Studies on *Ashwagandha Ghrita* with reference to murcchana process and storage conditions

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**ABSTRACT**

**Background:** *Withania somnifera* (L) (family-Solanaceae), known as ‘Indian ginseng’ or ‘Ashwagandha’ is acclaimed as an effective adaptogen, immunomodulator, aphrodisiac and sedative. *Ashwagandha ghrita* is a recognized ghee based Ayurvedic formulation. Few ancient texts suggest *murcchana* process for preparation of *Ashwagandha ghrita*.

**Objective:** The study was undertaken to evaluate probable effects of *murcchana* process on ghrita preparation with reference to time and storage conditions.

**Materials and Methods:** *Ashwagandha ghrita* samples were prepared separately using plain ghee (Indian cow’s ghee) and murcchana ghee. These formulations were stored separately in different glass bottles at room temperature and 400C/75%RH. Organoleptic characters (colour, odour, taste, texture and touch) and physicochemical parameters (acid value, peroxide value, iodine value, saponification value, unsaponifiable matter, refractive index and specific gravity) were determined after 3, 6, 9 and 12 months. Plain ghee and prepared ghrita were subjected to antioxidant evaluation by various in vitro methods.

**Results:** Changes were observed in organoleptic characters and physicochemical parameters of plain ghee and *Ashwagandha ghrita* formulations. Alterations in these parameters were more pronounced at high temperature and on long storage. *Ashwagandha ghrita* prepared with *murcchana* process exhibited better antioxidant potential in all in vitro methods.

**Conclusion:** The *murcchana* process was found to be beneficial towards quality of ghrita. Hence, *Ashwagandha ghrita* may be prepared along with *murcchana* herbs and stored in a good quality glass bottle to ensure improved shelf life of ghrita.

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1. Introduction

‘Panchgavya’ is a term used in *Ayurveda*, comprising five important substances obtained from cow, namely milk, ghee (clarified butter fat), curd, urine and dung. *Ayurveda* describes a good number of formulations containing ‘*panchgavya*’ components either individually as well as conjointly with other substances of herbal, mineral or animal origin. Several formulations based on each one of these five components are reported in Ayurvedic texts with medicinal claims [1–5].

Ghee, widely considered as the Indian name for clarified butterfat, is usually prepared from cow milk or buffalo milk or combination thereof. ‘*Ghrita*’, also known as clarified butter, is a traditional adjuvant/vehicle described in *Ayurveda* [6]. Charaka used word ‘*ahasaraveyra*’ for ghee and ‘*yogvahitwa*’ for ghrita by way of which it enhances the therapeutic efficacy and potency of plant ingredients it is processed with [3–5,7].

*Withania somnifera* belongs to the family Solanaceae, popularly known as ‘Ashwagandha’ is mentioned as herbal tonic and health food. It has been used in Ayurvedic and indigenous medicine for very long time to treat various kinds of diseases and human ailments. Among the Ayurvedic *rasayan* herbs (preparation that works as a health tonic to children, a medicine to middle aged persons and rejuvenator to the elderly), *Ashwagandha* holds an important place. It constitutes alkaloids and steroidal lactones...
namely; withanine, withanarine, somnine, sommiferine, sommiferine, pseudowithanine tropane, pseudo-tropane, choline, anal-ferine, anahydrine and isopelletierine [8–11]. Ashwagandha is widely claimed to have hepatoprotective [12,13], anxiolytic [14], antidepressant [14,15], nootropic [16], antimicrobial [17], anti-inflammatory [18,19], antioxidant [20], antistress [21], anticonvulsant [22], cardio-protective [23], antitumor [24–27], antigenotoxic [28], antiparkinsonian [29] and immunomodulatory [30] properties. Ashwagandha ghrita (AG) is an effective ghrita formulation beneficial for treatment of weakness, gynaecological disorders, general debility and infertility [31].

