroceptive behavioral model in adult mice.

MATERIALS AND METHOD:

1. Materials-

Animal strain: Swiss Albino mice
Test drug: *Mahapaishachik ghrita*
Induction drug: Diazepam
Standard Comparator: Rivastigmine
Parameter assessment instruments: 1) Morris water maze
2) Elevated plus maze

Methodology of the Assessment of Learning and Memory on EPM and MWM:

Elevated Plus Maze:-

In this experiment EPM served as exteroceptive behavioral model to assess learning and memory in mice. The apparatus consist of 2 open arm and 2 covered (closed) arms. The arm extended from a central platform and maze was elevated to height of 25 cm from the floor. In the experiment, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the closed arms with all its four legs. If the animal did not enter any of the closed arms within 90 sec., it was gently pushed into one of the two closed arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then returned to its home cage.

Morris Water Maze:

It consists of large circular pool of water. It is divided into 4 equal quadrants with E,W,S,N marking. The platform was kept in NE quadrant. The water was colored with chalk powder to hide the location of platform. Mouse was inserted in the pool in fixed quadrant opposite to the quadrant having platform. The round platform of 8 cm diameter was placed 1 cm below the surface of water in a constant position in the middle of the NE quadrant in the pool; the starting point was SW quadrant in all the trials. The mice try to find the safe place in the pool and climb the platform to escape from the necessity of swimming. To assess the learning potential of the mouse with a maximum time of 120 s (cut-off time) to find the hidden platform and the animals is allowed to stay on it for 30s. This time taken by the mouse to climb on platform was considered as TL.

Methodology Of Experiment:

The study protocol was approved by Institutional Research review board and permission of Institutional Animal Ethics Committee was obtained prior to initiation of experiment. The Registration No. of animal ethics committee is BVDUMC/1877/2018/002/006 date 22nd

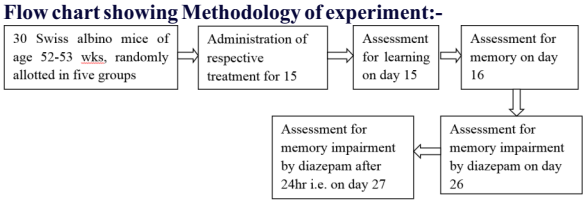
Nov 2018. The study site was CPCSEA approved Central Animal House, BVDU Medical College, Pune (Reg.no 258/CPCSEA/2000). The housing and feeding conditions were maintained throughout the experiment as per the standard guidelines. All the experiments were carried out during day light from 9 am to 4pm. Animals had free access to food and water.

30 Adult swiss albino mice of 52-53 wks of either sex of weighing 40-50gm in five groups consisting of six animals in each group were selected. Group I was treated as normal control group and received distilled water throughout the experiment. The animal of group II was served as a positive control and received Rivastigmine, a standard memory enhancer agent 2.4 mg/kg day p.o. for initial 15 days. Animals of group III & IV was given MG by oral route in extrapolated human dose of X – 5.2 gm /kg/p.o. and 2X – 10.4 gm / kg/p.o. respectively for initial 15 days. The animal of group V was used to serve a negative control for memory impairment by Diazepam in and received distilled water for initial 25 days. The animals of group V was subjected to all performance test done on EPM & MWM during the study to avoid training bias.

On 15th day, ninety minutes later the administration of respective treatment, drug/ distilled water, animals was subjected to the Elevated Plus Maze (EPM) and Morris Water Maze (MWM) to evaluate their learning performance. The same experiment was repeated after 24 h i.e. on day 16 to assess effect of respective drugs on memory of the animals. From day 16 to 26 the respective treatment drugs were continued. On day 26 memory impairment was induced by Diazepam (1mg/kg/i.p.) 45 min after the respective dose of the test drugs/distilled water to the animals of group II, III, IV and V. 45 min later the TL was assessed on EPM and MWM for assessment of effect of drug on memory and experiment was repeated after 24 h.

Dose calculation: The standard dose of medicated ghee is 40gm (1 pala) in human being. The standard dose of drugs was extrapolated in animals as per standard guideline given in Fundamentals of Experimental Pharmacology. The extrapolated dose was stated as X. Test drug MG was studied in 2 dose levels viz. X, 2X. The extrapolation factor for mice is 0.0026. Hence the dose level was given by oral route as: X – 5.2 gm /kg/p.o. and 2X – 10.4 gm / kg/p.o.

