Assessment of Nootropic Activity of Panchagavya Ghrita in Animal Models

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Abstract- Panchagavya ghrita [PGG] is one of the medicated ghee formulations, contains five cow products; cow ghee, cow milk, cow urine, cow dung juice and cow curd. PGG was prepared as per established SOP and compared with standard values. Nootropic activity of Panchagavya Ghrita (PGG) was studied using Diazepam induced amnesia in mice and Morris Water Maze test in rat model. PGG also reversed successfully the amnesia induced by Diazepam (1mg/kg, i.p.). PGG was administered in three dose levels of PGG as X/2 (2.5gm/kg), X (5gm/kg) and 2X (10gm/kg) in mice and X/2 (1.75gm/kg), X (3.5gm/kg) and 2X (7.0gm/kg) in rats.

In EPM test significant effect of 5gm/kg dose of PGG was determined and Piracetam and PGG at 3.5gm/kg drugs have significant memory enhancement action in MWM test.

Index Terms- Panchagavya ghrita, Elevated plus maze, Morris water maze, Nootropic

I. INTRODUCTION

WHO reported that worldwide 121 million people and 18 million people are suffering from Depression[1] and Alzheimer disease respectively. It is estimated that the number may increase three folds by 2050. Change in life style, peer pressures, stress and ageing are the trigger factors facilitate to alter human cognition. This may leads to develop cognitive disabilities viz Dementia, Depression and Alzheimer diseases. Thus it's a need to prevent the increase percentage of cognitive disorders with certain therapies and medicaments. Conventional therapy permits Nootropic drugs to manage these manifestations and to improve human cognitive abilities. Traditional ayurved system provides a group of herbal products named as Medhya Rasayanas to treat cognitive disabilities. Various herbal dosage forms such as Swaras, kalka, Choorna, Taila, Ghrita and Asav-aristha have been prescribed. Amongst them medicated ghee is the leading dosage form administered to treat different CNS disorders. Panchagavya ghrita [PGG] is one of the medicated ghee formulations, contains five cow products; cow ghee, cow milk, cow urine, cow dung juice and cow curd. Literature revealed that cow ghee, cow milk and cow urine possesses intellect and memory enhancing, rejuvenating and aphrodisiac activities[2, 3, 4], Cow dung juice has antibacterial[5] and cow curd has aphrodisiac[6] activity. Similarly various researches are reported on single cow products for their effects on CNS. Thus combination of these products may show cumulative desired effect of PGG on cognition i.e. improvement of learning and memory. Previously PGG has been assessed for anticonvulsant[7], hepatoprotective[8] and antiepileptic activities[9]; however no work has been carried out on assessment of nootropic activity of PGG. Therefore the present study is planned to assess nootropic activity of PGG using Elevated plus maze and Morris water maze animal models.

AIM:

Assessment of Nootropic activity of *Panchagavya Ghrita* in animal models

OBJECTIVES:

- To manufacture Panchagavya ghrita by following SOP
- To analyze Panchagavya ghrita in laboratory with standard parameters.
- To determine Nootropic activity of PGG by using Elevated plus maze and Morris water maze animal experimental models.

II. MATERIAL AND METHODOLOGY

Study was designed at two levels.
A) Pharmaceutical study
B) Experimental study

A] PHARMACEUTICAL STUDY:

MATERIALS[10]:

Each cow product i.e. Fresh Cow dung juice, Cow urine, Cow ghee, Cow milk and curd was taken 500 ml in quantity.

INSTRUMENTS:

Utensils, gas stove, muslin cloth, measuring cylinder, digital balance etc.

METHODOLOGY

SELECTION OF COW PRODUCTS:

Five cow products were collected from authentic source (Wai, dist Satara), where cows are of brown colored and of Deshi breed, they are provided with organic natural food and cradled in natural environment.

ANALYSIS OF ALL COW PRODUCTS OF PGG:

Organoleptic tests and analysis of all five cow products were done before preparation of Panchagavya ghrita with API parameters in the departmental laboratory and NAFARI laboratory. Values were matched with established standard values[9].

