Characterization and Analysis of Bhasma

¹H P E De Zoysa, ² C Jayaweera, ¹ M R P Dassanayake

¹Industrial Technology Institute, 363, Bauddhaloka Mawatha, Colombo-7 ²University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka

Abstract: Rasashastra is a branch of Ayurveda known as the Ayurvedic metallurgy which deals with medicinal properties of minerals, metals, animal products and poisonous plants. Bhasma belongs to this category which is prepared by utilizing metals and minerals of aquatic and soil origin. Bhasma have been used for treating various diseases and as preventive medicines. Traditionally, Bhasma are classified based on their color and appearance. Scientifically they are classified based on dominant metal and mineral groups. The adverse side effects caused by synthetic drugs become a factor that motivates mankind to go back to nature for safer remedies, which generates a considerable demand towards Ayurvedic drugs. Hence, rate of production of Bhasma is increased which leads to carry out inaccurate and inappropriate preparation protocols that lead to produce poor quality drugs and toxic substances. Adulteration and or substitution and presence of free particles affect on quality and safety of Bhasma. Due to lack of procedures for standardization, in spite of its excellent merits, Bhasma has yet remained as a traditional drug.

Therefore, both proper validation and standardization are equally important to prevent adverse health effects. It is important to analyze raw materials and end products of herbal- mineral formulations to control quality of Bhasma. In addition to that, standardization of manufacturing process and in- process quality control helps to produce reproducible quality products with minimal defects or variations. Preparation protocol for Bhasma varies from manufacturer to manufacturer and there is many Ayurvedic texts describing different methods and raw materials. Hence, it becomes obligatory to adopt modern analytical methodology to determine the important chemical constituents present in Bhasma qualitatively and quantitatively. But lack of procedures for standardization and chemical characterization, quality of herbal metallic products are questioned. Hence, it is essential to develop fingerprints for Bhasma by using modern analytical techniques.

Keywords: Bhasma, Characterization, Heavy metal toxicity, Thermogravimetry, Fourier transform infrared (FTIR) spectrometer, X-ray fluorescence (XRF) spectrophotometer.

1. INTRODUCTION

Traditional medicine is a science which has been practiced in most of the countries from generation to generation. Many of these countries have well established systems of traditional medicines which are supported by the government. Ayurvedic medicine which was originated within India and also practiced within nearby countries such as Sri Lanka can be considered as a system of treatment that promotes health while preventing and curing illnesses. There is an emerging market and demand for Ayurvedic medicine in other parts of the world.

In traditional medicine systems almost all the drugs are prepared by processing ingredients that could be found within environment. Plant, animal and mineral origin drugs are used in Ayurvedic system of medicine. Ayurvedic medicine can be divided into two broad disciplines based on the raw materials used for the preparation of drugs such as drugs consisting of herbal materials only and Rasashastra, in which metals are also being added to herbal materials.

Rasashastra is a branch of Ayurveda known as the Ayurvedic metallurgy. Rasashastra deals with medicinal properties of minerals, metals, animal products and poisonous plants. Bhasma belongs to this category which is prepared by utilizing metals and minerals of aquatic and soil origin. Bhasma gained their reputation as effective formulations for any disease

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compromising the aspects of nanotechnology (Wadekar,2005). During the process of production of Bhasma, the therapeutic value of metals is considered and modulate toxic metals into nontoxic formulations (Tripathi and Pandey, 2003).Bhasma are also referred as herbo-metallic preparations as they contain both metallic and herbal ingredients (Reddy, 2010).

Bhasma are made of tiny particles. Hence they can impart pharmacological efficacy effectively and efficiently within human body. Reduction in particle size facilitates absorption and assimilation of the Bhasma in the system (Sarkar and Chaudhary, 2010).Recent studies have claimed that the herbo-mineral formulations of Ayurveda constituting Bhasma to be equivalent and in tune with nanotechnology (Virupaksha et al., 2011). Bhasma have been used for treating various diseases and also as preventive medicines. Bhasma are classified based on their color and appearance. Scientifically they are classified based on their dominant metal and mineral groups, such as Rajata Bhasma (silver), Tamra Bhasma (copper), Loha Bhasma (iron), Pravala Bhasma (shells) etc.