Flow chart showing Methodology of experiment:-



RESULTS:

The assessment of parameter used in this study was transverse latency (TL) recorded on EPM and MWM. The animals were exposed to EPM and MWM for assessment on day 15, 16, 26, 27. For the analysis of obtained data 'Graphpad Prism 8' software was used and data was statistically analyzed with 'one way ANOVA' followed by 'Friedman' test

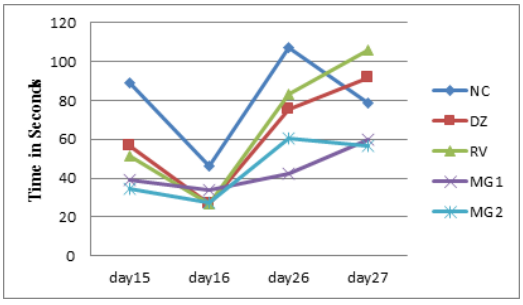


Figure showing the comparative TL findings on EPM:

TL observed for assessment of learning and memory in animals of RV group was comparable with MG treated group on day 15 & 16 respectively. But RV treated animals could not retain the memory after DZ induced memory impairment on day 26, further the animals of RV continued marked memory deficit after 24 hrs as compared to the MG receiving animals.

In MG1 & MG2 group animals, less TL was noted for learning and memory retention process as compared to all other groups. MG treated animals perform very efficiently to reduce memory impairment effect of DZ induced on day 26. Even after 24 hours on day 27 the memory retention in MG treated group was more as compared to all other groups.

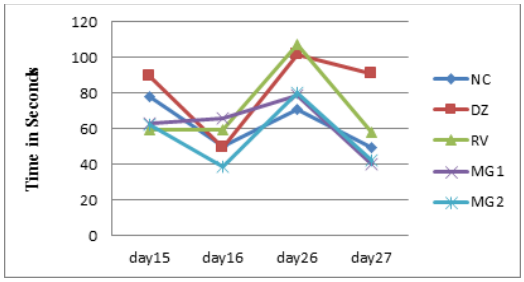


Figure showing the comparative TL findings on MWM:

During the experiment it was noted that RV treated animals maintained TL on day 16 due to memory enhancement effect of Rivastigmine. But after DZ induced memory impairment, RV treated animals could not retain memory, rather the animals showed marked memory deficit comparable to DZ group animals. It was also observed that this stiff memory impairment is further decreased and was comparable to MG receiving animals on day 27.

MG1 & MG2 treated animals showed less TL on day 15 indicating test drug improves the learning ability in mice which is comparable with standard group RV. The TL reading on day 16 showed that the memory in MG lower group is more than RV receiving animals but TL of MG2 group was decreased significantly, indicates better memory of mice receiving MG in higher dose. On day 26 after administration of diazepam, MG treated animals perform very efficiently with less TL indicating the effect of MG to reduce DZ induced memory impairment. Further on day 27 that is after 24 hours of induction of memory impairment, the decreased TL in both the MG treated groups showed the attainment of normal memory.

Thus from the behavioral animal experiment it can be concluded that the MG2 is more effective to enhance learning capacity of aged animals and it also reduces the effect of DZ induction memory impairment in both EPM And MWM exteroceptive behavioral model.

DISCUSSION:-

Learning and memory are the basic constituents of cognitive behavior. In traditional Ayurved science, lipid based medicines able to act on brain and have been prescribed to maintain healthy functioning of these components. Cognitive dysfunction is seen due to poor nutrition, old age, peer pressures and stressful life which primarily affect learning and memory process. The cognitive dysfunction expressed in the form of memory loss, mild cognitive impairment, amnesia, and dementia and further development of Alzheimer disease .

Old age is an undesirable and inevitable phase of human life. As per Ayurved philosophy in old age dominance of *Vatadosha* is seen. The average age of a human in contemporary era is increased leading to rise in elderly population. Therefore, the prevalence of specific Geriatric disease conditions is also increasing[20]. In Ayurved perspective the cause of all Geriatric disease *(Jarajanyavyadhi)* is degenerative changes of body elements (*Dhatuksaya)*. Hence this study was initiated with the hypothesis that, 'if start *Rasayana* drugs (capable to rejunivate and promotes healthy life) are started to administer in appropriate age group, may be effective to delay in the degenerative changes of old age'.