PREPARATION OF PGG:
Panchagavya ghrita was prepared as per follows

(I) PRE STEP[11] -:

1] PREPARATION OF COW DUNG JUICE: Fresh cow dung (750gm) was mixed with equal quantity of water (750ml) and homogeneous solution was prepared. It was then filtered through four fold *muslin cloth* to get clear liquid 600ml cow dung juice was obtained.

2] FILTRATION OF COW URINE: Fresh cow urine was filtered through *muslin cloth* to remove physical impurities. It was brown coloured clear liquid. The weight of cow urine was 500ml.

3] BOILING OF COW MILK: 500ml Cow milk was boiled and used without separating the cream and kept aside for cooling.

4] HEATING OF COW GHEE: 500ml Cow ghee was taken in frying pan and heated on low flame till fumes arise. Then it was allowed for cooling.

(II) MAIN PROCESS[9] -:

1] MIXING OF INGREDIENTS: Cow milk 500ml, cow dung juice 500ml, cow urine 500ml and cow curd was added in cow ghee and mixed thoroughly to form a homogeneous mixture.

2] BOILING: Whole mixture was boiled on low flame and stirred continuously. In process testing of the formulation was done wherein remnant could be easily rolled to form a wick which was not sticky. The foam on the supernatant part of the formulation was disappeared totally. When formulation was subjected to fire, no any crackling noise was observed which indicates total evaporation of water content from the formulation[12]. As the formulation fulfilled all the testing criteria, heat was cut off and formulation was set for cooling.

3] FILTRATION: The formulation was filtered through muslin cloth.

The final yield of the formulation was 90% [910 ml].

4] STORAGE: The formulation was packed in air tight glass container to protect from light and moisture.

ANALYSIS OF PGG:

TABLE: ANALYSIS OF PGG

Parameter	Observation
Sound	No sound after putting on fire
Touch	Oily
Color	Reddish brown
Taste	Sour
Odour	Specific odour of PGG

Parameter	Standard value	Prepared value
Ph	5	5
Specific gravity	0.9057	0.91
Moisture	0.15%	0.13%
Free fatty acid	0.62%	0.60%
Refractive index	42.1	42.2
Saponification value	291.72	290.80
Acid value	2.300	2.298
Wt/ml	0.9gm	0.9gm

Panchagavya ghrita was analyzed with organoleptic and physicochemical tests. It is resulted that obtained values were matched with established standard values.

B] EXPERIMENTAL STUDY:

The experimental protocol was approved by the Institutional Animal Ethical committee of BVDU medical college, Pune. Proposal no. was 1/2013, approval date was 18-feb-2013 and reference was bharati vidyapeeth medical college/1558/2012-13. The study was conducted at CPCSEA approved Central Animal House, Bharati Vidyapeeth University. Standard housing conditions were maintained throughout the study, Rodent pellet Diet & Water filtered through aqua guard ad libitum was provided. Animals were marked by using picric acid on different parts like Head, back, tail and so on. Present study was planned to assess Nootropic activity using two experimental models.

1. **DIAZEPAM INDUCED AMNESIA IN MICE**
2. **MORRIS WATER MAZE TEST**

ANIMALS: Either sex Swiss albino mice 18gm to 30gm and Wistar rats 160 gm to 200 gm were used in the present study.

DRUGS: Cow ghee, PGG and standard drug **[**Piracetam syrup (Nootropil, UCB India pvt. ltd.)] were used in the study. Induction drug used was Diazepam injection (Campose, Ranbaxy, India).

STUDY DESIGN: Swiss albino mice and Wistar rats were randomly distributed into 6 groups each containing 6 animals. Group I served as plain control and received only distilled water, Group II was treated with standard drug [Piracetam], Group III was treated with cow ghee and group IV, V and VI groups of PGG was divided into PGG X/2, PGG X and PGG 2X respectively.