In Ayurveda, ‘murchhana sansksara’ i.e. processing of cow ghee with antioxidant Ashwagandha herbs i.e. Emblica officinalis (Euphorbiaceae), Cyperus rotundus (Cyperaceae), Curcuma longa (Zingiberaceae), Terminalia chebula (Combretaceae) and Terminalia bellirica (Combretaceae) and Citrus medicus, is pondered as an important intermediate process in ‘ghrit-kalpaṇa’ to enhance the medicinal potency of ghrita and to get rid of bad odour and rancidity [32–37]. Despite the significance of murchhana sansksara in the processing of ghee, no scientific and systematic studies were conducted till date to delineate the advantage of murchhana sansksara with reference to storage conditions of ghrita. So, the present work was undertaken to verify some notions about incorporation of murchhana herbs in ghee by selecting A. ghrita as prototype of ghrita. Studies were also aimed towards investigation of effect of murchhana process and storage conditions on A. ghrita to ensure its effect on formulations.

2. Materials and methods

2.1. Preparation of AG using plain ghee

| Name                  | Family        | Common name | Voucher Specimen No. | Part of plant |
|-----------------------|---------------|-------------|----------------------|---------------|
| Withania somnifera   | Solanaceae    | Ashwagandha | 8961/21              | Roots         |
| Emblica officinalis   | Euphorbiaceae | Amlaki      | 8961/22              | Pericarp of fruits |
| Terminalia chebula    | Combretaceae  | Haritaki    | 8961/23              | Pericarp of fruits |
| Terminalia bellirica  | Combretaceae  | Bibhitaki   | 8961/24              | Pericarp of fruits |
| Cyperus rotundus Linn.| Cyperaceae    | Musta       | 8961/25              | Rhizomes      |
| Curcuma longa Linn.   | Zingiberaceae | Haridra     | 8961/26              | Rhizomes      |
| Citrus medica var.    | Rutaceae      | Matulunga   | 8961/27              | Juice         |

Table 1

Herbal composition of Ashwagandha ghrita.

The MG was prepared as per the procedure described in reference texts and published reports [32–34]. Briefly, initially stated amount of plain ghee (sixteen parts, 768 g) was melted in a vessel with moderate heating. A mixture of coarsely powdered five herbs; T. chebula (Combretaceae) fruits, Terminalia belerica (Combretaceae) fruits, C. rotundus (Cyperaceae) rhizomes, E. officinalis (Euphorbiaceae) fruits and C. longa (Zingiberaceae) rhizomes, in equal quantities (one part, 48 g) was ground with juice of C. medicus (one part) to form a smooth paste (kalka). The kalka was added to the molten ghee along with water (3.072 lit) and boiled on slow fire till complete evaporation of water. It was then strained through muslin cloth and stored in a well closed autoclaved glass bottle.

2.2. Preparation of murchhana ghee (MG)

During the study, additional sample of AG was also prepared at once using MG instead of plain ghee, following the same procedure and denoted as AGM i.e. A. ghrita processed with murchhana ghee [32]. Here also iron vessel was used during preparation. It was worthy to prepare separate formulations so the effects of murchhana herbs on composition of ghee during storage at different temperatures could also be estimated.

2.3. Preparation of AG using MG

The ghrita preparations were denoted as MG, AG and AGM for murchhana ghee, A. ghrita prepared with plain ghee, A. ghrita prepared with murchhana ghee respectively. The use of two different ghee samples i.e. plain ghee and murchhana ghee for preparation of AG allowed comparative assessment between AG and AGM. The samples were examined for various organoleptic properties, physicochemical parameters and antioxidant activity and then stored separately in well closed glass containers at room temperature (RT) and 40 °C/75%RH in humidity chamber (Newtronic, NEC 212 ET). Subsequently physicochemical and organoleptic evaluation of all samples was done after 3, 6 and 12 months of storage. All studies were performed in triplicate and mean values were recorded.

2.4. Evaluation of plain ghee, AG, MG and AGM

The ghrita preparations were denoted as MG, AG and AGM for murchhana ghee, A. ghrita prepared with plain ghee, A. ghrita prepared with murchhana ghee respectively. The use of two different ghee samples i.e. plain ghee and murchhana ghee for preparation of AG allowed comparative assessment between AG and AGM. The samples were examined for various organoleptic properties, physicochemical parameters and antioxidant activity and then stored separately in well closed glass containers at room temperature (RT) and 40 °C/75%RH in humidity chamber (Newtronic, NEC 212 ET). Subsequently physicochemical and organoleptic evaluation of all samples was done after 3, 6 and 12 months of storage. All studies were performed in triplicate and mean values were recorded.