2. TYPES OF BHASMA

| Name | Uses | Ingredients | |
|--|---|---|--|
| Tamra Bhasma | Helps in maintaining body circulation and tonicity, Used as an aphrodiasic agent | Cu, S, Hg | |
| Abhraka Bhasma (called as wonder drug due to its curative property in various ailments) | Treatment of hepatic dysfunction, Leukemia , Sex debility, azoospermia, cystic fibrosis, post encephalic dysfunction and cervical dysplasia | Biotite[K(Mg,Fe)3(AlSiO ₁₀)(OH) ₂], Ca, K, Si | |
| Lauha Bhasma | treatment of anemia due to iron deficiency | Fe ₂ O ₃ and Fe ₃ O ₄ | |
| Hiraka Bhasma | Excellent remedy for heart troubles, heart pains, contraction of veins and blood clotting. It is also a powerful tonic and antitumor agent | natural diamond carbon based drug that contains oxygen, sodium, magnesium, aluminum, silicon, phosphorus, sulfur, potassium, calcium, chromium and iron | |
| Naga Bhasma | used in treating diabetes, diarrhea, spleen and skin disorders. At high dosages Naga Bhasma causes cognitive dysfunctions and affects neuro- chemical parameters | Pb, arsenic disulfide | |
| Trivanga Bhasma | used to treat diabetes and as a diuretic drug | Pb, Zn, Sn | |

Table 01: Types of Bhasma

Tamra Bhasma:

Tamra Bhasma is known to have properties of maintaining body circulation, tonicity and used as an aphrodiasic agent. Purified copper, purified sulphur and purified mercury (Shodhit parad) are the main ingredients of Tamra Bhasma.

Abhraka Bhasma:

Abhraka Bhasma is produced from the mineral, mica (Biotite) $[K(Mg,Fe)3(AlSiO_{10})(OH)_2]$. It consists of Fe as a major element and Ca, K and Si in low concentrations. Abhraka Bhasma is widely used for the treatment of hepatic dysfunction, leukemia, sex debility, azoospermia, cystic fibrosis, post encephalic dysfunction and cervical dysplasia (Mookerji, 2001). Abhraka Bhasma is called as a wonder drug due to its curative property in various ailments.

Lauha Bhasma:

Lauha Bhasma is an Ayurvedic medicine prescribed for the treatment of anemia due to iron deficiency. It consists of Fe_2O_3 and Fe_3O_4 . Preparation of Lauha Bhasma involves several heating procedures those meant to convert the metal in to a fine, non-toxic and bio-available form.

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Hiraka Bhasma:

Hiraka Bhasma which is synthesized from natural diamond is an excellent remedy for heart troubles, heart pains, contraction of veins and blood clotting. It is also a powerful tonic and antitumor agent. It's a carbon based drug that contains oxygen, sodium, magnesium, aluminum, silicon, phosphorus, sulfur, potassium, calcium, chromium and iron other than carbon. During the process of formation of Hiraka Bhasma, Hiraka powder is heated in an iron ladle for a large number (approx. 108) of times. In this process diamond reacts with iron that after it triturated with plant juices (rose or aloe vera) and subjected to calcinations.

Naga bhasma:

Naga Bhasma is a lead based herbo-metalic preparation which has been used in treating diabetes, diarrhea, spleen and skin disorders. At high dosages, Naga Bhasma causes cognitive dysfunctions and affects neuro-chemical parameters. Purification of metal is done by quenching lead thrice sequentially in sesame oil, butter milk, cow's urine, sour rice gruel and horse gram decoction during the process of preparation of Naga Bhasma. In the next step purified lead is stirred well under heating with tamarind and peepal bark powder to reduce the metal to the powder form. The powdered lead is ground well with arsenic disulfide and other ingredients to obtain a texture which allows preparing round shaped discs. These discs are sun dried and subjected to control heating to obtain Naga Bhasma.

