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Neurobehavioral Evaluation of *Panchagavya Ghrita* Formulations in Rodent Models of Anxiety and Depression

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Abstract

Background: Depression and anxiety are prevalent neuropsychiatric disorders with limitations associated with current pharmacotherapies. *Panchagavya Ghrita* (PGG), an Ayurvedic medicated ghee prepared from five cow-derived products, has been suggested to possess neuropsychological benefits. **Aim:** To compare a ghee-dominant (PGG I) and an equal-proportion preparation (PGG II) regarding physicochemical characteristics, antidepressant and anxiolytic activity in rodents. **Methods:** Antidepressant activity was assessed in Wistar rats exposed to Chronic Unpredictable Mild Stress (CUMS) using sucrose preference, with imipramine as a standard drug (n=8); anxiolytic activity in mice was assessed using the Elevated Plus Maze (EPM) and Open-Field Tests (OFT), with diazepam as a standard drug (n=6 per group). **Results:** Both formulations met quality specifications, with pH 5.24–5.30, moisture ≤0.11%, saponification values 241.6- 244.9, and an identical thin-layer chromatography Retardation factor (Rf) of 0.73. In the EPM, mean time spent in the open arms increased from 99.8 s in controls to 113.8 s with PGG I, 129.0 s with PGG II, and 157.0 s with diazepam ($p < 0.01$). In EPM, significant group differences were observed in open arm entries ($F(3,20) = 19.84$, $p<0.001$) and time spent in open arms ($F(3,20) = 27.21$, $p<0.001$), with both PGG formulations increasing open arm exploration and reducing closed arm activity compared with control. In the OFT, mean central zone activity was 12.60, 10.56, 15.23, and 22.78 squares in the control, PGG I, PGG II, and diazepam groups, respectively. Significant effects were observed for ambulation ($F(3,20) = 6.26$, $p=0.004$), locomotion ($F(3,20) = 22.30$, $p<0.001$) and central square activity ($F(3,20) = 3.53$, $p=0.034$). PGG II showed greater efficacy than PGG I, although the effects were less pronounced than those of standard drugs. **Conclusion**: The findings demonstrate that both formulations of *Panchagavya Ghrita* possess significant anxiolytic and antidepressant-like activities in rodent models, with PGG II (equal-proportion formulation) showing comparatively superior efficacy.

Major Findings: *Panchagavya Ghrita* (PGG) formulations demonstrated significant anxiolytic activity in behavioural models. Both formulations increased open-arm activity and reduced anxiety-related behaviour in the EPM test. OFT results showed improved exploratory behaviour and increased central activity. In the CUMS model, PGG treatment improved depression-like behaviour. PGG II showed comparatively stronger antidepressant and anxiolytic effects than PGG I. The study reports the neurobehavioral potential of PGG formulations.

Keywords: Antidepressant Activity, Anxiolytic Activity, Ayurvedic Formulation, Chronic Unpredictable Mild Stress, *Panchagavya Ghrita*, Rodent Model

Abbreviations: PGG: *Panchagavya Ghrita;* CUMS: Chronic Unpredictable Mild Stress; OFT: Open Field Test; TLC- Thin Layer Chromatography

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1. Introduction

Depression and anxiety are among the most common psychiatric disorders worldwide. They contribute significantly to the global disease burden and pose a major challenge for modern medicine[1]. Depression is a major cause of disability and a common mental health problem globally[2]. In India, studies indicate that

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around 15% of people are reported to have some form of mental health disorder[3]. Among these, depressive disorders show a current prevalence of about 2.7%, while anxiety disorders affect nearly 2.9% of the adult population[4].

Apart from emotional distress, depression and anxiety are associated with reduced daily functioning, lower productivity, medical comorbidities, increased healthcare use, and higher mortality[5]. The National Mental Health Survey (NMHS) 2015–16 identifies a substantial treatment gap, showing that approximately 70–85% of people with common mental disorders are not provided with adequate care[6]. Despite the widespread use of medications that target monoaminergic pathways, their efficacy is sometimes hampered by side effects like slow onset, insufficient symptom alleviation, treatment resistance, and adverse effects that lower long-term adherence[7].

Given these limitations, increasing attention has turned toward traditional medicine systems. Ayurveda offers several psychoactive and rejuvenating formulations for treating psychiatric disorders[7]. Among these, PGG is repeatedly referenced in classical texts for its potential benefits in central nervous system disorders. PGG is a medicated ghee prepared using five cow products, namely curd, cow dung juice, urine, ghee, and milk[8].

