International Journal of Indian Medicine

www.ijim.co.in
ISSN: 2582-7634
Volume 4, Issue - 4

IJIM

INDEXED

April 2023

Original Article

eISSN : 2582 - 7634

International Journal of Indian Medicine, 2023, 4(4): 60-68 *ISSN: 2582-7634*

Access the article online

International Journal of Indian Medicine

International Category Code (ICC): ICC-1702 International Journal Address (IJA): IJA.ZONE/258276217634

Evaluation of the pharmacokinetic aspect of Brahmi Ghrita by oral and nasal route.

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Abstract:

Context: Nasya Karma is the main treatment for all the supra-clavicular diseases, i.e. disease of head, neck, mouth, eye, ear and nose. Administration of drugs by the route of nasal cavity is termed as Nasya Karma Nasal route of drug administration provides systemic absorption of drug and its fast action in low doses. But it is not clear till date the active principles of Ayurvedic formulation used in Nasya get absorbed in systemic circulation. **Aim**: To study comparative pharmacokinetic aspect of Brahmi Ghrita in experimental animals after nasal and oral administration and to explore the mode of action of Nasya. **Materials and Methods: For** pharmacokinetics study of Brahmi Ghrita same dose was use for nasal and oral administration in wistar strain albino rats. Bacoside A3 was used as marker compound and it's absorption in systemic circulation was assessed using HPTLC. **Results**: Bacoside A3 present in Brahmi Ghrita after nasal and oral administration was found to get absorbed in to systemic circulation. **Conclusion**: The intranasal administration of Brahmi Ghrita is an effective way for systemic availability of drugs and has extended drug absorption as compared to oral routes for same dose.

Key words: Brahmi Ghrita, Nasya, Bacoside A3

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How to cite this article: Gulhane CM, Thakar AB, Naria M. Evaluation of the pharmacokinetic aspect of Brahmi Ghrita by oral and nasal route. Int J Ind Med 2023;4(4):60-68

INTRODUCTION: Administration of drugs by the route of nasal cavity is termed as Nasya Karma[1]; it is the main treatment for all the supra- clavicular diseases, i.e. disease of head, neck, mouth, eye, ear and nose. *Acharya Charaka* included *Brahmi* in the list of *Shirovirechana Dravya* (medicine used for nasal administration).[2] *Brahmi* (*Bacopa moniera* Linn.) is a *Medhya* (cognitive) drug described in Ayurvedic classics.[3] *Bacopa moniera* (*Brahmi*) is a good Nootropic[4,5] (smart drugs, memory enhancers, and cognitive enhancers as well as intelligence enhancers). Its traditional use is as an important Ayurvedic traditional herbal medicine. It is used as a nerve and brain tonic to improve memory, learning and concentration. In addition, it repairs nerve damage, as well as stroke and brain injury. Some experimental studies show *Brahmi* Improves Learning and Memory in Mice.[6] Bacosides which are active ingredients of Brahmi repair damaged neurons by boosting kinase, which is the protein used to synthesize new neurons. It improves the synaptic activity and thus memory can be restored.[7]

Many experimental studies has been carried out in modern medicine which show that nasal delivery has been explored as an alternative administration route to target drugs directly to the brain.[8] But till date no experimental study is carried out using Ayurvedic preparation like *Brahmi Ghrita Nasya* and also it is not clear that when Nasya is given the active principles in the formulation gain entry in to the systemic circulation or not. The present study was planned to acquire some preliminary data with regards to the absorption of phytochemical constituents of the formulations when administered in the form of Nasya. The aim of this study was to explore the pharmacokinetic of *Brahmi Ghrita Nasya* using Bacoside A3 in experimental animals.

Aim and objective: To study comparative pharmacokinetic aspect of *Brahmi Ghrita* in experimental animals after nasal and oral administration.

MATERIAL & METHODS:

Test formulation

The fresh *Bhrahmi (Bacopa moniera Linn.*) was collected from Jamnagar and authenticated by pharmacognocy department. *Brahmi Ghrita*[9] *was* prepared in the pharmacy of ITRA, Gujarat Ayurved University, Jamnagar.