DRUG DOSE CALCULATION OF PGG: In ayurvedic science the recommended drug dose for medicated and cow ghee is 40 gm in human being[13].

Thus in **Diazepam induced amnesia model**, dose was extrapolated[14] for mice and treated as X dose. The study was carried on three dose level of PGG as X/2 (2.5gm/kg), PGG X (5gm/kg) and PGG 2X (10gm/kg). Similarly Cow ghee as vehicle control was administered in X dose (5gm/kg) to animals and induction drug diazepam was used in 1mg/kg by i.p. Similarly positive control group was treated with standard Piracetam drug with 7.8mg/kg dose.

In **Morris water maze model** drug doses were extrapolated[14] for Wistar rats. The study was carried with cow ghee (3.5gm/kg) and with three drug doses of PGG as X/2 (1.75gm/kg), PGG X (3.5gm/kg) and PGG 2X (7.0gm/kg). Group II was treated with standard Piracetam drug [270mg/kg].

METHODOLOGY

MODEL I] DIAZEPAM INDUCED AMNESIA IN MICE USING ELEVATED PLUS MAZE[15, 16]

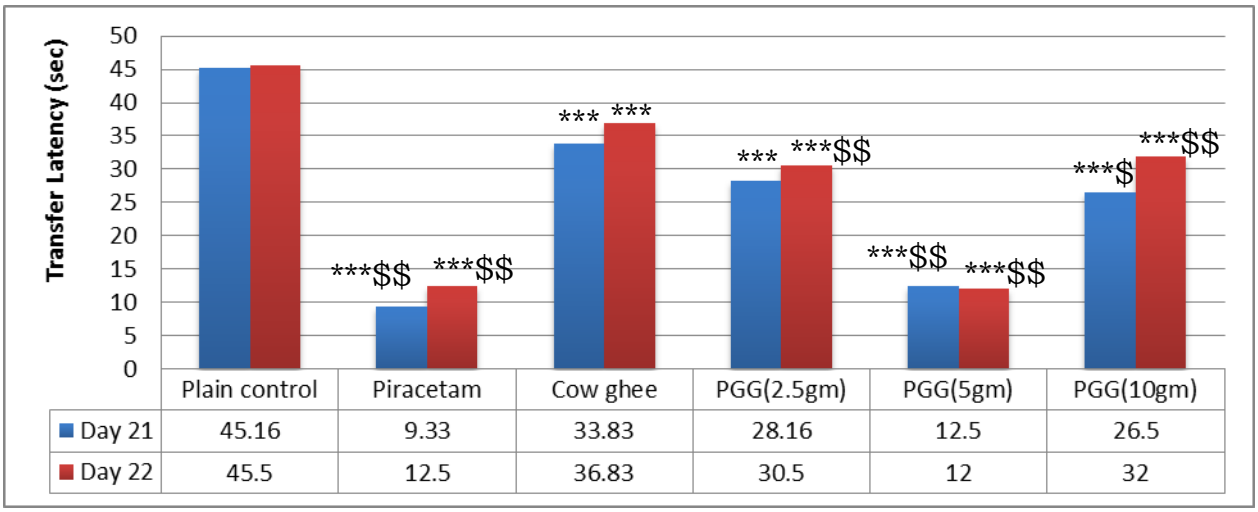
Elevated plus-maze apparatus consists of two open arms (16× 5 cm) and two covered arms (16× 5×12 cm). The arms extended from a central platform (5× 5cm) and the maze was elevated to a height of 25 cm from the floor.

On 0 day screening of all animals was done (animals that enter in one of the closed arms with all its four legs in 90 sec., were included in the study). Grouping of animals was done on same day. After grouping next day onwards, all animals from each group were treated with respective drugs for 21days. On 21st day, after 45 min of administration of the last dose, diazepam (i.p.) was induced to all animals. Transfer latency (TL) of each animal was recorded after 45 mins of administration of diazepam. Further TL was recorded after 24 hrs on 22nd day.