2.4.1. Organoleptic evaluation

Sensory (organoleptic) characters play an important role towards suitability of ghee containing formulations and are indicative of formulations rancidity [35]. These characters comprise of color, odour, taste, texture and touch. The plain ghee and ghritas were allowed to reach normal temperature for proper crystallization before testing. Initial specific observations and further changes in sensory characters after 3, 6 and 12 months of storage were noted carefully.
2.4.3.2. Nitric oxide radical scavenging assay. The nitric oxide radical scavenging assay was performed using a modified Greiss reaction [41, 42]. Briefly, 3 mL of 10–100 μg/mL of ghee/ghrita solutions and 1 mL of 0.1 mM solution of DPPH in ethanol were mixed together and after 30 min the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture specifies higher free radical-scavenging activity.

2.4.3.3. Hydrogen peroxide scavenging assay. The hydrogen peroxide scavenging assay was performed using a modified DPPH (1, 1-diphenyl-2-picryl-hydrazyl) method [41]. Briefly, 1.0 mL of 0.1 mM H2O2, 1.0 mL of 10 ghee/ghrita solutions, and 0.0 mL of 0.1 mM solution of DPPH in ethanol were mixed with sodium nitroprusside (5 mM) in phosphate-buffered saline and allowed to react for 150 min. Further these samples were reacted with Greiss reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The chromophore formed during the diazocoupling of nitrite with sulphanilamide and naphthylethylenediamine was subjected for absorbance measurement at 546 nm. The reaction mixture (without test sample) with equivalent quantity of distilled water served as control.

\[ \% \text{Inhibition} = \left( \frac{V_0 - V_1}{V_0} \right) \times 100 \]

where, \( V_0 \) was volume of sodium thiosulphate solution used to titrate the control sample in the presence of hydrogen peroxide (without ghee/ghrita) and \( V_1 \) was the volume of sodium thiosulphate solution used in the presence of the ghee/ghrita.

3. Results

3.1. Organoleptic evaluation

The plain ghee and ghrita preparations exhibited some specific peculiarities. The sensory characteristics were noted as soon as formulations were made. The taste and odour (aroma) were best observed when sample was warm and melted. Sensory characters of these samples of plain ghee and ghrita preparations were carefully observed initially (first day of preparation) and after 3, 6, 9 and 12 months of storage. During the course of study, it was observed that organoleptic characters demonstrated by plain ghee vary with that of ghrita formulations.

3.1.1. Color

It was observed that during storage of ghee, color got fade. Golden yellow color of plain ghee was changed to pale yellow after storage for 12 months at higher temperature whereas MG showed same color throughout investigation at both temperatures. The AG samples exhibited yellowish color while AGM appeared slightly brownish. On storage, color of ghee and ghritas showed changes which were mainly established after 6–9 months.

3.1.2. Odour and taste

Aromatic, pleasant and characteristic odour and taste of plain ghee and AG were gradually changed to slightly fragrant and bitter taste on storage at high temperature for 12 months duration. The MG and AGM revealed nearly identical odour (characteristic, aromatic and pleasant) and slightly astringent taste throughout study and at all storage conditions.

3.1.3. Touch and texture

Ghritas showed formation of small granules and loose layers when stored at room temperature. These layers and granule arrangements were hindered at high temperature. The observations revealed that the smooth, soft touch and texture of ghee, MG, AG and AGM samples were nearly unaffected during the study.

3.2. Physicochemical evaluation

Ghee undergoes physico-chemical changes, dependent primarily on the temperature of storage [44, 45]. Thus, it was thought worthwhile to study different physicochemical parameters of ghee and prepared ghritas which reflect corresponding changes in ‘ghee’ composition and are summarized in Table 2.

3.2.1. Acid value

Initially, acid values of AG and AGM were found to be more as compared to plain ghee and MG. All samples showed rise in acid value on storage. Samples of AG and AGM showed slow and gradual rise in acid values during period of storage. The rate of increase in acid value in sample containing murchana herbs was slower. The AGM samples stored at both temperatures were found to be protected to some extent as indicated by acid values.