Experimental studies have reported the antiepileptic[9], antidepressant[10], nootropic[11], hepatoprotective[12], antioxidant, and memory-enhancing effects of PGG and related cow product formulations[13]. A pre-clinical study found that PGG significantly delayed pentylenetetrazole-induced seizures, protected against tonic-clonic episodes, reduced oxidative stress, and improved cognition in rats[14]. Another investigation showed that PGG potentiated the effects of standard antiepileptic drugs without altering their serum levels, suggesting a possible synergistic pharmacodynamic interaction[15]. In addition to characterising *ghrita*-based preparations that increase the bioavailability of lipophilic substances across the blood-brain barrier, reports also discuss the bioactive contents of its components, particularly their immunomodulatory and antioxidant qualities[16–18]. Despite this, methodical studies using standardised PGG formulations, particularly in mood disorders, are still limited.

One important but relatively underexplored factor remains: No work has been reported on the comparison of PGG formulations prepared with different concentrations of cow products and their antidepressant and anxiolytic activities in animal models. The classical Ayurvedic literature describes the preparation of PGG using the five cow products in equal proportions, in a 1:1:1:1:1 ratio (PGG II)[19]. Earlier studies followed this approach, wherein cow dung juice was normally prepared by homogenising fresh dung in water[20]. However, in the opinion of specialists, PGG should be prepared using a *Ghrita* quantity equal to the combination of four cow products quantity (PGG I). Since ghee itself represents a lipid-based carrier that enhances the Central Nervous System (CNS) delivery of active constituents[21], such differences in approach may significantly alter the efficacy of PGG. This study was designed to compare the antidepressant and anxiolytic effects of PGG I and PGG II.

2. Materials and Methods

2.1 Ethical Considerations

Animal studies were performed following the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India, with prior approval from the Institutional Animal Ethics Committee (BiRD/ IAEC/ 2020/21/20, Dated, 21.03.2021).

2.2 Materials

The raw cow-derived ingredients used for the manufacture of PGG were cow dung juice (*Goshakruta Rasa*), sour curd (*Godadhi*), cow milk (*Gokshira*), cow urine (*Gomutra*), and cow ghee (*Goghrita*). They were sourced from authenticated local dairy sources. All cow products were sourced from disease-free, indigenous *Bos indicus* cows maintained under controlled husbandry conditions. Cow dung and urine were collected fresh from the same animals, while milk, curd, and ghee were sourced from untreated milk supplied by the same herd to ensure biological uniformity of the samples. Authentication of each ingredient was done by routine laboratory analysis based on the standards described in the Ayurvedic Pharmacopoeia of India (API)[20].

2.3 Methodology

2.3.1 Preparation of PGG Formulations

The formulations were prepared according to the standard *Snehpak-kalpana* procedures described in the API. The classical Ayurvedic tests for confirmation of the completion of heating during *ghrita* preparation include the wick test, foam disappearance, noise test, and specific colour and smell test.

2.3.1.1 Cow Dung Juice Preparation

Fresh cow dung and water were mixed in a 1:1 proportion and homogenised well[20]. The mixture was filtered through a fourfold muslin cloth to get a clear solution. The resultant extract was presented as a dark, turbid liquid with fine suspended particles.

2.3.1.2 PGG I (Ghee-Dominant Formulation)

For PGG I, the volumes of cow dung juice, curd, milk, and urine were each measured separately, and ghee was added in a volume equal to the total volume of those four components. First, the four non-ghee components were mixed and stirred well to make a uniform aqueous phase before adding the ghee. If the volume of each component was X mL, for instance, the volume of ghee added would be 4X mL.

The mixture was heated on a low flame for[22], and the process was continued till the formulation reached classical *ghrita*, characterised by the disappearance of froth, development of a characteristic aroma, and attainment of consistency. Finally, the formulation was cooled and stored in airtight glass containers.

2.3.1.3 PGG II (Equal-Proportion Formulation)

In general, PGG II was prepared by mixing cow dung juice, curd, milk, urine, and ghee in equal proportions. The only difference in its preparation from PGG I is that the proportion of cow ghee was taken in equal quantity to the other four cow-derived products as per API guidelines. The volume of each component was X ml for PGG II. It was heated gently till classical Ayurvedic end-points indicative of the formation of *ghrita* were attained. The formulation was then allowed to cool, after which it was gently decanted and filtered, if needed, to remove coarse particulate matter to get a clear medicated *ghrita*.

