ORIGINAL RESEARCH ARTICLE: EXPERIMENTAL STUDY

COMPARATIVE ASSESSMENT OF ANTIDEPRESSANT ACTION OF BRAHMI GHRITA PREPARED WITH FRESH AND TEN YEARS OLD COW GHEE ON CUMS ANIMAL MODEL

BHAVANA MENKUDLE1 MADHURI SADASHIV PAWAR2* JAYASHREE DAWANE3

ABSTRACT

Background: In traditional ayurved science, BrahmiGhrita [BG] is prescribed in the management of Unmada [depressive disorder]. It comprises of Brahmi, Ela and Puranghrita (10 years old clarified butter). However, generally Fresh Goghrita [Cow ghee] is utilized to prepare medicated ghee formulations. Till date antidepressant activity of BG in stress induced depression is not yet documented. Aims: Thus present study was conducted to assess antidepressant activity of two samples of BrahmiGhritaviz BG I and BG II in Chronic Unpredictable Mild Stress (CUMS) animal model.

Methods and material: Puran Ghrita, fresh Goghrita, BG I and BG II were administered orally at therapeutic dose [3.6gm/kg] along with application of specified stressors for 28 days. Animals were tested before and after treatment with Sucrose Preference and Force Swim test [FST]. Statistical test applied: ANOVA followed by Tuke’s test. Results: CUMS exposed animals showed depressive like behavior, with significant decrease in sucrose consumption and number of rotations in FST. Animals from standard drug [Imiprime], BG I and BG II have shown significant increase in rotations in FST [p≤0.0001] as compared to CUMS group. BG I and BG II demonstrated significant behavioral changes in animals as compared to Puranghrita and fresh Goghrita respectively [p≤0.05]. Despite that, the role of Puran Ghrita in preparation of Brahmi Ghrita couldn’t be evaluated as against Brahmi Ghrita of fresh Goghrita in CUMS induced depressive rats.

Conclusions: BrahmiGhrita prepared from ten years old and fresh cow ghee have confirmed antidepressant like effect in CUMS study.

Key Words: Brahmi[ SAT-F.449], PuranGhrita, BrahmiGhrita, antidepressant activity, CUMS

1 PG scholar, 2Associate Professor, Dept. of Rasashastra and Bhaishajyakalpana, Bharati Vidyappeth [deemed to be university] college of Ayurved, Pune (INDIA)

3 Associate Professor of Dept. of Pharmacology, Medical College, BVDU, Pune (INDIA)

Corresponding Email id: drdalvimadhu08@gmail.com Access this article online: www.jahm.in

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INTRODUCTION
Change in lifestyle and work pattern, excess commitments and peer pressures encounter stressful life in healthy person. Acute and chronic stress plays an important role to develop fatigue, obsession, anxiety and depression. The manifestations for these disorders comprise sadness, guilt, physical and mental slowing, psychomotor retardation, loss of interest, pleasure and self-destructive ideations [1].

Traditional ayurvedic science has given immense importance to use various medicinal plants to treat depressive like symptoms. Medicated ghee formulations are the drug of choices to treat such conditions. Herbal drugs are processed with lipid bases for selective extraction of potent bio-components in these formulations.

Brahmi Ghrita [BG] a medicated ghee formulation comprises of Brahmi[ Bacopa monnieri], Ela [Elettaria cardamomum] and Puran Ghrita [10 years old clarified butter] which is prescribed to treat depressive disorders [EM-1][2]. Brahmi, a medicinal herb revealed to have therapeutic potential to treat various mental disorders [3]. Anti-stress, anti-oxidant, anti-depressant and cognitive enhancement activities of Brahmi were reported in previous studies [4,5,6,7]. Ela has also been reported to possess anti-oxidant [8], anti-depressant [9] and cognitive enhancement actions [10]. Puran ghritas indicated to treat psychosis, epilepsy, unconsciousness and has cognitive enhancer action [11]. Hence it is hypothesized that the combination of Brahmi, Ela and Puran ghrita in preparation of BG might be useful to produce antidepressant action. Conversely, in routine pharmaceutical practice fresh Goghrita[Cow ghee] is used to prepare medicated ghee formulations. Thus, present study was planned to prepare two samples of Brahmi Ghrita [BGI and BGII] by using Puran Ghrita and fresh Goghrita. Till date no any scientific data for beneficial effects of Brahmi Ghrita of such combinations is available. Therefore the attempt was made to find out and compare the action of two formulations of Brahmiin CUMS induced depressive animals.

MATERIALS AND METHODS [Study Design]

A] Pharmaceuticals of Brahmi Ghrita

B] CUMS experimental study: Assessment of antidepressant effect of BGI and BGII

Preparation of Brahmi Ghrita [BG I and BG II]:
BG I: Brahmi (Bacopa monniera), Ela (Elettaria cardamomum), Puran Goghrita, potable water
BGII: Brahmi (Bacopa monniera), Ela (Elettaria cardamomum), Fresh Goghrita, potable water

Procedure: Soft paste of fresh plant [SAT-F.165] Brahmi [70gm] and fine powder of Ela [30gm] were mixed together to form a homogeneous mass. 400gm Puran ghrita [SAT-F.246] was heated on low flame till fumes arise and then it was allowed to cool. Soft and fine paste of
herbal drugs [100gm] and potable water [1600 ml] was added to Puran Ghrita and mixed appropriately. That mixture was heated on low flame till total water content gets evaporated and extraction of bio-components of herbal drugs in lipid base was attained. The prepared formulation [BG I] was filtered and stored in airtight containers. Similar protocol was followed to prepare BG II wherein instead of ten year clarified butter, fresh cow ghee was used. Both formulations were further analyzed for organoleptic and physico-chemical tests. The estimation of BGI and BGII is given in Table 1.

Table 1: Organoleptic and Physico-chemical analysis of BG I and BG II

<table>
<thead>
<tr>
<th>Tests</th>
<th>BGI Description</th>
<th>BGII Description</th>
<th>Parameters</th>
<th>BGI Value</th>
<th>BGII Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Touch</td>
<td>Oily</td>
<td>Oily</td>
<td>Specific gravity</td>
<td>0.639</td>
<td>0.948</td>
</tr>
<tr>
<td>Colour</td>
<td>Dark Yellow</td>
<td>Yellowish + brown</td>
<td>Moisture Content</td>
<td>0.18</td>
<td>0.12</td>
</tr>
<tr>
<td>Taste</td>
<td>Astringent, Pungent</td>
<td>Bitter</td>
<td>pH</td>
<td>6.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Odour</td>
<td>Strong Characteristic</td>
<td>Sweet fragrance</td>
<td>Refractive Index</td>
<td>1.4537</td>
<td>1.4529</td>
</tr>
<tr>
<td>Appearance</td>
<td>Clear oil</td>
<td>Clear oil</td>
<td>Acid Value</td>
<td>1.43</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Iodine Value</td>
<td>34.28</td>
<td>34.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saponification Value</td>
<td>227.82</td>
<td>228.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peroxide Value</td>
<td>7.10</td>
<td>7.35</td>
</tr>
</tbody>
</table>

Experimental study: CUMS study was followed as described by Kumar B et al., 2010 and Lin Zang et al., 2014, with minor modifications[12].

Animals: Wistar rats of either sex having average weight of 180-250g were used for the study. Housing was done in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines in standard cages. The animals were given food and water ad libitum and were exposed to 12 hours light and dark cycle. Study started after obtaining the approval (IAEC/BVDUMC/3301/2016/015/002) from Institutional Animal Ethics Committee of the Bharati Vidyapeeth University Medical College, Pune, India.

Treatment drugs:
BGI, BGII, Puran Ghrita, fresh Goghrita and standard antidepressant drug [Imiprimine] were administered in the therapeutic dose.
Table 2 indicates detailing about drug doses and duration of treatment.

**Experimental Method:**

**Baseline Sucrose Preference Test:** This test was employed to test Anhedonia in rats. This procedure is composed of training and testing courses. Rats were trained to consume 1% sucrose solution before starting the actual experimentation (stress exposure and dosing). In this training course, rats were deprived of food and water for 48 hours and were exposed to only 1% sucrose solution. After training, animals were kept on normal food and water for next three days.

On day 7, food and water deprivation was done to animals for further 23 hours. After the completion of 23 hours, sucrose preference test was performed to record baseline readings. In this test, rats were exposed to two pre-weighed bottles kept in each cage, one bottle of 1% sucrose solution and another bottle contained potable water. The consumed quantity of sucrose solution and water was recorded; sucrose preference was calculated with the formula given below.

\[
SP = \frac{\text{Sucrose intake (g)}}{[\text{Sucrose intake (g)} + \text{Water intake (g)}]} \times 100.
\]

**Table No 2: Animal groups with drug doses of respective drugs**

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Adult dose (human dose)</th>
<th>Extrapolated dose per 200 gm</th>
<th>Duration of treatment</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Plain control</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>II</td>
<td>CUMS (non-treated)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>III</td>
<td>Positive control (imipramine hydrochloride)</td>
<td>10mg/kg</td>
<td>2mg/200g</td>
<td>28 days</td>
<td>Oral</td>
</tr>
<tr>
<td>IV</td>
<td>Vehicle control (Puran Ghrita)</td>
<td>40 g</td>
<td>3.6gm/kg (0.72gm/200g)</td>
<td>28 days</td>
<td>Oral</td>
</tr>
<tr>
<td>V</td>
<td>Test drug (BG I)</td>
<td>40 g</td>
<td>3.6gm/kg (0.72gm/200g)</td>
<td>28 days</td>
<td>Oral</td>
</tr>
<tr>
<td>VI</td>
<td>Vehicle control (Fresh Goghrita)</td>
<td>40 g</td>
<td>3.6gm/kg (0.72gm/200g)</td>
<td>28 days</td>
<td>Oral</td>
</tr>
<tr>
<td>VII</td>
<td>Test drug (BG II)</td>
<td>40 g</td>
<td>3.6gm/kg (0.72gm/200g)</td>
<td>28 days</td>
<td>Oral</td>
</tr>
</tbody>
</table>
Administration of drugs and application of stressors: From day eight up to day thirty-fifth, over the period of 28 days, animals were subjected to stress paradigm once daily along with drug treatments. Drugs were administered orally one hour prior to induction of the stress. Rats of plain control group (non-stressed animals) were housed in different cage, in separate room and did not have any contact with the stressed group.

Stressors were randomly scheduled daily and repeated over period of 4 weeks. Stressors used in CUMS model include, keeping animal in empty bottle for one hour, swimming in cold water, tail pinch, separation for 24 hours, high density housing, 45° cage tilt, overnight illumination, no bedding, soiled cage, and food-water deprivation.

Behavioral Test: After completion of 28 days experimental protocol, Forced Swim Test (FST) and Sucrose Preference Test were conducted to assess anti-depressant action of drug treatments.

Forced Swim Test: An apparatus consists a water tank (30×20×15 cm) with a rotating wheel (25 cm diameter) at its centre was used in this test. The tank was filled with water up to height of 13 cm maintained at 25±1°C. Each rat was placed in the rotating wheel; it was locked so that rat could not escape out. In an effort to escape out of water, rat tried to swim and end up with rotating the wheel. Animals from all groups were exposed for 5 minutes and the rotations of wheel displayed on the digital counter were recorded. Rats were removed after five minutes, wiped, dried and returned to their respective cages.

Sucrose Preference Test: It was again performed in the similar manner as done for base-line readings. From 36th day animals were deprived for food and water for next 23 hours, thus remaining one hour on 37th day sucrose preference test was conducted. The consumed quantity of sucrose solution was recorded and percentage of sucrose preference was calculated with given formula.

Statistical analysis: Data collected from sucrose preference test and Force Swim Test was analyzed with One way ANOVA followed by Tukey’s test using Graph pad prism 6 version. p<0.05 was considered statistically significant.

RESULTS

Rats from CUMS group exhibited significant (p ≤ 0.0001) decrease in number of rotations in FST test, indicating generation of depressive phase in comparison to other drug treated groups. Animals treated with Imiprimine, Puran ghrita, BG I, fresh Goghrita and BG II showed significant increase in number of rotations as compared to CUMS group (p≤ 0.0001). However, BG I and BG II has shown significant (p≤ 0.05) difference in number of rotations as compared to Puran ghrita and fresh Goghrita treated groups. Thus it implies that BG I and BG...
II have significant anti-depressant activity than vehical control groups [Table 3]. Moreover, substantial difference was not evaluated in relation to number of rotations in BGI as compared to BG II.

Table 3: Effect of Brahmi Ghrita I and Brahmi Ghrita II on number of rotations in Force swim test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal group</th>
<th>CUMS group</th>
<th>Standard drug group</th>
<th>Puran Ghrita Group</th>
<th>BG I group</th>
<th>Fresh Goghrita</th>
<th>BGII group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rotations</td>
<td>82.67±5.715</td>
<td>36.17±2.714 ***</td>
<td>79±2.098 ###</td>
<td>70.33±3.077 ###</td>
<td>79.5±6.221 ###</td>
<td>63.83±5.456 ###</td>
<td>72±4.195 ###</td>
</tr>
</tbody>
</table>

Data is expressed as Means ±SEM (n=6/per each group). BG I compared with Puran Ghrita and BGII compared with Fresh Goghrita [* p<0.05]. Comparison of CUMS with plain control [***p<0.0001] and CUMS with all treated groups [### p<0.0001]

In Sucrose preference test, CUMS exposed group (non-treated) showed significant (p<0.001) decrease in consumption of sucrose solution as compared to plain control group (non-stressed), indicates development of depressive phase in animals of CUMS group. Twenty eight days treatment with Standard drug, Puran ghrita, BG I, fresh Goghrita and BG II showed significant (p<0.001) increase in sucrose consumption as compared to CUMS group [Table 4]. In this test we couldn’t find statistical significant difference in all treatment groups.

Table 4: Effect of Brahmi Ghrita I and Brahmi Ghrita II on percentage of sucrose consumption in CUMS induced rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plain control</th>
<th>CUMS</th>
<th>Standard drug</th>
<th>Puran Ghrita</th>
<th>BG I</th>
<th>Fresh Goghrita</th>
<th>BGII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of Sucrose consumption</td>
<td>84.18 ± 2.00</td>
<td>54.25 ± 13.75 ***</td>
<td>82.13 ± 5.70 ###</td>
<td>81.48 ± 5.88 ###</td>
<td>83.1 ± 4.59 ###</td>
<td>78.42 ± 3.50 ###</td>
<td>82.04 ± 6.21 ###</td>
</tr>
</tbody>
</table>

Data is expressed as Means ±SD (n=6/each group), ***/ ### p<0.001. *** indicates comparison of CUMS group with plain control group, ### indicates comparison of CUMS group with other drug treated groups.

DISCUSSION:
The etiology of depressive disorders is to be different depending on the different factors like genetic tendency, threatening life events and some of the social impacts, specific stress factors and imbalanced HPA axis. Stress...
induced depression might be ranging between mild to moderate type, hampers working memory of sufferers; characterized by mental and physical fatigue, disturbed sleep, irritability, hypersensitivity, retarded movements, emotional and cognitive imbalance [13]. Pharmacological and psychological treatments are employed to reduce depressive like conditions in humans. Many of antidepressant drugs have shown positive effects and are also associated with known side effects [14]. Consequently alternative supportive medicines are to be searched and documented.

Brahmi Ghrita, traditional ayurvedic medicine comprising of three ingredients [Brahmi, Ela and lipid base] is claimed to have therapeutic effects to treat mental illnesses. Thus, present study was attempted to evaluate antidepressant like effect of Brahmi Ghrita prepared from ten year old cow ghee as BGI and from fresh cow ghee as BG II by using Chronic Unpredictable Mild Stress animal model.

Chronic stress serves as a factor to produce depressive-like behavior in animals as evidenced by behavioral measurements of anhedonia (Sucrose preference test) or despair (forced swim test). The present study results of sucrose preference test specified that the animals from CUMS group showed decrease in sucrose preference after 28 days of duration, suggestive of anhedonic effect of chronic stress regime. Decrease in percentage of sucrose consumption indicates reduction in ability to receive reward related behavior in animals referring to anhedonia (Ali et al., 2015) which is one of the core symptoms of depression. Standard drug, vehical control drugs and both the test drugs administered in therapeutic dose for twenty eight days have shown significant reversal of decreased sucrose consumption, specifying positive effect of all drugs for reversal of anhedonic effect as against CUMS group.

In humans and animals, chronic stress response starts with decreased monoamine neurotransmitters [serotonin and non adrenaline] and dysfunction of HPA axis which governs multitude activities and takes part in depression as well. Antidepressant drugs increase monoaminergic neurotransmission and reverse some of the changes at HPA axis [15]. It is well known that endothelial cells of brain capillaries do not permit bulk passage of water and solutes between the endothelial cells. The tight junction of endothelial cells i.e. blood brain barrier which merely allows free passage of lipid soluble drugs [16]. Thus considering the available data and comparable antidepressant like effect of BG I and BG II to standard drug Imiprimine, it can be postulated that lipoid based test drugs might be crossing BBR and acts by reversing depleted levels of
neurotransmitters through their neuroprotective actions. In previously reported studies on instrumental analysis of different extracts of Brahmi *Bacopa monnieri* revealed the identity of functional entities such as bacoside A, bacoside B, saponins, glycosides, bacogenins, ascorbic acids etc. Antidepressant, antistress, anxyolitic and antioxidant activity of *Bacopa monnieri* have been also reported.[17] Similarly, various pharmacological actions viz anti-inflammatory, analgesic, anti-depression and anticonvulsant have been reported to Ela *E. cardamomum*. Many phytochemical studies have shown presence of flavanoids, tannis, saponins, alkaloids in this drug and interpreted that these phytoconstituents play important role to attribute anxyolytic-antidepressant activity of extract of *E. cardamomum*.[18]

Ayurved has also explained importance of synergism in designing of ayurvedic medicaments wherein active components of herbal drugs are extracted in lipid bases with the specific preparation method,[19] facilitates to break pathogenesis of certain CNS disorders. Thus the synergistic action of combinations of potent herbal drugs [Brahmi and Ela] with lipid bases might be responsible to attribute antidepressant like activity of test drugs [BG I and BG II].

**Limitations:** This report on Brahmi Ghrita of fresh cow ghee and ten year old Cow ghee in *vivo* antidepressant study on CUMS -induced depression in rats was carried out as baseline study. Further phyto-chemical screening, pharmacological and histopathological investigations are to be planned to find out specific active constituents responsible for antidepressant activity and the exact mechanism of action of test drugs.

**CONCLUSION:**

The present investigation has provided the scientific evidence for the traditional herbal formulations, Brahmi Ghrita prepared from ten years old and fresh cow ghee as antidepressants in CUMS induced depression. However, comparative significant difference in the effects of BG I against BG II is not elucidated through this experimental animal study.

**REFERENCES:**


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