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PHARMACEUTICO – ANALAYTICAL AND IN-VITRO STUDY CYTOTOXIC EFFECT OF *MEDHOARBUDAHARA ARKA*

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ABSTRACT
Arka Kalpana is a liquid dosage form in Ayurvedic pharmaceutics. Various methods of preparation are mentioned in *Arka Prakāśa*, depending on the nature of the drugs. However, comparatively few classical references are available regarding Arka preparation. In *Arka Prakāśa* (6th Śataka), *Medoarbudhahara Arka* is mentioned under the indication *Medoarbuda*. In todays practice, many Vaidyas follow the preparation method prescribed in the Ayurvedic Formulary of India (AFI). To compare them, a pharmaceutical, analytical, and in vitro cytotoxicity study was conducted on the MCF-7 and OP9 cell lines. Two batches were prepared: one according to classical references, and the other following the AFI method.

KEYWORDS: Arka, Medoarbudhahara arka, Manashila, MTT Assay.

INTRODUCTION

Ayurveda describes many different dosage forms, many of which contain chiefly herbo-mineral components. These dosage forms include liquids, solids, semisolids, etc *Arka* falls under the liquid dosage form. In *Arka Prakasha*, Lankapati Ravana declares Arka to be one of the *Panchavidha Kashaya Kalpana*.[1] While explaining its importance he mentioned that even from stones that is *Rasa Dravyas* also we can extract the *Arka*. He mentioned that *Arka* has *Sarva Dhatu Vedaka*[2] action that is penetration into deeper tissues and leads to strengthening of the tissues. *Arbuda* mentioned in our classics is a *Tridoshaja Vyadhi*, which involves *Rakta, Mamsa, and Meda Dhatu*. According to *Acharya Sushruta, Medoja Arbuda is a Sadhya Vyadhi*.[3]

In *Arka Prakasha* author mentioned *Medhoarbudahara Arka*[4] prepared with ingredients *Shudha Manashila, Gruhadhooma, Patanga, Lodra, Haridra* and *Madhu. Shudha Manahshila*[5] having the *Sarvarasayanagra Lekhana, Patanga*[6] having property like *Rakta Shodhaka Pitta Kaphahara, Lodra*[7] having *Shotahara Karma* and *Haridra*[8] having *Raktashodhak, Shotahara Karma* and *Madhu*[9] having *Lekhana* and *Kaphahara Karma, Grahadhuma* being the carbon compound[10] it is believed to have action similar to activated charcoal probably binds with the poison to act as physical antidote and surface adsorbent.

Medhoarbhudahara Arka[4], an *Arka* preparation containing Rasa dravya, was not available in the market. It could be easily prepared and administered to patients. *Medhoarbhudahara* Arka is primarily indicated for *Medaja Arbhuda*. A comparative study was conducted to analyse the cytotoxic effect of *Medhoarbhudahara* Arka on human breast cancer cells (MCF-7) and normal cells (OP9), focusing on its involvement with *Medo Dhatu*, using the MTT assay."

MATERIALS AND METHOD

Table -1: List of ingredients of Medoarbhudahara Arka.

Sl no	Dravya	Botanical name	Part used	Quantity
1	*Haridra*	Curcuma longa *linn*	Coarse powder	35g
2	*Lodra*	Symplocos racemosa *roxb*	Coarse powder	35g
3	*Patanga*	Caesalpinia sappan *linn*	Coarse powder	35g
4	*Gruhadooma*	soot	Coarse powder	35g
5	*Manashila*	Arsenic disulphide	Fine powder	35g
6	*madhu*	Honey		35g
7	*Jala*			2250 ml

PROCEDURE

Two batches of *Medoarbudhara Arka* were prepared — the 1st batch according to Arka Prakāśa and the 2nd batch according to Ayurvedic Formulary of India references."

Manashila shodhana[11]

Manashila shodhana was performed by *bhāvanā* with Adraka svarasa for seven times.

Batch -1 method of preparation[4]

- *Haridra*, *Lodhra*, and *Pattanga* were individually taken and coarsely powdered (*Yavakuta Choorna*) separately."
- The coarse powder of *Haridra,Lodra,Patanga,Gruhadooma* and fine powdered of Manashila were weighed each 35 g were taken and mixed well.
- Then, 350 mL of water was added to the mixture and kept for soaking 8 *praharas* under sunlight (*Sūrya kīraṇa*) and 8 *praharas* under moonlight (*Chandra kīraṇa).*
- After that 35 g of honey were added to it, and mixed thoroughly.2100 kg of water was added to mixture.
- The mixture was transfer to the distillation apparatus. Started the distillation. the initially the temperature was kept 60-70℃ when boiling was stated after 60 minutes after that temperature was reduced 30-40℃ and distillate stared collecting after 90 minutes. first few drops were discarded
- 1260 ml of *Medoarabudahara* was collected and stored in air tight container.