2.3.1.4 Physico-chemical Evaluation of Formulations

Both formulations were subsequently subjected to complete physicochemical analysis as per API and Food Safety and Standards Authority of India (FSSAI) methodologies. Ayurvedic quality assessments in the

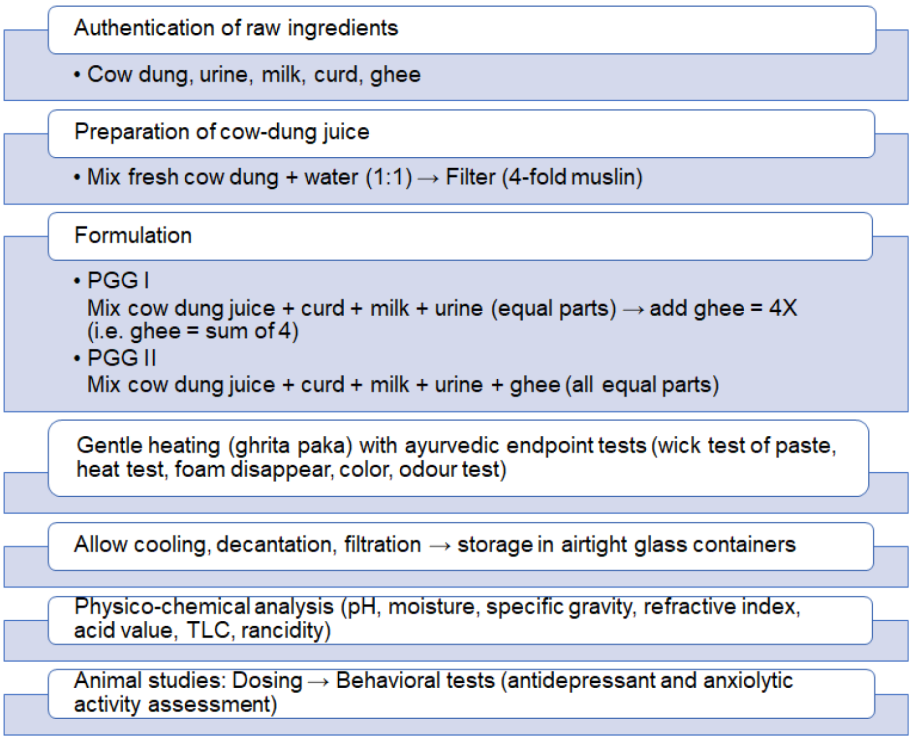


Figure 1. Study design.

form of *Rasa Ashtaka Pariksha* (colour, odour, taste, touch, and clarity) were also conducted to ensure that the *ghrita* met traditional standards.

The analytical tests conducted were organoleptic evaluation, pH determination, moisture content, specific gravity, refractive index, butyro-refractometer reading, acid value, per cent free fatty acids, saponification value, iodine value, alcohol-soluble and hexane-soluble extractive values, Thin Layer Chromatography (TLC), and rancidity test. Figure 1 summarises the methodology for this study.

2.3.1.5 Animal Study Protocol

The CUMS model was used to determine antidepressant activity, whereas the EPM and OFT were employed for assessing anxiolytic activity. A total of 40 adult Wistar albino rats (180- 250 g) were used for the antidepressant study (CUMS model), with 8 rats per group in five groups. For the anxiolytic studies (EPM and OFT), 24 adult Swiss albino mice (22- 25 g) were used, with 6 mice per group across four groups. Animals were kept under controlled conditions at about 24 ± 1 °C, with 45-55% relative humidity and a 12:12 h light–dark schedule, and were provided free access to standard chow and water.

2.3.1.6 Dosing and Drug Administration

The human therapeutic dose of *ghrita* (40g/70kg) was converted to animal doses using body surface area normalisation[22]. The resulting doses were 3.6g/kg for rats and 5g/kg for mice[23]. Normal saline served as the vehicle control and was administered at 1 mL/kg orally in rats and 10 mL/kg orally in mice. The standard drugs used were: Imipramine (10 mg/kg, oral) for antidepressant activity[24] and Diazepam (1 mg/kg, intraperitoneal) for anxiolytic activity[25,26].

For the CUMS study, rats were administered their respective treatments daily for 28 days in parallel with the stress induction protocol. For the EPM and OFT studies, mice were treated once daily for 7 days before behavioural evaluation. All test formulations and vehicles were administered orally. Adult healthy male Wistar rats were used for the antidepressant study, and adult healthy male Swiss albino mice were used for the anxiolytic study.