Swarna makshika:

Swarna makshika is used for the treatment of anemia, insomnia, convulsions, and for skin diseases (Sharma, 2004). Swarna makshika contains iron (Fe), Copper (Cu) and sulfur (S) as major elements.

Ayakandha Chenduram:

Ayakandha Chenduram consists of iron oxide, cinnabar, juice of Karisalai (*Ecliptaprostata*) as mentioned in Yaakoobu Vaidhya Chinthamani-70 Ayakandha Chenduram has been traditionally used to cure anemia.

Trivanga Bhasma:

Trivanga Bhasma is a calcinated metal and mineral based preparation which is used to treat diabetes and as a diuretic drug (Arvindet al., 2010). Lead, zinc and tin are the metals used in the preparation of Trivanga Bhasma along with plant materials. The preparation of Trivanga Bhasma involves two steps, sodhana and marana processes. In sodhana, naga [lead], vang [tin] and yasad [zinc] (26 g each) was detoxified by adding madhuka thaila. The compounds were then triturated with small amounts of herbal powders till a homogenous paste was formed. The obtained mixture is then subjected to marana process, and transferred to an earthen crucible covered with a lid and sealed with sealing clay. Finally it was kept for calcination and then is blended with kumariswars [Aloevera juice] to form a cake. The cake on drying obtained yellow colour which is Trivanga Bhasma (Panditet al, 1999)

3. SYNTHESIS OF BHASMA

Traditional process of manufacturing metallic Bhasma consists of several procedures including satvapatana(extraction of metal) and bhasmikarana (conversion in to the form of non toxic) ,sodhana (purification), marana (conversion to non toxic fine powder), mardana (preparation of intermediate mixture) and putapak (reactions at high temperature).

Traditional approach of synthesis of Bhasma consist of time-consuming and complicated processes such as purification and grinding together with number of herbs, juices, along with metal and or metallic compounds following calcinations in a complicated procedure to get the dry powder of Bhasma. Novel approaches such as sono-chemical technique could shorten the processing time; reduce the number of chemicals used and energy requirement leading to reduction in the cost of making the traditional Bhasma.

S. Sivasankaran et al carried out the synthesis of palladium based metal oxides by using a simple procedure which requires water, ethanol and palladium as chemicals, the synthesis could be carried out in any kind of sonication equipments such as the probe-type direct sonicator or the bath type indirect sonicator. These metal oxides can be used as aqueous slurries or in powder form. By using ultrasound energy, a series of palladium based mono and multi metal oxides such as Pd-Cu-O, Pd-Fe-O, Pd-Co-O, Pd-Mn-O, Pd-Cu-Mn-O, Pd-Cu-Ni-O etc can be produced. The synthesis

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of Pd- based metal oxides by this sonochemical process is a novel, facile, green, faster and inherently safer process as compared to the existing processes for the synthesis of metal oxides.

4. REQUIREMENT OF THE ANALYSIS OF THE QUALITY OF BHASMA

The adverse side effects caused by the synthetic drugs become a factor that motivates mankind to go back to nature for safer remedies which generates a considerable demand towards Ayurvedic drugs. As a result of huge demand, rate of production of traditional medicine is increased which leads to carry out inaccurate and inappropriate preparation protocols that may lead to produce poor quality drugs as well as toxic substances.

Adulteration and or substitution as well as presence of free particles have a significant effect on the quality and safety of the drug. Hence proper validation and standardization is important to prevent adverse health effects which can be caused by poor quality products. It is important to analyze raw materials as well as the end product of herbal– mineral formulations to control the quality of products. In addition to that, standardization of manufacturing process and in process quality control will help to produce reproducible quality products with minimal defects or variations.

Preparation protocol for Bhasma varies from manufacturer to manufacturer and there is many Ayurvedic texts describing different methods and raw materials. Hence it is required to develop fingerprints for Bhasma.