Animals: Healthy Wistar strain albino rats weighing between 280 ± 10g were of either sex were used for the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC/10/2012/04) in accordance with the guideline formulated by CPCSE, India.

Husbandry conditions: Animals were kept in polypropylene cage with top stainless steel grill. Peddy husk was used as bedding material for animals. The animals were exposed to 12 hours light and 12 hours dark cycle with the relative humidity of 50 to 70% and the ambient

temperature was 22 ± 03°C. All animals were kept on same environmental conditions**.** The selected animals were fasted over night before experiment.

Diet: Amrut brand rat pellet feed supplied by Pranav Agro Ltd. was provided throughout the study period. Drinking water was given *ad libitum* in polypropylene bottles with stainless steel sipper tube. The animals were fasted overnight before experimentation.

Acclimatization period: All the selected animals were kept under acclimatization for seven days before experimentation.

Numbering & Identification: The animals were marked with saturated picric acid for proper identification.

Dose fixation: Dose of the drug was calculated by extrapolating the human therapeutic dose to rat and mice on the basis of body surface area ratio (conversion factor 0.018 for rat) by referring to the table of Paget and Barnes (1964)[10]

Brahmi Ghrita and Plain *Ghrita :* The adult dose of *Ghrita* is 16ml[11,12] for nasal and oral administration.

Rat dose = Therapeutic human dose × conversion factor for rat of 200 g

= 16 ml × 0.018 = 0.288 ml for 200 g rat = 1.44 ml/kg body weight of rat

Route of drug administration: The *Ghrita* administered according to the body weight of the animals by oral route with the help of oral feeding canula and nasal administration by micropipette. The test drug and vehicle were administered between 08:30 am to 09:30 am.

Instruments used: Weighing scale, micro-titre plates, serological water bath, digital Plethysmograph, burette, needle, syringe, surgical instruments, centrifuge machine, refrigerator, water bath etc.

Experimental protocol: The selected animals were grouped randomly irrespective of sex into two groups each consist of three animals. Group I received *Brahmi Ghrita* through *Nasya* route (1.44 ml/kg) and Group II received *Brahmi Ghrita* through *oral* route (1.44 ml/kg). In the morning initial blood sample was collected through retro-orbital puncture under light ether anaesthesia. Afterwards the *Brahmi Ghrita* was administered through oral and nasal route to respective groups. Blood samples were again taken at regular interval of post-dose (after drug administration), 30 mins, 60 mins, 120 mins and 180 mins for estimation of biomarker in the serum through HPTLC method. From the blood samples, serum was separated by centrifuging at 3000 rpm for 10 minutes. The serum samples were subjected to HPTLC analysis to know absorption of *Brahmi Ghrita* through nasal and oral route in to systemic circulation by using Bacoside A3 as marker compounds.

Chromatographic conditions:

- Application mode : CAMAG Linomat V Hamilton Syringe

- Development chamber : CAMAG Twin trough chamber (20 x 10 cm^2)
- Plates : Precoated silica gel GF_{254} plates
- Chamber saturation : 30 min
- Development distance : 8 cm
- Development time : 30 min
- Scanner : CAMAG TLC Scanner III
- Scanning mode : Linear at 254 nm and 366 nm
- Detection : Deuterium lamp, Mercury lamp
- Photo documentation : CAMAG reprostar
- Data system : CATS software (Ver. 3.17)
- Drying device : Oven
- U.V. Spectrum : 200 nm to 700 nm

Steps involved in HPTLC:

- Selection of chromatographic layer.
- Sample and standard preparation.
- Layer pre-washing, Layer pre-conditioning.
- Application of sample and standard.
- Chromatographic development.
- Detection of spots.
- Scanning.
- Documentation of chromatic plate.