2.3.1.7 Experimental Design and Grouping

2.3.1.7.1 EPM and OFT Studies in Mice (n = 6 per group)

Animals were randomised into 4 groups as follows: Normal saline, PGG I, PGG II, and Diazepam. Normal saline was administered as a 10mL/kg dose orally. PGG I and PGG II were given orally at doses of 5g/kg each, once daily for 7 days. Diazepam was administered intraperitoneally 30 minutes before behavioural testing, whereas PGG formulations and vehicle were administered orally once daily according to the experimental protocol before behavioural assessment. All behavioural assessments were performed during the same period of the light cycle to minimise circadian variation and ensure experimental consistency. Animals were randomly allocated into experimental groups using a randomisation procedure before initiation of treatment. Furthermore, investigators performing behavioural scoring and outcome assessments were blinded to treatment allocation throughout the study.

2.3.1.7.2 CUMS Study in Rats (n = 8 per group)

Animals were randomised into 5 groups as follows: Normal control (vehicle only), CUMS control (vehicle + CUMS), Imipramine + CUMS, PGG I + CUMS, and PGG II + CUMS. Behavioural scoring and biochemical analyses were performed by investigators blinded to treatment allocation. Normal saline was administered at a 1mL/kg dose orally to the normal control and CUMS control groups. Imipramine was given at a 10mg/kg dose. PGG I and PGG II were administered at doses of 3.6g/kg each, once daily for 28 days.

2.3.2 Behavioural Assessments

2.3.2.1 Sucrose Preference Test

Anhedonia was assessed using a two-bottle choice test. The animals were given a 1% sucrose solution for 24 hours to get them used to it, and during the next 24 hours, they had access to both the sucrose solution and water. After a 24-h deprivation period, sucrose preference was measured over a 3-h test period. The preference percentage was calculated as:

$$Sucrose\ \Pr eference(\%) = \frac{Sucrose\ \text{int}ake}{Sucrose\ \text{int}ake + Water\ \text{int}ake} \times 100 \quad (1)$$

2.3.2.2 Chronic Unpredictable Mild Stress (CUMS) Protocol

The CUMS protocol was applied for 29 days (Day 8–36) and involved the random presentation of mild stressors so that animals could not predict their occurrence[24]. The stress protocol included placing an empty water bottle for 1 hour, overnight illumination, 24 hours of

social isolation, and deprivation of food and water for 24 hours. Additional stressors involved inescapable electric shocks (1.5 mA; 15 s on, 150 s off), cold-water swimming at 4°C, wet bedding for 24 hours, tail pinch for 10 minutes, high-density housing for 24 hours, and tilting the cage at a 45° angle.

2.3.2.3 Elevated Plus Maze

The apparatus consisted of two open arms (50 × 10cm) and 2 closed arms (50 × 10 × 40cm) extending from a central platform (10 × 10cm), raised 50cm above the floor. Each animal was placed on the central platform facing an open arm and observed for 5 minutes. The number of entries into the open and closed arms, as well as the time spent in each arm, was recorded. An increase in the percentage of open-arm entries and time spent in the open arms was considered indicative of anxiolytic activity. The maze was cleaned with 70% ethanol between trials to eliminate olfactory cues.

2.3.2.4 Open Field Test (OFT)

The apparatus consisted of a square area (68 × 68 × 45cm) divided into 16 equal squares and illuminated with a 40-W bulb, and was used to study locomotor activity and anxiety-like behaviour. Each mouse was placed gently in the centre of the area and allowed to explore for 5 minutes. The total movement, ambulation, and time spent in the central squares were noted. Increased locomotor activity and time spent in the centre of the arena, along with reduced grooming behaviour, were considered indicators of anxiolytic activity. The apparatus was cleaned with 70% ethanol between observations to prevent interference from odour cues.

2.3.3 Statistical Analysis

Results are presented as mean ± Standard Error (SE). All the statistical analyses were performed using the SPSS/PC+ package (Version 20, Chicago, IL). Statistical differences between means were determined using One-Way Analysis of Variance (ANOVA) followed by the Fisher Least Significant Difference (LSD) test. A p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1 Physico-chemical Properties

The physico-chemical values for raw materials and for PGG I and PGG II are listed in Table1. Minor differences were observed between PGG I and PGG II in specific gravity, acid value, free fatty acids, and

Table 1. Physico-chemical characteristics of raw materials and *Panchagavya Ghrita* samples