OBSERVATION

- Patanga is difficult to pound compare to haridra and lodra.
- Water content was completely evapouated.
- Drugs become soft and absorbed the water. And reduced in weight around 180 g.
- *Manashila* was settled in the bottom of the vessel.
- Boiling was started after 60 mins
- Distillation was started collecting after 90 minutes
- The oil drops were seen in the upper surface of the Arka
- The total 6 hours were taken for the distillation.

Table no -2: Temperature chart for *surya kiran /Chandra kirana.*

DATE	TIME	TEMP
2/1/2025	11:30 am	26℃
2/1/2025	3:00 pm	27℃
2/1/2025	7:00 pm	23℃
2/1/2025	10:00 pm	19℃
3/1/2025	12:00 am	17℃
3/1/2025	2:00am	16℃
3/1/2025	5:00 am	18℃
3/1/2025	7:00am	19℃
3/1/2025	9:00am	20℃
3/1/2025	12:00pm	26℃
3/1/2025	2:00pm	29℃
3/1/2025	4:00pm	27℃
3/1/2025	8:00pm	21℃
3/1/2025	11:00pm	19℃
4/1/2025	1:00am	16℃
4/1/2025	4:00am	15℃
4/1/2025	6:00 am	15℃
4/1/2025	10:00am	19℃
4/1/2025	11:00am	21℃
4/1/2025	1:00pm	25℃
4/1/2025	3:00pm	23℃
4/1/2025	5:00pm	21℃
4/1/2025	8:00pm	19℃
4/1/2025	10:00pm	17℃
4/1/2025	11:00pm	18℃
5/1/2025	6:00am	16℃
5/1/2025	10:00am	20℃
5/1/2025	1:00pm	27℃
5/1/2025	4:00pm	24℃

Batch -2 method of preparation[12,13]

PROCEDURE

- The coarse powder of *Haridra*, *Lodra*, *Patanga*, *Gruhadooma* and fine powdered of *Manashila* were weighed each 35 g were taken and mixed well.
- Then, 250 mL of water was added to the mixture and kept for soaking.(6hrs)
- After that 35 g of honey were added to it, and mixed thoroughly.2100 kg of water was added to mixture.
- The mixture was transfer to the distillation apparatus. Started the distillation. the initially the temperature was kept 60-70℃ when boiling was stated after 70 minutes after that temperature was

reduced 30-40℃ and distillate stared collecting after 1 hr 14 minutes. first few drops were discarded.
- 1260 ml of *Medoarabudahara* was collected and stored in air tight container.

OBSERVATION
- Water content was remain the vessel.
- Drugs become soft, swollen and absorbed the water .weighed around 420g
- Manashila was settled in the bottom of the vessel.
- Boiling was started after 70 mins.
- Distillation was started collecting after 1hrs 14 min minutes
- The oil drops were seen in the upper surface of the Arka
- The total 6hours 38 minutes were taken for the distillation.

PRECAUTIONS
- Drugs are coarsely powdered.
- Soaked well in water and covered vessel mouth with bandage cloth.
- Distillation apparatus cleanly through and dried well.
- The collecting flask mouth was closed with aluminium foil.
- First few drops were discarded.
- Temperature was maintained.

IN VITRO STUDY BY MTT ASSAY
RESULTS
Table – 3: Organoleptic characters Medoarbhudahara Arka.

Organoleptic character	MAA B-1	MAAB-2
Colour	Colour less	colourless
Odor	Pungent	Pungent
Consistency	Liquid	Liquid
Taste	Tasteless	Tasteless

Table -4: Refractive index Medoarbhudahara Arka.

Sl no	Name of sample	Temperature (℃)	Refractive index(nD)
1	MAA B-1	20℃	1.3332
		25℃	1.3327
2	MAA B- 2	20℃	1.3342
		25℃	1.3337

Table -4: Physio-chemical analysis Medoarbhudahara Arka.