Serum sample preparation:

- 1ml serum was adjusted to pH 2.5 with 300µl of 1 mol potassium dihydrogen phosphate solution & 30µl phosphoric acid.
- 5ml of acetonitrile was added and vertex mixed for 1min. It forms a precipitate.
- It was centrifuged at 3500 r.p.m. for 15min at 8-10^{0}C.
- Supernatant liquid is evaporated to dryness at 37^{0}C.
- Residue reconstituted in 1 ml methanol.
- The solution was filtered through Whatman filter paper No. 41 in a dry stoppered tube & stored at 0-4^{0}C for further analysis. This solution was used for HPTLC analysis.

Standard preparation: Reference standard of Bacoside A3 were provided by M/s Natural remedies vt.Ltd. Bangalore India. Standard was prepared in alcohol (Std.S s 1mg/ml)

Sample for HPTLC

For HPTLC study following samples were titled as track 1-13

Track-1 : Processed serum sample of before nasal administration of *Brahmi Ghrita*

Track-2 : Processed serum sample of before oral administration of *Brahmi Ghrita*

Track-3: standard sample of Bacoside A3 2.5 µg (Std.S s 1mg/ml)

Track-4: standard sample of Bacoside A3 5.0 µg

Track-5 : standard sample of Bacoside A3 7.5 µg

Track-6: Processed serum sample of after 30 min of nasal administration of *Brahmi Ghrita*

Track-7: Processed serum sample of after 30min of oral administration of *Brahmi Ghrita*

Track-8: Processed serum sample of after 60 min of nasal administration of *Brahmi Ghrita*

Track-9: Processed serum sample of after 60 min of oral administration of *Brahmi Ghrita*

Track-10: Processed serum sample of after 120 min of nasal administration of *Brahmi Ghrita*

Track-11: Processed serum sample of after 120 min of oral administration of *Brahmi Ghrita*

Track-12: Processed serum sample of after 180 min of nasal administration of *Brahmi Ghrita*

Track-13: Processed serum sample of after 180 min of oral administration of *Brahmi Ghrita*

Selection of mobile phase

In liquid chromatography, the solute retention is governed by the solute distribution factor, which reflects the different interactions of the solute - stationary phase, the solute - mobile phase and the mobile phase – stationary phase. For a given stationary phase, the retention of the given solute depends directly upon the mobile phase, the nature and the composition of which has to be judiciously selected in order to get appropriate and required solute retention. The mobile phase has to be adapted in terms of elution strength (solute retention) and solvent selectivity (solute separation). Solvent polarity is the key word in chromatographic separations since a polar mobile phase will give rise to low solute retention in normal phase and high solute retention in reversed phase LC.

Mobile phase for present study:

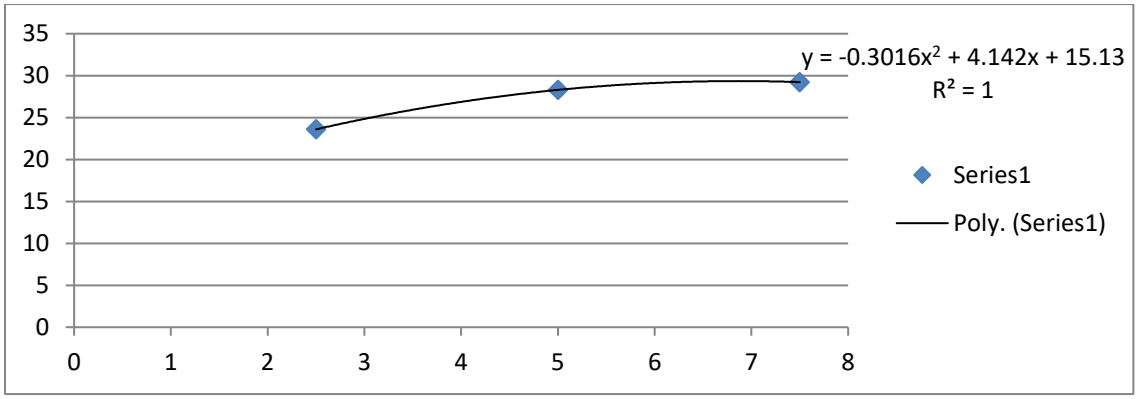
Mobile phase: Dichloromethane (85) : Methanol (15) : Acetic acid (5 V/V)

Procedure and Data Analysis:

The *Chromatographic System* was set up as above. Separately injected equal volumes (about 20µl) of the Standard preparation and the serum sample preparation into the chromatograph, the chromatograph for qualitative percentage of Bacoside A3 were recorded and any peaks from the Mobile phase preparation were discarded. Quantitative determination was carried out with previously used external standard method[13] using Microsoft excel sheet. Maximum serum concentration was determined by visual inspection of the Data.