ASSESSMENT PARAMETER: Transfer Latency (TL) - The time taken by mouse to reach into any one of the closed arms with all its four legs.

STATISTICAL ANALYSIS: The data was analyzed using ANOVA followed by Dunnet test.

GRAPH I: THE EFFECT OF PGG ON COGNITION ON 21ST AND 22ND DAY.



	Plain control	Piracetam	Cow ghee	PGG(2.5gm)	PGG(5gm)	PGG(10gm)
Day 21	45.16	9.33	33.83	28.16	12.5	26.5
Day 22	45.5	12.5	36.83	30.5	12	32

*** Indicates plain control group compared with other groups.**

$ Indicates Goghrita control group compared with other groups.

RESULTS: Piracetam, Cow ghee and PGG three doses (2.5gm, 5gm and 10gm/kg) groups showed significant ($p<0.001$) decrease in TL on 21st day and 22nd day, when compared with plain control group. Animals treated with Piracetam and PGG (5gm/kg) showed significant ($p<0.001$) decrease in TL on 21st day and 22nd day when compared with cow ghee. While PGG (2.5gm/kg) and (10gm/kg) groups didn't show significant decrease in TL when compared with cow ghee. As per expected result diazepam treated animals showed increase in TL in plain control group on 21st day.

MODEL II] MORRIS WATER MAZE TEST[17]:

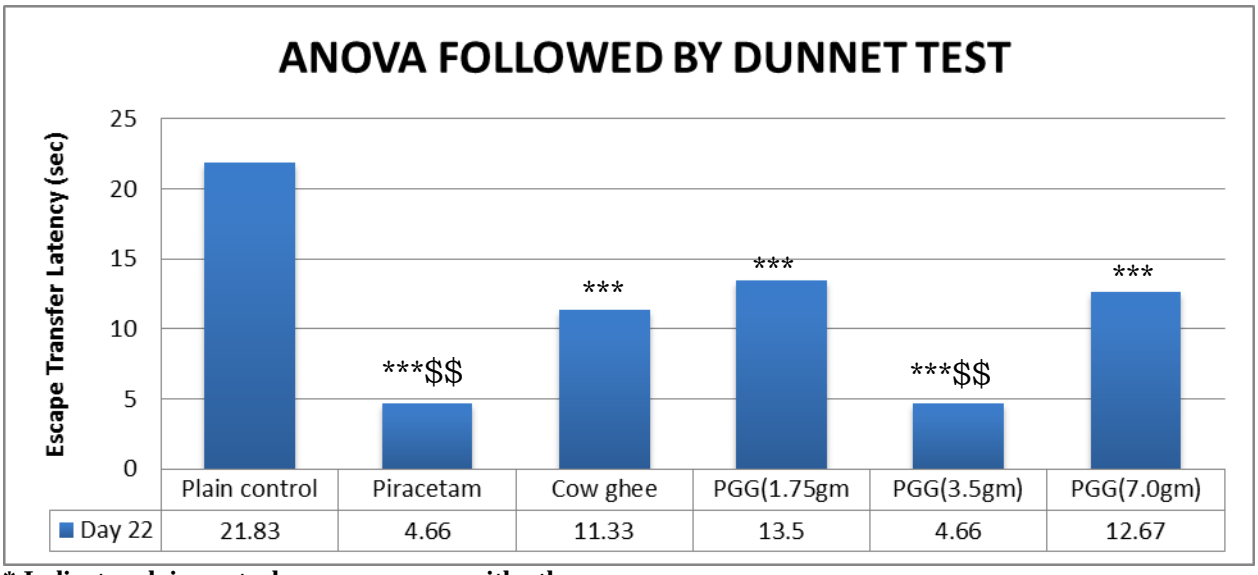
This model is widely used to study spatial learning and memory. It consists of circular water tank, 6 feet diameter and 3 feet in depth which is filled with opaque water at 26^0C to 28^0 C temperatures. Platform is circular in shape with 20 cm in diameter and 1.5 feet in height which was fixed in water tank at one of the quadrant.