World Health Organisation has defined the old age above 60 yrs. Different phases of life have been described in Ayurvedic philosophy. Ayurved classical literature *Charak Samhita* has described the span of human age as 100 yrs and has divided in three phases as*: Balyavstha*(Childhood- 0 to 30years), *Madhyavastha*(Young- 30 to 60 years) and *Jirnavastha*(Old- above 60 years). In *Sushrut Samhita,* the 100 yrs of human age has been divided in three phases as well but with more specification. *Sushrut* labelled *Balyavastha* till age of 15yrs and *Tarunavastha* from 16 to 70 yrs with further sub classification. The span between16-20 is *Vridhi* where only growth of body constituents may be observed. From 21-30 years of age group is labelled as *Yoauvan*

where growth of body constituents and maturity of some of the body constituents is achieved. In the next subclass of *Sampurnata* (31-40yrs) all the body constituents achieve maturity. In next age group of 41-70 yrs of age (*Parihani*)the already matured body constituents tends to move gradually towards the wear and tear processes and ageing starts with few degenerative changes of bodily tissues *(Dhatu*). After 70 years(*Vriddhavstha)* gross degeneration of body tissues are witnessed in the form of diminution of tissue, sense faculties, strength, vitality, grey hairs, baldness, dyspnoea etc.

In conventional health science, therapeutic drugs used for cognitive disabilities or maintenance of memory functions of old age demonstrate positive effects with certain limitations. Ayurved science advocated variety of herbal formulations to maintain the cognitive functions in normalcy and treat cognitive disabilities. Herbal formulations grouped under nootropic or memory enhancer (*Medhya Rasayan*) group, emphasized their use in maintenance of cognitive abilities of human being in healthy state and standard guidelines have been laid by classical text of Ayurved for its administration. The test drug of present study *Mahapaishachik Ghrita*(MG) is a medicated fat based formulation advocated to increase intellect and memory (*Medhya* and *Smritivardhak)* and belongs to the class of *Medhya* and *Rasayan* formulations[9].

The data collected in present study support the finding of several recent studies stating successful utility of Elevated Plus Maze and Morris Water Maze apparatus in assessment of nootropic and anti-amnesic agents. In this experiment animals were assessed for learning and memory after 15 days pretreatment of test drug MG. Also effect of Test drug was tested on Diazepam induced memory deficit.

During the experiment it was noted that all the animals of all group have comparable weight gain throughout the experiment. Also it was noted that in female mice less TL was observed as compared to male mice of the same group. Hence it may be stated that female mice have greater ability to learn quickly. Also DZ induced memory impairment was prominent in male mice as compared to female mice in every group. After induction of diazepam, mice showed more TL in MWM than EPM in every group this implies that in spite of pretreatment of nootropic agents the DZ induces considerable memory loss.

The higher dose of MG showed less TL, compared to standard (Rivastigmin)and diseases (Diazepam) control group. MG also demonstrated significant ameliorative effect in Diazepam induction amnesia. *Mahapaishachic ghrita* (MG) is a *Goghrita* based herbal formulation prescribed for mental disorder with indication of enhancement of learning and memory. *Ghrita* is considered best among all the *fat base medicated preparations*. It has been well established that cow ghee promotes memory, intellect, power of digestion[9]. When ghee is prepared with certain specific drugs in accordance with prescribed procedure, its potency and utilities increase many fold. This is because no other misogynistic substance except ghee has such tremendous capacity to absorb the properties of the drugs mixed with it. Besides *Goghrita* provides lipid base to the ingredients of MG which may facilitates them to reach up to the level of neuron and cross easily blood brain barrier. The obtained results may be contributed to the nootropic ingredients of the MG. The ingredients of MG have been proven effective as Anti-depressant and Anti-anxiolytic agents on CNS disorders and as well to improve memory[29]. The prime ingredients of *Mahapaishachik Ghrita* are *Mucuna Pruriens* (*Kapikacchu acts as* Anti-depressant, Antioxidant*), Nordostachys Jatamansi (Jatamansi used as* Memory retention and learning enhancer)*[29], Bacopa Monneri (Brahmi as* Anxiolytic, Antidepressent*)[29], Terminalia Chebula (Haritaki* Enhance the learning and memory recall ability, Antioxidant)*[31], Convolvulus pluricaulis (Shankhapushpi as* Antidepressant)*[30], Convolvulus pluricaulis (Vacha as* Antioxidant, Neuroprotective*), Desmodium Geneticum (Shalparni as* Antioxidant, Nootropic*), Commiphora Mukul (Guggul as* Antioxidant, acts on impairment in learning and memory*), Asparagus Racemosus (Shatavari has* Anti-stess activity, Enhances memory, Antidepressant, Antioxidant*).*

CONCLUSION:

Thus from the collected data it can be concluded that MG in higher dose demonstrates nootropic effect in adult mice and also prevents Diazepam induction amnesia.

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