It becomes obligatory to adopt modern analytical methodology to determine the important chemical constituents present in the drug qualitatively and quantitatively. But lack of procedures for standardization and chemical characterization, the quality of the herbal metallic products are questioned. Due to lack of procedures for standardization, in spite of its excellent merits and routine since ancient times, Bhasma has yet remained as a traditional drug.

Conventional studies to evaluate the quality of Bhasma:

To evaluate the final product of Bhasma, several test methods were used during ancient times. These methods are relatively simple and mostly based on physical characteristics. Hence the accuracy of the test results may be questionable. Some of them are described in table 02.

| Name of test | Procedure in brief | Results | Interpretation of result |
|------------------------------|---------------------------------------|------------------------|---|
| Nishchandratva | Check whether there is | Bhasma should not | Every particle of mineral is |
| (physical test) | any(luster) "chandrika" | contain luster | incinerated and converted in to |
| | in metal as metal reflects | | Bhasma form. |
| | light when fall on it. | | |
| Rekhapurnatvam | study sukshmata | Bhasma particles | Rate of absorption of drug is |
| (organoleptic method) | (Fineness) of Bhasma by | should be minimum in | directly proportional to particle |
| | fingertips by thumb and index finger. | size | size. Finer the particle size, quicker is the absorption. |
| Varitaratvam or Varitara | study lightness | Bhasma | Indicates uniformity of the size |
| test | and fineness of Bhasma | particles should float | of particles and fineness of |
| | based on floating | over surface of water | particles |
| | character of Bhasma on | | Functions |
| | stagnant water surface | | |
| Unama test- based | Study fineness of | Grain should remain | Indicates uniformity of size of |
| On surface tension | Bhasma by keeping a | on water without | particles and fineness of particles |
| | grain of rice on layer of | sinking | |
| | Bhasma that float on | | |
| | water | | |
| Nisvadutam test- based | Check taste of Bhasma | Bhasma should be | Indicates conversion of mineral |
| on organoleptic | | tasteless | into Bhasma form |
| properties of Bhasma | | | |
| Niruttha test or Rajat test- | Check whether there is | Mass of silver should | Conversion of metal into its |
| based on chemical | any significant difference | not be increased | oxide form prevents formation |
| properties of Bhasma | of mass of a piece of | | of alloy |
| | silver after heating it | | |
| | with prepared Bhasma to | | |

Table 02: Traditional tests those use to evaluate quality of Bhasma

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| Slakshnatvam- based on physical property | red hot for 5 minutes and cooling back to room temperature Evaluate quality of Bhasma based on sensation produced by simple touch with finger | Indicates level of incineration of Bhasma | Indicates ability of Bhasma to absorb and assimilate within body without producing any irritation to mucous membrane |
|--|---|---|--|
| Dantagreakachikachitatva -based on organoleptic property of Bhasma | tips Check whether there is typical 'kach-kach' sound when Bhasma is put in to mouth and pressed in between teeth | There should not be any typical 'kach-kach' sound | of gastrointestinal tract Lack of 'kach-kach' Sound means particles are finer so that there are no any hard or sand like materials |
| Vishishtavarnotpatti- based on physical property of Bhasma | Compare color of Bhasma at the end of Marana process with the color in the classical reference | Color should match to the mentioned color in the classical reference | Indicates the appropriate synthesis of Bhasma |

For an example, lead-silver alloys are known to form at a temperature of 304 -579 $^{\circ}$ C. When samples of Naga Bhasma are subjected to conventional niruttha test, no losing the weight in the temperature range of 304 -579 $^{\circ}$ C suggests the absence of metallic lead.

Modern analytical techniques to evaluate the quality of Bhasma:

Analytical instruments such as thermo gravimetric Analyzer, Fourier transform infrared spectrometer (FTIR), X-ray fluorescence spectrophotometer (XRF), particle size analyzer, surface area analyzer, scanning electron microscope with energy dispersive analysis of X-rays and X- ray diffractometer can be used to evaluate the quality of herbo metallic drugs.