Parameter	Cow Curd	Cow Milk	Cow Ghee	Cow Urine	Cow Dung Juice	PGG I	PGG II
pH	3.71	7.10	4.45	8.36	6.50	5.30	5.24
Moisture (%)	86.95	87.74	0.15	94.98	97.54	0.05	0.11
Protein (%)	3.35	3.79	–	–	–	–	–
Fat (%)	4.30	3.65	–	–	–	–	–
Total Solids (%)	13.05	12.26	–	5.02	2.46	–	–
Specific Gravity	1.0254	1.0368	0.9100	1.0401	1.0253	0.9198	0.9296
Butyro-refractometer (40 °C)	–	–	41.1 (ghee)	–	–	41.5	41.5
Refractive Index (40 °C)	–	–	1.4532	–	–	1.4535	1.4535
Weight per mL (g/cm^3)	–	–	0.9071	–	–	0.9142	0.9240
Acid Value	–	–	1.03	–	–	1.27	1.02
Free Fatty Acids (%)	–	–	0.52	–	–	0.64	0.51
Saponification Value	–	–	242.2	–	–	244.9	241.6
Iodine value	–	–	29.8	–	–	30.4	30.8
Rf (TLC)	–	–	0.73	–	–	0.73	0.73
Rancidity	–	–	Absent	–	–	Absent	Absent

PGG - *Panchagavya Ghrita*, TLC- Thin layer chromatography

iodine value, although both formulations met standard quality criteria for medicated *Ghrita*.

3.2 CUMS Sucrose Preference Test in Wistar rats

3.2.1 Sucrose Preference (Index of anhedonia)

Sucrose preference index (%) before and after the CUMS protocol is shown in Table 2. The values in the figure indicate the absolute change [After CUMS (%)-Before CUMS (%)].

All groups showed a decline in sucrose preference after exposure to the CUMS protocol. The largest absolute declines were observed in the Standard and Test groups. The Control group also showed a decline, but of smaller magnitude.

3.3 Elevated Plus Maze in Mice

One-way ANOVA revealed significant treatment effects across all behavioural parameters assessed. In the EPM, significant differences were observed for number of open arm entries [$F(3,20) = 19.84$, $p < 0.001$], time spent in open arms [$F(3,20) = 27.22$, $p < 0.001$], number of closed arm entries [$F(3,20) = 4.04$, $p = 0.021$], and time spent in closed arms [$F(3,20) = 115.49$, $p < 0.001$] (Table 3).

Both PGG I ($p < 0.05$) and PGG II ($p < 0.01$) increased the number of open-arm entries and the time spent in open arms compared to the control group. Diazepam (a positive control) produced the largest increase in open-arm parameters relative to control ($p<0.01$). Additionally, PGG II significantly increased the duration spent in open arms compared to control ($p< 0.05$) (Figure 2).

For closed-arm behaviour, both PGG I showed reductions in entries ($p< 0.05$) and time spent ($p < 0.01$) in closed arms compared to control. Diazepam significantly decreased both closed-arm entries and time relative to control ($p<0.001$). Furthermore, PGG II exhibited a significantly lower closed-arm time than PGG I ($p < 0.05$) (Table 2).

3.4 Open Field Test in Mice

OFT parameters showed significant group effects for peripheral ambulation [$F(3,20) = 6.26$, $p = 0.004$], total locomotion [$F(3,20) = 22.3$, $p < 0.001$], and central zone activity [$F(3,20) = 3.53$, $p = 0.034$] (Table 3).

Compared with Control, PGG I ($p<0.05$) and PGG II ($p<0.01$) show reductions in periphery ambulation. Diazepam caused a marked reduction in ambulation compared to control ($p<0.01$), as well as PGG I($p<0.05$). No significant difference was found between PGG II and Diazepam. Total locomotor activity was significantly reduced in the PGG II group compared to the control ($p < 0.05$). Diazepam also decreased total locomotion relative to the control, PGG I, and PGG II groups ($p < 0.01$ for all). There was a trend for lower locomotor activity ($p = 0.065$) in the PGG II group compared to the PGG I group (Figure 3).

PGG I and II groups did not alter central activity compared to the control. Diazepam produced higher

Table 2. Sucrose preference test before Chronic Unpredictable Mild Stress (CUMS) induction