3.2.2. Peroxide value

In case of plain ghee, AG, MG and AGM, peroxide value showed gradual increase during storage at both temperatures. Results clearly revealed that plain ghee showed steady and maximum oxidative damage (1.581–5.982), mainly at high temperature and after twelve months storage. MG and AGM also showed rise in peroxide value at high temperature during storage period.

3.2.3. Iodine value

Iodine value of plain ghee, MG, AG and AGM samples showed gradual decrease on storage. Changes in iodine value were strikingly different at various temperatures. It was observed that initially iodine value dropped slowly but declined significantly at higher temperature, mainly with samples stored for 9–12 months duration. The AG showed large fall in iodine value (42.10–32.34) as compared to AGM (45.71 To 38.34) on storage for 12 months duration at 40 °C/75%RH.
The results indicate the changes in saponification value of ghee and ghrita formulations during storage. In case of plain ghee, saponification value was decreased up to nine months but then surprisingly it showed upward shift in some samples. In AG and MG samples stored in glass container, saponification value got decreased at all temperatures throughout the period of study. As shown in Table 2, AGM stored at both temperatures initially showed increase in saponification value after three months i.e. 33.23, whereas plain ghee showed slight changes in RI after nine months storage.

During the present study it was observed that the RI of plain ghee was lower i.e. 1.4448 than MG, AG and AGM. AG sample exhibited sharp rise in RI (1.4550–1.4565) at high temperature for 12 months storage whereas MG and AGM showed slight changes in RI after nine months storage.

All ghrita samples i.e. MG, AG and AGM showed gradual increase in specific gravity with time at both temperatures. Comparatively plain ghee showed less increase in specific gravity after three months to 12 months at 40 °C i.e. 0.925 to 0.953. In case of AGM, sample kept at 40 °C showed sudden rise in specific gravity i.e. 0.952 to 1.471 after twelve months storage.

In case of in-vitro antioxidant evaluation, all test samples i.e. plain ghee, MG, AG and AGM exhibited concentration dependent (10–100 μg ml⁻¹) free radical scavenging activity. The IC₅₀ of AGM by the DPPH method was found to be 22.97 μg ml⁻¹, whereas plain ghee, MG and AG showed IC₅₀ values as 40.76, 28.42 and 33.23 μg ml⁻¹, respectively. In NO method, IC₅₀ for AGM was found to be 24.56 μg ml⁻¹, whereas plain ghee, MG and AG showed IC₅₀ value as 41.43, 28.22 and 35.12 μg ml⁻¹ respectively. Plain ghee, MG, AG and AGM demonstrated dose dependent H₂O₂ scavenging activity with the IC₅₀ 43.13, 31.03, 38.76 and 25.98 μg/ml respectively (Table 3). Ascorbic acid revealed excellent antioxidant activity in all in-vitro methods.

### 3.2.4. Saponification value

### 3.2.5. Unsaponifiable matter

### 3.2.6. Refractive index

### 3.2.7. Specific gravity

### 3.3. Antioxidant evaluation

### Table 2

| P | M | Plain ghee | MG | AG | AGM |
|---|---|------------|----|----|-----|
| 0 | 0 | 3 | 6 | 9 | 12 | 0 | 0 | 3 | 6 | 9 | 12 | 0 | 0 | 3 | 6 | 9 | 12 |
| AV | RT | 0.335 | 0.336 | 0.468 | 0.538 | 0.649 | 1.307 | 1.310 | 1.315 | 1.382 | 1.423 | 1.450 | 1.451 | 1.452 | 1.680 | 2.250 | 2.342 | 2.343 | 2.345 | 2.351 | 2.362 |
| PV | RT | 1.581 | 1.581 | 1.586 | 1.589 | 3.590 | 1.339 | 1.341 | 1.386 | 1.410 | 1.416 | 1.822 | 1.826 | 1.845 | 1.921 | 2.87 | 2.062 | 2.072 | 2.075 | 2.252 | 2.260 |
| SV | RT | 22.71 | 22.72 | 22.73 | 21.83 | 21.96 | 217.8 | 216.2 | 213.3 | 211.5 | 209.6 | 12.410 | 41.420 | 42.556 | 40.22 | 41.71 | 44.32 | 44.20 | 42.56 | 40.22 |
| RI | RT | 1.4448 | 1.4449 | 1.4449 | 1.4449 | 1.4449 | 1.453 | 1.4530 | 1.453 | 1.453 | 1.453 | 1.4535 | 1.4550 | 1.4552 | 1.4555 | 1.4557 | 1.4549 | 1.454 | 1.454 | 1.454 | 1.454 | 1.454 |
| SG | RT | 0.925 | 0.931 | 0.931 | 0.941 | 0.941 | 0.950 | 0.950 | 0.966 | 0.967 | 0.971 | 2.041 | 2.031 | 2.031 | 2.031 | 2.031 | 0.952 | 0.956 | 0.974 | 0.983 | 1.250 |