OBSERVATION & RESULTS:

Fig-1: Linearity curve for standard Bacoside A3 (scatter plot)



Calibration curve at graph area

Fig no.2 *in situ* spectrum of samples shows presence of Bacoside A3 in all sample after *Nasya* and oral administration of *Brahmi Ghrita*.

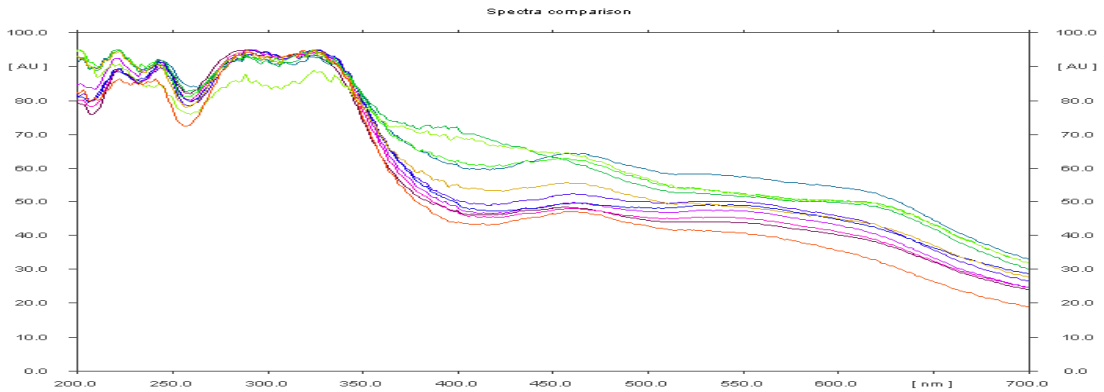
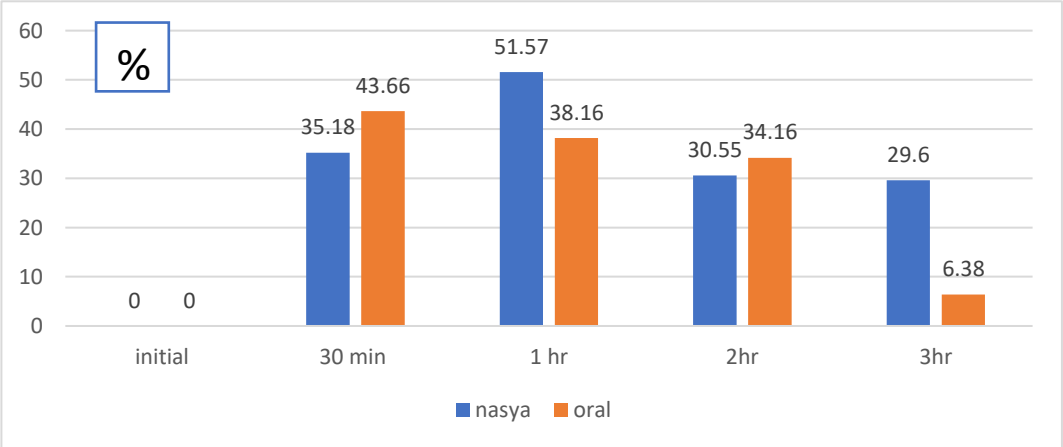


Fig no. 3: % area under curve (Short U.V. 254 nm) of Bacoside A3 in serum HPTLC after oral and nasal administration of *Brahmi Ghrita* in experimental animals.