Physico-chemical analysis	MAA B-1	MAA B-2
pH @ 25℃	4.82	4.92
Specific gravity @25℃(g/cm^3)	1.0172	1.0178
Volatile matter	7.92%	8.64%
Non -Volatile matter	92.08%	91.36%
ICPOES -arsenic	<O.1ppm	0.25ppm

MTT Assay Results- MTT assay was analysed by for the given research samples using MCF 07 cell line for cytotoxicity study. The results depicted a overall inhibition of 71 and 69 % for MAAB I and MAAB II respectively. Further when the same was compared to normal cell the inhibition was found to be 44 and 46 % of MAAB I and MAAB II indicating the less toxicity profile compare to cancer cell line.

IMAGES

Figure -1: ingredients of Medoarbudahara Arka

Figure -2: mixing the ingredients

Figure -3: soaking in double quantity of water

Figure -4: soaking in surya kirana

Figure -5: soaking in Chandra kirana

Figure -6: adding Madhu

Figure -8: prepared medoarbudahara arka

Figure -7: distillation set up

DISCUSSION

Discussion on selection of the topic

Medoarbudahara Arka is an Arka preparation containing *Rasaousadhi* that is not currently available in the market and has not been researched to date. No prior research has been conducted on any *Arka* formulation containing *Rasaousadh*i. This study therefore presents a unique opportunity to introduce clinicians and researchers to this novel formulation, made to study the cytotoxic affect of *Medoarbudahara Arka*[4] on human breast cancers cells and normal cells having involvement of *Medo Dhatu* using MTT Assay.

Discussion on Ingredients

Haridra, Lodhra, Patanga, Manashila, Gruhaduma, and Madhu mainly have katu, tikta, and kashaya rasas and ushna veerya, and they primarily act on Kapha and Vata doshas, and due to their lekhana, raktaśodhaka, and rasāyana karmas, they will act on Arbuda

Pharmaceutical Discussion

Medhoarbhudāhara Arka, mentioned in the sixth śataka of *Arka-prakāśa*[4] under *Auṣhad-Arka Yoga*, is a rare formulation with no other known references in classical texts. Quantity of IngredientsThe śloka does not specify proportions. According to the *Ādhārabhūta Siddhānta of Bhaishajya Kalpanā,* when no explicit quantity is given, each ingredient should be used in equal measure Additionally, because these are considered *kaṭina dravyas*, the methodology for preparation must align with the classical principles governing such materials.

Manashila shodhana[14] was done with adraka swarasa Arsenic poisoning reduces the level of glutathione in the blood. It has been shown that, ginger acts as an antidote by reducing fall in the amount of glutathione in the blood.

- Studies have shown that Ginger juice decreased the elevated character of NFκB and TNF-α. Anti-inflammatory activities: so it helps in anti-cancer activity. So, this ingredient was selected for the *Bhavana.*
- *Ardraka* is acidic in nature with a pH of 3.6107; hence, it may help to combat the *Tikshnatva* of *Manahshila. Manahshila* is highly alkaline with a pH of 8.3; so, ginger is an optimal medium to balance the pH *of manahshila* - Neutralization of alkalinity.

Probable Reason for Using *Yavakuta Choorna* in Preparation[15]

Yavakuta Choorna, which passes through sieve number 10/ 44, was considered a coarse powder suitable for the preparation.

1. **Optimal Extraction of Active Constituents:** The coarse texture of *Yavakuta Choorna* allows for effective extraction of medicinal properties during the boiling process, ensuring a potent arka.
2. **Enhanced Surface Area for Solvent Interaction:** A finer particle size increases the surface area, facilitating better interaction with the solvent (water), which aids in the efficient transfer of active compounds.

Importance of Exposing to Surya-Kirana / Candra-Kirana

Activation by Light / Photochemical Effects
Sterilization / Disinfection
Change in Properties
Enhancing Volatile Component Liberation
Stability / Potency Enhancement
Probable Cause of Manashila Settling at the Bottom
It is heavy in nature and has a significantly higher specific gravity compared to other substances
Addition of Honey in Arka Preparations at last Since honey is a liquid, it need not be soaked with other drugs in the Arka preparation; it can be added directly.

Addition of 10 parts water in Arka Preparation After exposing the drugs to Suryakirana and Chandra kirana, all the water content was evaporated, making it impossible to extract the Arka from the drug. Therefore, as per AFI, 10 parts of water were added to the drug to facilitate the extraction of Arka.