Note: All values are mean of three replications.

1. Parameter, M= Months; T= Temperature; RT= Room Temperature; AV= Acid Value; PV= Peroxide Value; SV= Saponification Value; UM= Unsaponifiable Matter; RI= Refractive Index; SG= Specific Gravity; MG= Murcchana ghee; AG= Ashwagandha ghrita; AGM= Ashwagandha ghrita processed with murcchana ghee.

### 4. Discussion

Multicomponent formulations are a common practice in Ayurveda. Generally, many ingredients in different forms are processed together/separately to get maximum collaborative therapeutic effect (sometimes additive effects) or to minimize side effects or to make it more acceptable by patient. For instance, AG contains Ashwagandha roots processed with cow ghee. In some Ayurvedic scripts it is mentioned that before making any ghrita, ghee should be processed with ‘murcchana kriya’, with the use of some herbs to suppress, if any rancid (bad) smell present in ghee [32]. This renders the ghee clear, devoid of any bad effects and smell and prevents ghee from spoilage [33].

In present study, AG was selected as a prototype. In Ayurveda Sarsangraha, MG is mentioned for AG preparation [32]. Some other reference texts lack mention of specific process i.e. ‘murcchana process’ to be carried out during preparation of AG. Therefore, this study was an attempt to unlock the ambiguity regarding use of murcchana herbs during preparation of AG.

### 4.1. Organoleptic evaluation

Sensory evaluation is a scientific discipline used to measure, analyse and interpret reactions to organoleptic characteristics of foods and materials perceived by the senses of sight, smell, taste, touch and hearing [46]. Timely discussions with Ayurvedic experts revealed that some sensory properties of ghee and ghrita formulations manifest themselves optimally at different temperatures.
processing [44,48–50]. The plain ghee possesses a characteristic aroma which is due to presence of free fatty acids, carboxyls and lactones contributing to ghee flavour [51]. The taste and aroma were altered due to presence of various phytoconstituents in MG, AG and AGM. The tannin rich murcchana herbs seemed to be the important compounds influencing the astringent taste and flavour of MG and AGM samples at both temperatures.

Organoleptic evaluation of touch and texture of plain ghee and all ghrita formulations failed to reveal any significant inference.

4.2. Physicochemical evaluation

Acid value is a measure of the amount of carboxylic acid groups in fatty acid compounds. As oil/ fat rancify, over a period of time, triglycerides get converted into fatty acids and glycerol, causing an increase in amount of acids. It can be considered as catalytic effect of iron (from manufacturing vessel) or presence of acidic phytoconstituents or generation of free fatty acids from triglycerides present in ghee or all these may be responsible for higher acid values of ghrita preparations as compared to plain ghee [52].

Lipid peroxidation depends on fatty acid composition and storage conditions of fat or oil [53]. Extent of lipid peroxidation i.e. auto-oxidation is measured in terms of peroxide value, giving initial evidence of rancidity in unsaturated fats and oils. Free unsaturated acids are oxidized more easily and quickly than the same acids in intact glycerides and thus high acidity signifies high peroxide value [54]. It can be assumed that catalytic action of iron and/or high temperature must be responsible for maximum oxidative deterioration of ghee [52,55]. The murcchana herbs seemed to be offering some protection against this catalytic and oxidative damage or peroxide formation at room temperature only.

Iodine value indicates quantity of iodine absorbed at unsaturation which expresses amount of unsaturation in a fat. Large fall in iodine value of AG as compared to AGM can be corroborated with more oxidation due to high unsaturation in AG whereas in AGM murcchana herbs might have shown their protective antioxidant effect.

Saponification value is an index of mean molecular weight (or chain length) of all the fatty acids present and is directly proportional to the fatty matter content. It indicates the number of reactive terminal acid groups in the fat. More the fatty matter content or more the carboxylic functional group per unit mass, there will be more chances of rancidity factor and less will be the shelf life and therapeutic value [55–58]. In present investigation, changes in saponification value failed to reveal any significant inference and these variations could be attributable to interactions between different ghee components and phytoconstituents.

The unsaponifiable matter consists of substances (lipids of natural origin such as sterols, pigments, vitamins, higher aliphatic alcohols and hydrocarbons as well as any non-volatile foreign organic matter) present in oils or fats which are not saponifiable by alkali hydroxides. Possible interactions between phytoconstituents and ghee components might have resulted in insignificant changes in unsaponifiable matter of samples stored at both temperatures throughout study.

Refractive index, the ratio of the velocity of light in vacuum to its velocity in the substance, is a fundamental physical property of a substance. The RI is often used to ascertain a particular substance, check its purity, or measure its concentration. If RI is more, there will be more concentration of light which facilitates rancidification of ghrita i.e. decomposition of ghrita [53–58]. Slight changes in RI of AGM and MG showed reduction in rancidity indicating the antioxidant effect of murcchana herbs.

Specific gravity of ghrita is an indication of the solid to liquid ratio in ghrita. In case of liquid and semi-solid preparations, ongoing chemical processes change their consistency and are responsible for conversion of liquid contents into solid or vice-versa [55]. Sudden rise in specific gravity of AGM after twelve months storage could be due to the solid extracts originated from the added herbs during the formulation process. Increase in specific gravity revealed increase in solid contents compared to liquid contents with respect to all ghrita formulations. Less proportion of liquid contents in preparation increases life span of formulations [55–58].

Antioxidant potential of test samples by all in-vitro methods was found in increasing order i.e. plain ghee < AG < MG < AGM. Various tannin-rich herbs used in preparation of MG might be responsible for potent antioxidant activity. There could be synergistic effect of antioxidant herbs from MG and Ashwagandha, which resulted in highest antioxidant potential of AGM.

From the physicochemical evaluation of plain ghee and all ghrita samples, it can be interpreted that plain ghee and AG had undergone certain major physicochemical changes during storage as compared to MG and AGM (Table 2). It can be assumed that oxidation is the main reason towards alterations in physicochemical properties and ultimately rancidity of ghee-based formulations. Murcchana herbs i.e. T. chebula, T. belerica, C. rotundus, E. officinalis and C. longa are sources of polyphenolic compounds comprising phenolic acids (gallic acid, ellagic acid, chebulinic acid), flavonoids, coumarins, tannins with proven free radical scavenging potential [59–63]. These antioxidant principles of murcchana herbs must have contributed towards protection against oxidative damage and hence AGM performed better as compared to all other ghrita preparations. Our findings confirm the earlier reports of significance of ‘murcchana samskara’ of ghrita for minimizing rancidity and increasing stability.

5. Conclusion

In a nutshell, the observations and results suggest that, murcchana process is prerequisite for preparation of A. ghrita to ensure its maximum acceptability, stability and better shelf life. However, it is also established that murcchana herbs are not much effective against oxidative damage occurred at high temperature.

| Table 3 |
| --- |
| In-vitro antioxidant evaluation of Plain ghee, MG, AG and AGM. |

| Method     | Plain ghee | MG      | AG       | AGM      | Ascorbic Acid |
|------------|------------|---------|----------|----------|---------------|
| DPPH Method | 40.76 (0.9877) | 26.42 (0.9904) | 33.23 (0.9556) | 22.97 (0.9822) | 14.38 (0.9762) |
| NO Method   | 41.43 (0.9732) | 28.22 (0.9621) | 35.12 (0.9859) | 24.56 (0.9459) | 17.82 (0.9826) |
| H$_2$O$_2$ Method | 43.13 (0.9839) | 31.03 (0.9732) | 38.76 (0.9733) | 25.98 (0.9912) | 20.21 (0.9458) |

Note: All values are mean of three replications, Values in bracket indicate regression coefficient of variability.
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Conflicts of interest

None.

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