Four consecutive learning trials were given to all animals from three different quadrants with platform on 1st, 2nd and 3rd day. On 4th day screening was done without platform using probe test (animals that found the platform in 60 sec., were included in the study). Grouping was done on same day. On the same day animals from each group were treated with respective drugs for 21days. On 22nd day assessment of memory was done of each group in similar way.

ASSESSMENT PARAMETER: Escape transfer latency (ETL) - The time taken by rat to reach the platform site for the first time in 60 sec.

STATISTICAL ANALYSIS: The data was analyzed with Anova followed by Dunnet test.

GRAPH II: ASSESSMENT OF MEMORY ON 22ND DAY.



*** Indicates plain control group compare with other groups.**

$ Indicates Goghrita control group compare with other groups.

III. RESULTS

The data was analyzed through Anova followed by Dunnet test it is resulted that Piracetam, cow ghee and three doses of PGG (1.75gm, 3.5gm and 7.0gm/kg) groups showed significant ($p<0.001$) decrease in ETL on 22nd day, compared with plain control group. Piracetam and PGG (3.5gm/kg) groups showed significant ($p<0.001$) decrease in ETL on 22nd day compared to Cow ghee. While PGG (1.75gm/kg) and (7.0gm/kg) groups didn't show significant decrease in ETL compared to cow ghee.

IV. DISCUSSION

PGG is a medicated ghee formulation prepared from the authentic cow products.

Pharmaceutical study revealed that organoleptic tests and physico-chemical constants of cow products and PGG were alike with the standard established values inferred that PGG was formulated with standard established method and manufacturing process is validated.

In EPM test; amnesia was induced with diazepam (benzodiazepine). The short-term use of benzodiazepines affects multiple areas of cognition and the pharmacological action of diazepam enhances the effect of the neurotransmitter GABA by binding to the benzodiazepine site on the GABA receptor leading to develop amnesia and depression. In present study, plain control group showed increase in TL indicates development of amnesia as expected. While PGG treated groups showed decrease in TL compared to plain negative control ($p\leq0.001$) indicating inhibition of the action of Diazepam. Significant decrease in TL was confirmed in Piracetam drug group ($p\leq0.001$) while significant activity of PGG at 5gm/kg dose ($p\leq0.001$) was also determined compared to cow ghee. PGG at lower dose (2.5gm/kg) and higher dose (10gm/kg) didn't show significant decrease in TL compared to cow ghee. Thus attenuation of amnesia produced by induction of diazepam is resulted in standard (Piracetam) and (5gm/kg) groups. This indicates that both drugs are having learning and memory enhancing activity.

In MWM test acquisition trials were given to animals with platform and then retention of memory was tested without platform after 21 days of treatment. In this study retention of memory was seen in Piracetam and PGG at 3.5gm/kg groups wherein decrease in ETL was resulted than plain and cow ghee control groups. PGG at lower dose (1.35gm/kg) and higher dose (7.0gm/kg) didn't show decrease in ETL compared with cow ghee group ($p\leq0.001$). Animals treated with Piracetam and PGG (3.5gm/kg) dose were able to locate the platform site indicating increase in spatial memory after 21 days of treatment.

In earlier researches (Vernon M.W., 2014(12) etal) Piracetam may act through cholinergic neurotransmission. In the study PGG and Piracetam drugs showed comparable memory enhancement activity in MWM test thus it can be depicted that PGG formulation might be acting through cholinergic neurotransmission.

V. CONCLUSION

It is concluded that Nootropic activity of PGG at (5gm/kg and 3.5gm/kg) is determined in Diazepam induced amnesia and Morris water maze models respectively. With these results, it is interpreted that PGG might be helpful to treat cognitive disabilities such as amnesia, dementia etc and can be used as an adjuvant drug.

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सिद्धं पिबेदपस्मारकामलाज्वरनाशनम् ॥ इति पञ्चगव्यं घृतम् ॥ (च.चि.१०/१७)

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