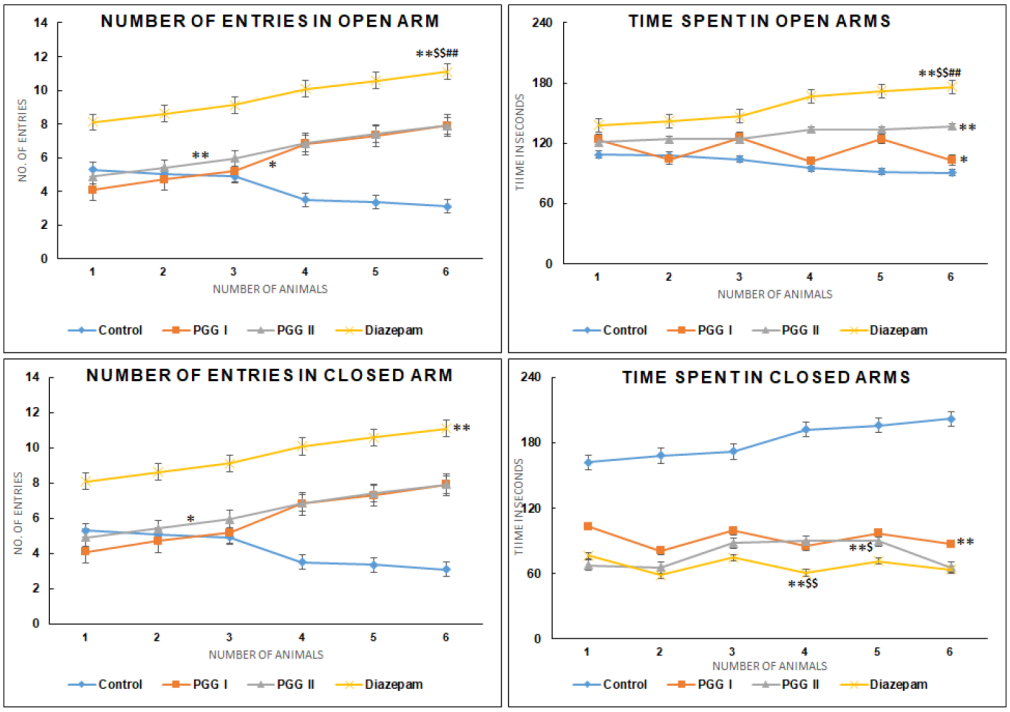
	Control	Standard	PGGI+CUMS	PGG II+CUMS
Sucrose preference test before CUMS				
Sucrose	85	80	85	85
Water	25	30	35	30
Index of Anhedonia (%)	77.27	72.72	70.53	73.91
Sucrose preference test after CUMS				
Sucrose	80	45	50	55
Water	40	55	60	60
Index of Anhedonia (%)	66.66	45	45.45	47.82
Index before and after CUMS (%)				
Index (%)	-10.61%	-27.72%	-25.08%	-26.09%

Data represented as %
PGG = *Panchagavya Ghrita*.

Table 3. One-way ANOVA results for behavioural parameters in rodent models of antidepressant and anxiolytic activity

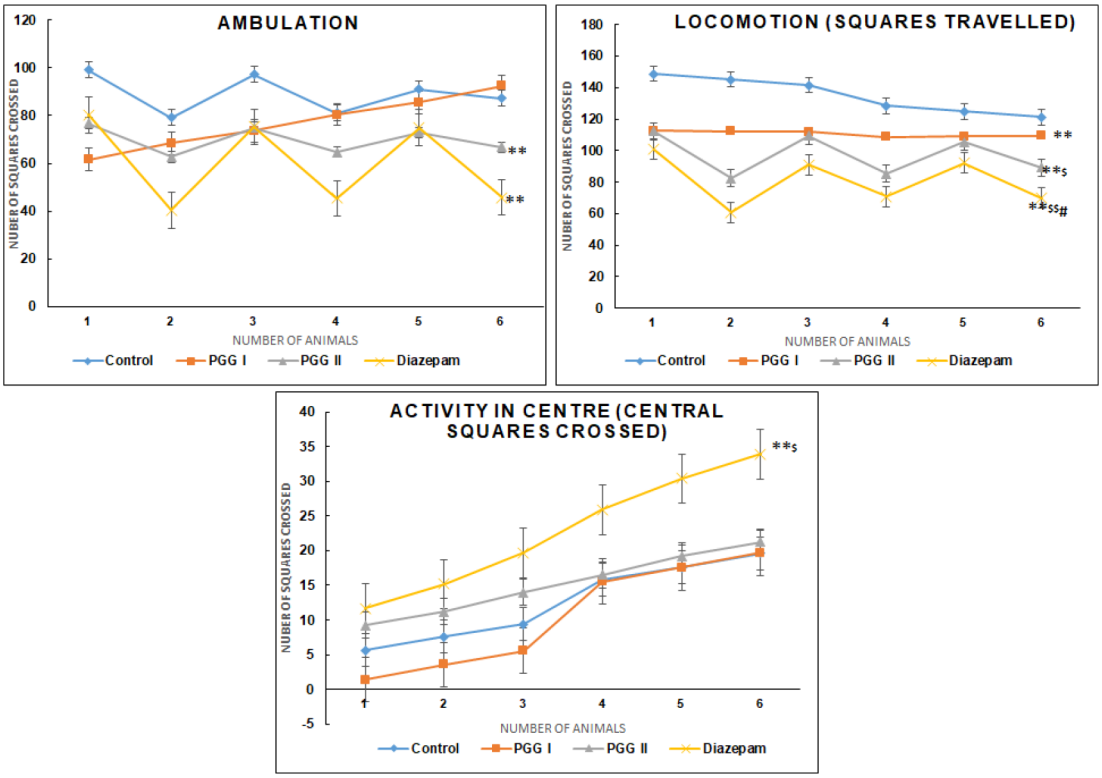
Parameters		Sum of Squares	Degrees of freedom (df)	Mean Square	F	Significance
No. of entries in open arm	Between Groups	90.900	3	30.300	19.840	.000
	Within Groups	30.544	20	1.527		
	Total	121.444	23			
Time spent in open arm	Between Groups	10803.282	3	3601.094	27.215	.000
	Within Groups	2646.413	20	132.321		
	Total	13449.695	23			
No. of entries in closed arm	Between Groups	50.160	3	16.720	4.038	.021
	Within Groups	82.815	20	4.141		
	Total	132.975	23			
Time spent in closed arm	Between Groups	49388.400	3	16462.800	115.485	.000
	Within Groups	2851.064	20	142.553		
	Total	52239.464	23			
Ambulation (periphery squares crossed)	Between Groups	2652.024	3	884.008	6.262	.004
	Within Groups	2823.537	20	141.177		
	Total	5475.561	23			
Locomotion	Between Groups	9388.447	3	3129.482	22.300	.000
	Within Groups	2806.699	20	140.335		
	Total	12195.146	23			
Activity in centre (central squares crossed)	Between Groups	514.276	3	171.425	3.527	.034
	Within Groups	972.023	20	48.601		
	Total	1486.299	23			