In Short U.V. (254 nm) maximum concentration of Bacoside A3 at Rf value 0.95 and 0.94 was observed in standard sample of Bacoside A3. Serum samples of collected from both groups also showed the same Rf values for maximum concentration of Bacoside A3. From the above shown graph, it is clear the Bacoside A3 were present in the plasma of both group samples after oral and nasal administration of *Brahmi Ghrita* collected at various time intervals. *Brahmi Ghrita* through oral route showed that maximum concentration of Bacoside A3 was appeared in serum sample after 30 mins (43.66%) and thereafter the concentration was gradually decrease after 1 hr (38.16%), 2 hrs (34.16%) and 3 hrs (6.38%). *Brahmi Ghrita* through nasal route showed that maximum concentration of Bacoside A3 was appeared in serum sample after 1 hr and thereafter the concentration was gradually decrease after 2 hrs (30.55%) and 3 hrs (29.60%).

DISCUSSION

Intranasal route has promising approaches for delivery of drugs to the brain. The delivery of drugs to the CNS from the nasal route may occur via olfactory neuroepithelium. The intranasal administration of drugs is an effective way for systemic availability of drugs as compared to oral and intravascular routes. Actually, it seems to present fast and extended drug absorption.[14] This experimental study is carried out for to study the pharmacokinetic *Brahmi Ghrita* after nasal and oral administration with same dose. Bacoside A3 was present in the serum sample of rats after 30 mins of drug administration through oral and nasal routes. This indicate that drug have fast absorption though both routes in experimental animals. *Brahmi Ghrita* through oral route showed that maximum concentration of Bacoside A3 in serum was observed after 30 min and thereafter gradually decrease after 1 hr, 2 hrs. and 3 hrs of drug administration. After 3 hrs the concentration of Bacoside A3 was almost decrease which indicate the diminution of active component of *Brahmi Ghrita* through oral route after 3 hrs. *Brahmi Ghrita* through nasal route showed that concentration of Bacoside A3 in serum was increased from 30 mins to 1 hr. at maximum level and thereafter gradually decreases after 2 hrs and 3 hrs of drug administration. After 3 hrs, the concentration of Bacoside A3 was still 26.9% in serum which indicates that drug through nasal route may have extended efficacy compare to oral route.

Lipophilic drugs are well absorbed from the nasal cavity, exhibiting pharmacokinetic profiles similar to those obtained after intravenous administration. These drugs are absorbed quickly and efficiently across the nasal membrane via trans cellular mechanisms. This observation is true for lipophilic compounds having molecular weight lower than 1 kDa. On the other hand, the rate and degree of nasal absorption of polar drugs is low and highly dependent of the molecular

weight. Drug absorption is expected to be diminished with decrease lipophilicity because the nasal membrane is lipophilic. Whenever lipophilicity is too high, the drug permeation through the wall may be reduced because drug does not dissolve easily in the aqueous environment of nasal cavity.[15] Oil and Ghee preparations are more viscous in nation it is proved that formulation with higher viscosity has a better contact time thus increases the absorption. At the same time, high viscosity enhanced the permeability of drugs.[16]

Clinically also nasal administration of *Brahmi Ghrita* effective against oral same dose. Relatively low doses are effective when administered through nasal route with less systemic toxic effects. It is proved that the intranasal administration of drugs is an effective way for systemic availability of drugs as compared to oral and intravascular routes. Actually, it seems to present fast and extended drug absorption, and it has been supported by many studies planned to compare intranasal drug delivery against oral and parenteral administration.[17]

CONCUSSION:

From the present study, it is concluded that *Brahmi Ghrita* is absorbed in systemic circulation from oral and nasal route in rats. The intranasal administration of drugs is an effective way for systemic availability of drugs as compared to oral and intravascular routes for same dose. Actually, it seems to present fast and extended drug absorption. The intranasal route is an accessible alternative route for *Brahmi Ghrita* drug administration. This route provides future potential for several drugs through the development of safe and efficacious formulations for simple, painless and long-term therapy. From this route drugs can be directly target to the brain in order to attain a good therapeutic effect in CNS with reduced systemic side effects.

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Source of Support : None declared
Conflict of interest : Nil

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