- Medoarbhudahara arka :Batch -2 .This batch was prepared as per the AFI references to validate the change

Analytical Discussion

Discussion on Organoleptic characters

There is little difference in the organoleptic characteristics compared to the first batch. However, the second batch has a stronger pungent odor and taste.
In arka prakasha it mentioned that smell should be pleasant but in medoarbhuda hara arka it pungent smell it may be because of presences of manashil.
In Manashila, the two sulfur atoms are bonded together; that is what causes the strong, pungent smell initially. Even Gandhaka is called by names like *Puti-Gandha* and *Ugra-Gandha.*[16] After some time has passed, the smell becomes much milder as the substance settles or the volatile components dissipate**.**

Discussion pH

- The pH of MAA B-2 is 0.10 units higher than that of MAA B-1 (4.92 − 4.82 = 0.10). This means that MAA B-2 is slightly less acidic than MAA B-1**.**Acidic pH increases cytotoxicity and is associated with changes in cell metabolism, altered Akt kinase and NF-κB activity, and increased reactive oxygen species production even acidic increases the Bioavailability.[17,18]

Discussion specific gravity

- MAA B-2 is marginally denser than MAA B-1. This could mean that B-2 has a slightly higher concentration of solutes/solids (dissolved substances, suspended particles, salts, etc.). If these are formulations or solutions, B-2 may have more dissolved material (or less diluent), making it heavier per unit volume.

- **Discussion Volatile matter and non -volatile matter**

Volatile matter in B-2 is higher by 0.72 percentage points. Because the first batch was kept under Surya Kírana and Chandra Kírana, some volatile matter may have evaporated. The volatile matter present in the formulation is due to Haridra. Even Manahshila contains negligible volatile matter because Shodhana was performed using Adraka Swarasa.

- **Discussion on refractive index**

MAA B-2 shows a slightly higher refractive index than MAA B-1, with a difference of 0.0010, suggesting slight variations in composition or structure between the two batches.

Discussion on ICPOES –arsenic.

- The first batch contains lower arsenic levels compared to the second batch due to prolonged soaking in water. Consequently, some volatile compounds of *Manashila* may have evaporated or changed form."

- **Discussion on** TLC

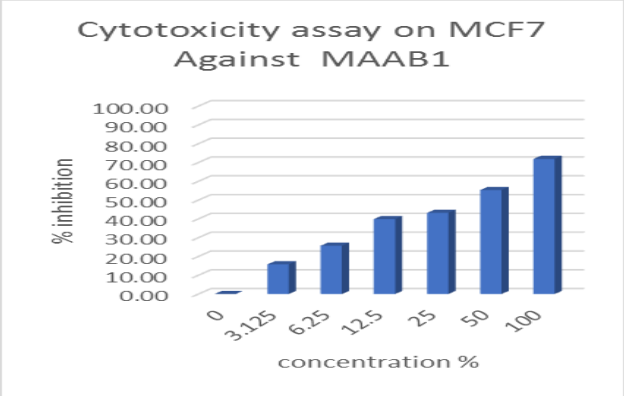
Both samples exhibited the presence of flavonoids, tannins, curcumin, desmethoxycurcumin, bisdemethoxycurcumin, brazilin, and sappanchalcone, with slight variations between them. These compounds are attributed to the inclusion of Haridra (Curcuma longa), Lodra (Symplocos racemosa), Patanga, and honey in the formulations.

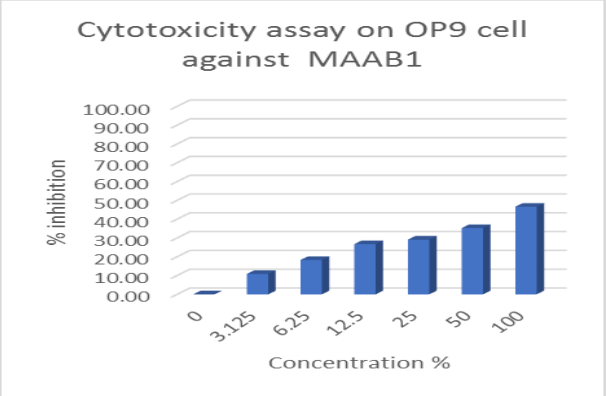
The MTT assay results demonstrate that both MAAB1 and MAAB2 exhibit significant cytotoxic effects on the MCF-7 breast cancer cell line, with inhibition percentages of 71.90% and 69.43%, respectively, at the highest concentration tested (100%). These findings suggest that MAAB1 and MAAB2 possess potential anticancer properties, as indicated by their ability to reduce cell viability in MCF-7 cells.