Data were analysed using ANOVA.



Data are shown as mean ± SEM. Data were analysed using ANOVA and the LSD test. Significance: $^{*}p < 0.05$, $^{**}p < 0.01$ versus control; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus PGG I; $^{\#}p < 0.05$ versus PGG II. PGG = *Panchagavya Ghrita*.

Figure 2. Elevated plus maze test in different groups.



Data are shown as mean ± SEM. Significance indicators: $^{*}p < 0.05$, $^{**}p < 0.01$ versus control; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ versus PGG I; $^{\#}p < 0.05$ versus PGG II. PGG = *Panchagavya Ghrita*.

Figure 3. Open field test in different groups.

central activity compared to the PGG I group ($p < 0.01$), with no difference between Diazepam and PGG II groups.

4. Discussion

This study investigated the physicochemical parameters and behavioural properties of the PGG formulations. PGG I is predominantly a ghee-based preparation, and PGG II is the one with an equal ratio of all five ingredients. These formulations were assessed for their antidepressant and anxiolytic properties using standardised models of induced stress, depression, and anxiety. The findings indicate that both formulations meet acceptable pharmaceutical standards and that they do exhibit definite antidepressant and anxiolytic properties.

The physicochemical analysis showed that both formulations satisfied the pharmacopeial standards prescribed for medicated *ghrita* preparations. The pH values ranged between 5.24 and 5.30, the moisture content was extremely low (0.05–0.11%), and no signs of rancidity were detected, indicating good thermal stability and minimal risk of lipid degradation. These findings are in line with the standards described in the API[20] and are comparable with previously reported profiles of PGG[19]. All samples showed a consistent TLC Rf value of 0.73, suggesting uniform lipid composition. Minor differences in specific gravity, acid value, and free fatty acid content between PGG I and PGG II are expected due to variations in ingredient proportions, yet all values remained within acceptable pharmaceutical limits[12].

The EPM was used to assess anxiolytic-like behaviour by measuring the number of entries and time spent in the open and closed arms. Compared to the control group, animals treated with PGG and PGG II showed a significant increase in the number of entries into the open arms and time spent in the open arms, along with a corresponding reduction in closed arm entries and time spent in closed arms. These behavioural changes indicate a reduction in anxiety-like behaviour with *Ghrita*. The diazepam-treated group exhibited the maximum increase in open

arm exploration and the largest decrease in closed arm activity, confirming the sensitivity and validity of the EPM model. When the two test formulations were compared, PGG II consistently produced greater open arm entries and longer open arm residence time than PGG I, indicating a stronger anxiolytic-like effect. Similar behavioural effects have been reported earlier for herbal and *ghrita*-based preparations evaluated in stress-related and neurobehavioural models[16].