Thermo gravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have been employed to study any physical or chemical changes in drugs. For an example, TGA and DTA analysis of mercury based Indian traditional metallic herbal drug Ras-sindoor indicated the presence of mercury sulphide based on a sharp peak at 354°C which corresponded to melting temperature of mercury sulphide.

Scanning Electron Microscope Analysis (SEM) is another technique that can be used to evaluate Bhasma. High-energy electron beam is focused to the sample which is kept inside the microscope's vacuum column evaporator. Because of the interactions of electrons and the surface of the sample, emission of electrons or photons is taken place which is collected by an appropriate detector. The electrons are counted by the detector and the signals are sent to the amplifier. The resultant image shows the number of electrons dispersed from each spot of the sample. The micrographs consist of data about topography of a sample.

Energy Dispersive X-ray Analysis (EDAX) helps to carry out compositional analysis of Bhasma. The data produced by the EDX analysis consists of the spectra containing the elements present in the given sample which is being analyzed.

Differential pulse polarography (DPP) can be used to study trace amounts of chemicals with detection limits on the order of 10⁻⁸ molL⁻¹. Metals including Pb, Cd, Zn, Cu and Fe were successfully identified and determined in herbal products by using DPP. A DPP method can be applied to the different types of drugs such as drops, tablets and capsules in various buffer systems over the pH range of 3.5 to 10.0.

Characterization of Abraga Bhasma:

Shree Devi MS et al carried out experimental analysis to standardize the Abraga Bhasma based on its chemical composition and particle size. They concluded that Abraga Bhasma is preferably non-toxic to humans in its therapeutic dose. The SEM micrograph confirmed the presence of nano particle of size 87 – 95 nm and the EDAX results confirms the presence of carbon, oxygen, zinc, magnesium, calcium, potassium and iron which is necessary for its therapeutic effect. Abraga Bhasma consists of nano particles, which leads to have increased bio availability, better absorption and attainment of maximum therapeutic effect even at minimum dose level.

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Synthesis and characterization of lead based herbo-metallic preparations:

Surya Nagarajan et al (2012) found the presence of oxide, sulfate, carbonate and arsenate forms of lead as a mixture in commercially procured Naga Bhasma samples of different manufacturers. Singh et al. (2010) found that Naga Bhasma contained lead sulfide in crystalline form along with the organic contents which are incorporated during preparation. According to the findings of Surya Nagarajan et al (2012) the thermo gram of Naga Bhasma samples, which were obtained in the presence of Nitrogen gas rich atmosphere shows degradation profile above 800°C indicating proper bhasmikarana (incineration) as it proves the absence of free metal or organic matter in comparison with the reported melting point and decomposition temperature of herbal components of nagabhasma.

FTIR spectra can be used to confirm the absence of organic matter and also the presence of Lead-Oxygen bond at 500-650 cm-1. The scanning electron micrographs of nagabhasma samples point out that they form irregular aggregates of distorted nano particles of various sizes and shapes. In accordance with XRF results and energy dispersive x-ray analysis presence of arsenic is confirmed. Other elements such as calcium, tin, molybdenum and potassium are observed in the samples of nagabhasma. Surya Nagarajan et al (2012) confirmed that X- ray diffraction patterns of commercial batches of nagabhasma show the presence of lead oxide (Pb2O3).

Analytical evaluation of Cu based herbal metallic preparations:

Tamra Bhasma contains copper in the form of oxide. Dr. Kanchan, Chitnis and Miss. Ashley Stanley chemically evaluated the Tamra Bhasma to estimate the amount of copper present in Tamra Bhasma of different batches, different manufacturers and formulations by atomic absorption spectroscopy, spectrophotometric and X- ray diffraction analysis. It was seen that samples from different batches and different manufacturers contained varying amounts of Copper. Spectrophotometric analysis showed that copper was present predominantly in its cupric state. X- Ray Diffraction analysis showed that the copper in the Bhasma was present in its sulphide form. Analysis of formulations showed variations in the copper content from the label claim.