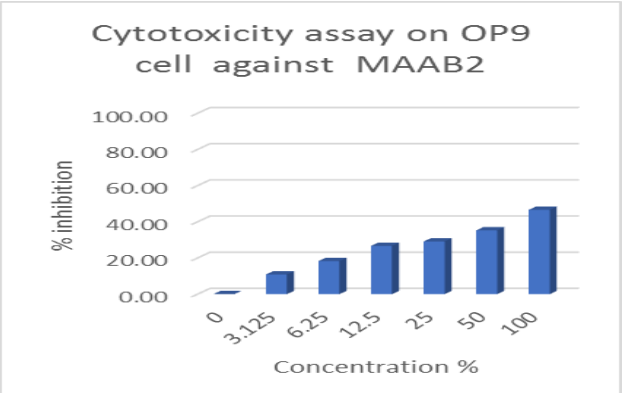
In contrast, when tested on the OP9 murine stromal cell line, which serves as a model for normal cells, the inhibition percentages were lower: 44.18% for MAAB1 and46.60% for MAAB2 at the same concentration. This indicates that while MAAB1 and MAAB2 exhibit some level of cytotoxicity towards normal cells, their effects are less pronounced compared to cancerous cells.

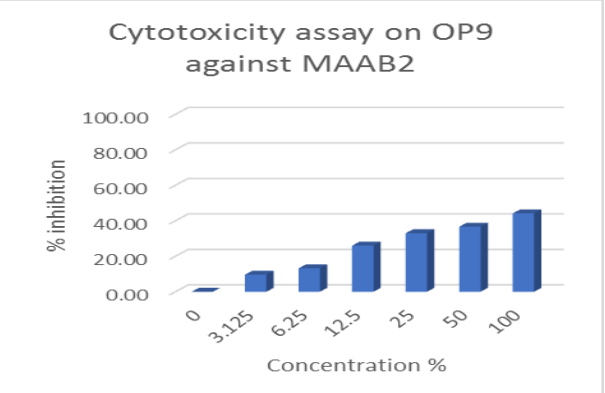
The differential cytotoxicity observed between cancerous and normal cells suggests that MAAB1 and MAAB2 may selectively target cancer cells, sparing normal cells to some extent. This selectivity is a desirable characteristic in anticancer agents, as it may reduce the side effects associated with conventional chemotherapy, which often affects both cancerous and normal cells.

However, it is important to note that the observed cytotoxicity in normal cells, although lower than in cancer cells, still warrants further investigation. Additional studies are needed to assess the long-term effects, potential toxicity at lower concentrations, and the underlying mechanisms of action of MAAB1 and MAAB2.









CONCLUSION

Medoarbhudahara formulation is mentioned in *Arka Prakasha*, in the sixth Śataka. It contains the ingredients Haridra, Lodra, Patanga, Gruhadhuma, Manashila, and Madhu.

- It is an Arka preparation that includes a Rasa-Aushadha. It is indicated in *Medo Arbhuda*."
- "Manashila *śodhana* was done with *adraka swāraṣa bhāvana* seven times. Since adraka also has some anticancer activity, this purification enhances its medicinal potential.
- Two batches of *Medoarbhudahara Arka* were prepared.
- The first was made according to classical references: the material was soaked in two parts water, exposed to sun and moon rays for eight prahara, then the Arka was extracted.
- The second was made according to AFI (Ayurvedic Formulary of India) references: the material was soaked in a sufficient quantity of water, left for 9-10 hours, then the Arka was extracted. Both batches were compared through analytical methods and MTT assay.
- All analytical parameters were within limits. Both samples had acidic pH. The arsenic level by ICP-OES was < 0.1 ppm in the first batch, and 0.25 ppm in the second batch, respectively.
- MTT assay was analysed both samples using MCF 07 cell line for cytotoxicity study. The results depicted a overall inhibition of 71 and 69 % for MAAB I and MAAB II respectively. Further when the same was compared to normal cell the inhibition was found to be 44 and 46 % of MAAB I and MAAB II indicating the less toxicity profile compare to cancer cell line.
- It can be concluded that the two *Medoarbhudahara Arka* samples showed strong cytotoxicity against MCF-7 breast cancer cells. Although some toxicity was observed in normal cells, indicating they may not be safe for long-term use, the first batch showed better results than the second batch.

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