The open field test was employed to evaluate locomotor activity and anxiety-related behaviour by measuring ambulation, total locomotion and activity in the central zone. Compared to the control group, animals treated with PGG I and PGG II showed a reduction in ambulation and total locomotion, indicating a decrease in peripheral exploratory activity. This reduction was more pronounced in the diazepam-treated group, consistent with its known sedative and anxiolytic effects. Despite reduced overall locomotion, both formulations produced a marked increase in activity in the central squares compared with the control group. Increased central activity is considered an index of reduced anxiety as rodents naturally avoid the centre of an open arena. Among the two formulations, PGG II elicited greater central zone exploration than PGG I, suggesting comparatively stronger anxiolytic-like activity. The ability of PGG II to reduce anxiety without marked motor suppression suggests a more favourable anxiolytic profile than benzodiazepines, which often cause sedation at therapeutic doses. This behavioural effect is consistent with earlier evidence indicating that *Panchagavya*-based formulations may exert neuromodulatory or adaptogenic effects without impairing motor function[13,17,18]. This enhanced performance may be attributed to its balanced formulation, which could support improved solubilisation, transport, and metabolic availability of bioactive components. A ghee-rich formulation, PGG I, may deliver higher concentrations of lipophilic constituents to the brain compared to the traditional equal ratio formulation, PGG II, with potentially enhanced antidepressant or anxiolytic effects.

In the CUMS model, a reduction in sucrose preference was observed in all groups, including unstressed controls, confirming both the sensitivity of the test and the effectiveness of the stress-induction protocol. Similar reductions in sucrose preference have been widely reported in chronic stress studies used for antidepressant screening[10]. The decrease was more pronounced in the imipramine-treated and test formulation groups; A pattern commonly observed in CUMS models, where antidepressants administered during ongoing stress do not fully prevent early anhedonic responses. Instead, their effects become more evident in subsequent behavioural tests that assess motivation and active coping[24]. CUMS exposure significantly reduced wheel rotations relative to normal animals, indicating the development of depression-like behavioural despair. Imipramine markedly reversed this effect, which is consistent with its well-established antidepressant activity[24]. Both PGG I and PGG II also significantly increased wheel rotations relative to the CUMS control group, indicating antidepressant like effects of *Ghrita*. Among the two, PGG II consistently produced higher rotation counts than PGG I, suggesting comparatively greater antidepressant efficacy. However, the magnitude of improvement remained lower than that observed with imipramine. These findings are in agreement with previous reports demonstrating neuroprotective, antioxidant, and CNS-modulating effects of *Panchagavya*-based formulations in models of stress, cognition, and seizures[9, 13–15]. The comparatively stronger effect of PGG II suggests that its balanced composition may facilitate better synergistic action or enhanced bioavailability of active constituents.

The antidepressant and anxiolytic effects of PGG may be attributed to its rich lipid matrix, particularly omega-3 polyunsaturated fatty acids from cow ghee and milk, which are known to modulate neurotransmitter systems, reduce neuroinflammation, and regulate the hypothalamic-pituitary-adrenal axis involved in stress responses[27]. Additionally, milk-derived proteins and amino acids such as tryptophan may enhance serotonergic signalling, while antioxidant and neuroprotective components reported in *Panchagavya* constituents may mitigate oxidative stress associated with depression and anxiety[9,16]. The superior efficacy of PGG II may result from improved synergistic interactions and bioavailability of these bioactive components due to its balanced formulation.

The findings of the experimental models demonstrate that both PGG formulations exhibit antidepressant- and anxiolytic-like effects, but PGG II shows superior efficacy across multiple behavioural parameters. The combination of good physicochemical stability and

significant behavioural efficacy supports the potential of PGG, particularly PGG II, for further investigation in stress-related and affective disorders. Further studies are required to clarify the underlying neurochemical mechanisms, assess oxidative stress parameters, and understand pharmacokinetic behaviour. Additional work on dose optimisation, standardised toxicology, and safety evaluation will also be necessary before considering clinical application.

5. Conclusion

This comparative study shows that both PGG I and PGG II produce antidepressant- and anxiolytic-like effects in rodent models. Physicochemical testing confirmed that both formulations are stable and chemically consistent. In behavioural studies, both formulations improved behaviour in the following CUMS exposure and increased open-arm exploration in the EPM test. PGG II consistently demonstrated greater behavioural improvement than PGG I, including higher wheel rotations and increased central zone exploration in the OFT. These findings suggest that the equal-proportion formulation (PGG II) may possess comparatively enhanced neurobehavioral activity under the experimental conditions employed. However, further studies investigating the underlying molecular mechanisms, biochemical markers, toxicity profile, and clinical efficacy are warranted before translating these findings to human applications.

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Author Contribution

Amit Raghunath Pawar: Literature review, data curation, manuscript drafting, writing-original draft; validation, final approval of the version to be published; Madhuri Sadashiv Pawar: Conceptualisation, Validation, Supervision, critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript.