Introduction

In ayurvedic traditional science Go-ghrita (cow ghee) based formulations are said to be excellent due to its ability to carry the therapeutic properties of the herbs to the specific target site. Thus these are showing specific pharmacological actions towards particular body tissues (Kajaria et al., 2013). The quality assessment of medicated ghee formulations is of supreme importance in order to justify their acceptability in modern system of medicine (Sachan et al., 2016). Establishment of quality standards for medicine is having a great value to reproduce the desired therapeutic effect.

Brahmi Ghrita, one of the medicated ghee formulations, comprises of Brahmi (*Bacopa monnieri*), Ela (*Elettaria cardamomum*) and Puran ghrita (10 years old cow ghee). It is prescribed to minimize the episodes of Unmada and Apasmara (mental and behavioural disorders) (Shastri, 2006).

In accordance to classical formula, Brahmi ghrita should be prepared with Puran ghrita. However, Puran ghrita is not available in ample quantity and in existing pharmaceutical practice medicated ghee formulations are prepared with fresh go-ghrita (cow ghee). Though the potential of BG is having specific target action on brain tissue, thus to produce desired effect it should be prepared with certain standard operating procedures. Thus before initiating *in vivo* study, validation of manufacture process and laboratory analysis of Brahmi Ghrita prepared from Puran ghrita and fresh go-ghrita is essential.

Materials and methods: In accordance to ayurvedic standard protocols, two samples of Brahmi ghrita were prepared with Puran Ghrita (BG I) and fresh cow ghee (BG II). Further validation and comparative physico-chemical analysis was done for both samples. Results: The physico-chemical parameters such as specific gravity, moisture content, acid value, iodine value, saponification value and peroxide value of BG I and BG II samples were almost alike. Conclusion: The process of manufacture and analytical values of BG I and BG II samples are validated and established in the study.

Keywords: Medicated ghee, Brahmi ghrita, Puran ghrita, fresh go-ghrita (cow ghee), Standardization

Abstract

**Objective:** Brahmi Ghrita (BG) is a medicated ghee formulation, comprises of Brahmi (*Bacopa monnieri*), Ela (*Elettaria cardamomum*) and Puran ghrita (10 years old cow ghee). It is recommended to treat broadly described mental illnesses especially in Unmad (Psychosis) and Apasmar (Epilepsy). However, Puran ghrita is not available in ample quantity and in current practice medicated ghee formulations are prepared with fresh go-ghrita (cow ghee). Though the potential of BG is having specific target action on brain tissue, thus to produce desired effect it should be prepared with certain standard operating procedures. Thus before initiating *in vivo* study, validation of manufacture process and laboratory analysis of Brahmi Ghrita prepared from Puran ghrita and fresh go-ghrita is essential.

Materials and methods: In accordance to ayurvedic standard protocols, two samples of Brahmi ghrita were prepared with Puran Ghrita (BG I) and fresh cow ghee (BG II). Further validation and comparative physico-chemical analysis was done for both samples. Results: The physico-chemical parameters such as specific gravity, moisture content, acid value, iodine value, saponification value and peroxide value of BG I and BG II samples were almost alike. Conclusion: The process of manufacture and analytical values of BG I and BG II samples are validated and established in the study.

**Keywords:** Medicated ghee, Brahmi ghrita, Puran ghrita, fresh go-ghrita (cow ghee), Standardization

In accordance to classical formula, Brahmi ghrita should be prepared with Puran ghrita. However, Puran ghrita is not available in ample quantity and in existing pharmaceutical practice medicated ghee formulations are prepared with fresh cow ghee. Thus there is a need to develop and generate specific quality standard values for Brahmi ghrita prepared from Puran ghrita and fresh go-ghrita along with paste of Brahmi and Ela. Previously, studies have been carried out on standardization (Yadav et al., 2013) on different formulations of Brahmi ghrita prepared with different components. Likewise experimental and clinical trials have also been investigated (Chandola, 2008; Gubbannavar et al., 2012). However, till date no work has been carried out to explore laboratory quality parameters of Brahmi ghrita prepared with Brahmi, Ela, Puran ghrita as well as with fresh go-ghrita. Hence in the present study, preparation method of two samples of Brahmi Ghrita is developed and validated through physico-chemical analysis.

Materials and methods

**Materials**

Fresh whole plant of Brahmi (*Bacopa monnieri*) was
collected and identified through the ayurvedic experts. Authentication of the sample of whole plant of Brahmi was done in research laboratory using API parameters to assess purity and quality of the raw drug.

Five samples of Ela (Elettaria cardamomum) collected from different vendors were undergone through consensus method and further the ideal sample of Ela was identified and selected by using ayurvedic parameters. Authentication of Ela was done with total ash, moisture content, water and alcohol soluble extractives and volatile oil determination.

Ten year old Puran ghrita was collected from a renowned ayurvedic physician, as an authentic source drug. Plain cow ghee was kept in airtight container for ten years under his proper control and care. Fresh go-ghrita was collected from renowned dairy unit, where ‘Deshi’ (domestic) cows are cradled in natural environment and it is prepared according to traditional method. Fresh go-ghrita and Puran ghrita were analysed in a renowned analytical laboratory with API (Ayurvedic Pharmacopoeia of India) parameters viz pH, specific gravity, moisture content, acid value, iodine value, saponification value and peroxide value. (Table 1 and 2)

**Physico-Chemical Analysis**

BG I and BG II were analyzed with organoleptic tests as stated in table 1 and Physico-chemical analysis was carried out in research laboratory with API parameters as stated in table 2. The different physico-chemical parameters such as specific gravity, pH, moisture content, acid value, iodine value, saponification value and peroxide value were determined by standard procedures.

**Results and discussion**

Ayurvedic medicated ghee formulations differs in contents, as per the therapeutic indices it is observed that synergism of the specific components and application of ayurvedic pharmaceutical procedures is done in formulations and thus it reflects to produce different pharmacological actions. To reduce the batch variation in production, there is a need to develop certain quality standards for those formulations. In earlier research works some of the medicated ghee formulations have been studied in regards to their standardization by means of modern techniques.

Brahmi ghrita is a specific formulation narrated in classical text, gives clear guideline about use of Puran ghrita (as a lipid base) in preparation. In accordance to ayurvedic treatment principles also to treat specific CNS disease conditions, Puran ghrita has shown added potential than fresh cow ghee (Shastri, 2006). However, till date any data has not been available about Brahmi ghrita prepared from Brahmi, Ela and Puran ghrita, as well Brahmi ghrita prepared from Brahmi, Ela and fresh go-ghrita.

In present study, two formulations of Brahmi ghrita i.e.BG I and BG II were tested with organoleptic tests and analyzed with physico-chemical tests to provide standard values for individual formulations (Table 1). Analytical values of Puran ghrita against BG I and fresh go-ghrita against BG II were also compared and discussed.

It is observed that before processing, Puran ghrita was sticky in touch, yellowish creamy in colour and after processing with herbal drugs it was changed to non-sticky and darker yellow in BG I. The odour of Puran ghrita was strong, unpleasant and taste was astringent-bitter, after

<table>
<thead>
<tr>
<th>Tests Parameters</th>
<th>Puran ghrita</th>
<th>BG I</th>
<th>fresh go-ghrita</th>
<th>BG II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Touch</td>
<td>Oily, Sticky</td>
<td>Oily</td>
<td>Oily, Granular</td>
<td>Oily</td>
</tr>
<tr>
<td>Color, appearance</td>
<td>Yellowish, Creamy</td>
<td>Dark Yellow</td>
<td>Semisolid, Pale, Yellowish</td>
<td>Yellowish + brown</td>
</tr>
<tr>
<td>Taste</td>
<td>Astringent, Bitter</td>
<td>Astringent, Pungent</td>
<td>Sweet</td>
<td>Bitter</td>
</tr>
<tr>
<td>Odour</td>
<td>Strong +++</td>
<td>Strong Characteristic +</td>
<td>Mild pleasant</td>
<td>Sweet- fragrant smell</td>
</tr>
</tbody>
</table>

www.ajpp.in
Table 2. Comparative analysis of Puran ghrita, BG I and fresh go-ghrita, BG II

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Puran ghrita</th>
<th>BG I</th>
<th>fresh go-ghrita</th>
<th>BG II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>0.748</td>
<td>0.639</td>
<td>0.965</td>
<td>0.948</td>
</tr>
<tr>
<td>Moisture Content</td>
<td>0.16</td>
<td>0.18</td>
<td>1.35</td>
<td>0.12</td>
</tr>
<tr>
<td>pH</td>
<td>4.95</td>
<td>6.5</td>
<td>5.44</td>
<td>3.8</td>
</tr>
<tr>
<td>Acid Value</td>
<td>1.06</td>
<td>1.43</td>
<td>1.50</td>
<td>2.59</td>
</tr>
<tr>
<td>Iodine Value</td>
<td>32.19</td>
<td>34.28</td>
<td>30.10</td>
<td>34.93</td>
</tr>
<tr>
<td>Saponification Value</td>
<td>223.23</td>
<td>227.82</td>
<td>224.4</td>
<td>228.49</td>
</tr>
<tr>
<td>Peroxide Value</td>
<td>4.11</td>
<td>7.10</td>
<td>7.65</td>
<td>7.35</td>
</tr>
</tbody>
</table>

physically it was changed to less strong and pungent in taste. Whereas fresh go-ghrita was oily - not sticky, sweet and light yellowish in appearance and after processing, it was darkened to yellowish brown in colour and bitter in taste. The sweet pleasant smell was remained unchanged. These changes might have been taken place due to synergism of herbal drugs and extraction of phyto-constituents in lipid bases with the aid of heating and water.

Physico-chemical analysis of the present study drugs are compared with each other and thus interpreted as below (Table 2).

Specific gravity is a ratio of weight of material in reference to weight of water for constant volume. The weight of lipid material is affected by basic constitution, dissolved constituents used during the processing of formulation. It also changes due to the effect of temperature during the process. (Haldar et al., 2013; Sharma et al., 2016). In present study no significant change in specific gravity was observed between Puran Ghrita and BG I as well fresh go-ghrita and BG II.

pH is the measure of hydrogen ion concentration; a measure of the acidity or alkalinity of a solution. The pH scale ranges from 0 to 14. pH less than seven are acidic, while those with pH greater than seven are basic or alkaline. Puran ghrita might be slightly acidic than fresh go-ghrita as its pH value was 4.95 which is lesser than the value of fresh go-ghrita. While it was observed that pH of BG I was greater than Puran ghrita, indicating BG I is less acidic in nature as compared to Puran ghrita. Whereas, pH of BG II was reduced as compared to fresh go-ghrita, which signifies that after processing alkaline nature of BG II was increased.

Moisture content indicates the presence and percentage of moisture in the substance. Higher value of moisture suggests that go-ghrita is more susceptible for getting contaminated. (Kadibagil and Sarashetti, 2017; Haldar et al., 2013) The moisture content in all the ghrita samples were within permissible limit, indicating that they were free from contamination.

Acid value is called as neutralization number. This is the amount of Potassium hydroxide required in milligram to neutralize the acid present with any compound. Thus this value indicates the Free Fatty Acids (FFAs) present in go-ghrita. It is related to its quality, stability and shelf life. Formation of FFAs might be the important factor for rancidity of ghrita. FFAs is formed due to the hydrolysis of triglycerides and may be promoted by the reaction with moisture present in Cow ghee (Frega et al., 1999; Reddy et al., 2013; Haldar et al., 2013). We found that sample of fresh go-ghrita is more acidic than Puran ghrita. After processing the acid values of BG I and BG II were increased. Increase in acid value might be due to extraction of phyto-consituents (Bacoside A, Bacoside B, Saponinis, Terpenin etc) of Brahmi and Ela in both the formulations, which might be promoted by the reaction of triglycerides present in the fats. However, after processing of ghrita, the increase in FFAs might be less stable and show low shelf life as compared to raw ghrita samples (fresh go-ghrita and Puran ghrita).

Iodine value: This value determines the amount of unsaturated fatty acids in the form of double bond which reacts with iodine. High iodine value indicates fats are rich in poly-unsaturated fatty acids (PUFA) which are helpful in reducing LDL cholesterol level. (Ewers et al., 2009; Sharma et al., 2016). It was observed that the values of Puran ghrita, BG I and BG II are almost alike and much greater than Fresh go-ghrita. So it can be said that Puran ghrita, BG I and BG II have high amount of PUFA. BG I and BG II (medicated ghee formulations) might be helpful to reduce LDL cholesterol level when administered to human being. Though these are lipid base formulations, due to presence of PUFA, those formulations would not alter or change physiological lipid profiles of human being.

Saponification value: It is the measure of average molecular weight (or chain length) of all fatty acids present in the fats (Kadibagil and Sarashetti, 2017). It is said that in manufacture of medicated ghee formulations, due to process of hydrolysis, liberation of low molecular weight SCFA (short chain fatty acids) is carried out. It might be happening in the presence of alkaline nature of paste of
herbal drugs and liquid media. SCFA are recognised as an essential fuel source for colonocytes, particularly in distal colon. The histological, endoscopic and metabolic similarities between diversion colitis and ulcerative colitis suggests that nutritional SCFA deficiency state may play an important role in pathogenesis of these diseases (Rabassa, Roger, 1992). SCFA are readily absorbed and may play an important role as a protective effect for distal colon (Wong et al., 2006). It was observed that the saponification values of BG I and BG II formulations were greater than the Puran ghrita and fresh go-ghrita. Thus it can be assumed that BG I and BG II have more percent of SCFA than Puran ghrita and fresh go-ghrita. Hence, inferred that BG I and BG II may improve colonic health as it might be getting easily absorbed and digested. It could possibly play a protective role as compared to plain Puran ghrita and fresh go-ghrita.

**Peroxide value:** Peroxides (O$_2^-$) are intermediate products of fat oxidation. Rapid breakdown is taken place to Aldehydes, Ketones and other products. This value is an indicator of fat oxidation. It is used to assess the stability or rancidity of fats by measuring lipid peroxides and hydro-peroxides formed during the initial stage of oxidation. (Sharma et al., 2016; Haldar et al., 2013) In present study peroxide value of Puran ghrita is comparably lesser than fresh go-ghrita, BG I and BG II. However with the obtained values we can't interpret firmly about the rancidity of BG I and BGII.

It was observed that obtained values for the parameters such as specific gravity, moisture content, pH, acid value, iodine value and saponification value are alike to all four samples. However, peroxide value of Puran ghrita was comparatively lesser than fresh go-ghrita, BG I and BG II.

**Conclusion**

In the present study analytical values for Puran ghrita, BG I and BG II are established. Analytical data generated for Brahmi ghrita prepared from Puran ghrita and Brahmi ghrita prepared from fresh go-ghrita possibly will be considered as quality standard. Further researchers can use these analytical values for BG I and BGII as standard values. A detail further research is needed for scientific validation and further establishment of data by using higher instrumental techniques.

**Conflict of interest**

The authors are declaring no conflict of interest.

**References**