Analytical evaluation of Ayakandhaachenduram:

K. Rajamaheswari et al carried out the analysis of Ayakandhaachenduram by using FT-Raman Spectrometer. To evaluate grain size, particle size, material homogeneity and inner metallic distributions, SEM and XRD studies were carried out (Wei etal2008).The FT-IR peaks indicated that Si-O bond and Fe-O bond formation exists in the compound. In addition to that, most of the peaks were well matched with FT-IR spectral of alpha-Iron oxide. The peak at 1100 cm⁻¹ indicates the presence of siliceous-iron based compound. The SEM images clearly indicated the presence of aggregated particle formation with alpha-iron oxide as a major phase. The materials showed good crystalline in nature with particle size of below 500 nm, which was also predicted and confirmed by SEM analysis. The X-ray diffraction pattern of the prepared Siddha medicine Ayakandhachendhooram existed with major alpha-iron oxide pattern and its major peaks exactly matched with the JCPDS data card number (JCPDS-33-0664). Elemental composition of Ayakandhachenduram was determined by EDX analysis. It was found that iron content was major component of the herbal formulation with respect to all other minor elements such as carbon and siliceous materials, which existed in minor quantity.

Characterization and Comparative Evaluation of Trivanga Bhasma

Arun Rasheed et al carried out comparative analysis of prepared Trivanga Bhasma vs samples those obtain from the market. The physical examination using conventional methods showed both samples are yellow in color, tasteless, having a characteristic odor and pH are 3.7. Both the formulation was found floating when sprinkled on the surface of water. Bhasma entered into the lines of the fingers when rubbed between the fingers so passing the fineness test. The loss of metallic luster was confirmed when exposed to sunlight as there were no brilliance and shine of metal. The particle size determination was conducted by mesh test and the results showed that both Bhasma came under very fine powder category.

The FTIR spectra showed no peak for any organic molecule or bond corresponding it, there by confirming the absence of organic matter and external organic contamination. The AAS study was conducted to determine the concentration of elements present in both formulations. The results showed that the elements Lead, Zinc and Tin were seen in major concentrations in both forms of Bhasma. SEM images of two different categories showed difference in size and agglomeration of the particles. The market samples showed well-defined plate like structures while the formulated 'Trivanga Bhasma' showed spongy, relatively compact micro crystalline aggregates with loss of grain boundaries.

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Agglomeration of the particles is due to repeated cycles of calcinations involved in preparation. The influence of method of preparation on morphology, particularly the calcinations temperature and duration, has also been reported in similar studies also (Wadekar*et al.*, 2006).

For TGA, formulated and marketed Bhasma were heated in presence of air. The thermo grams confirmed the presence of sulphide forms of lead, zinc and tin. The XRD pattern of both samples suggested that the drug is present in crystalline form. The size of crystallites in Trivanga Bhasma was calculated from the XRD pattern using the Scherrer formula and found to be in the range 52.7 nm. Since Scherrer equation is not always a reliable measure of particle size. AFM analysis was done. It confirmed that both formulations have spherical morphology with an average particle size of 60nm. The spherical morphology was due to the aggregation of the nano crystals of the metallic oxides.

Characterisation of bulk metal oxides prepared by sonochemical process

S. Sivasankaran et al characterized dry Pd-based metal oxides using SEM-EDX, XRD and DLS methods for the determination of chemical composition, crystal structure, average particle size and distribution. Results obtained from DLS technique by CILAS 1064 particle size analyzer confirms the formation of both nano and micro particles.SEM micrograph for 0.8 micron size particles showed flower like repeating pattern while EDX spectra showed the elemental composition of surface was made up of atoms of palladium, copper and oxygen. The XRD spectra confirmed the phases as palladium and copper (II) oxide.

5. CONCLUSION

Physicochemical characterization is essential for drug standardization. Analysis of marketed formulation of Bhasma through modern analytical procedures such as SEM, EDAX lead to finds out the variations in composition. Ayurvedic literature emphasizes the use of heavy metals in their formulations for curing and prevention of diseases. It is essential to evaluate Ayurvedic formulations for their content due to the possibility of the occurrence of heavy metal toxicity. Heavy metals in Ayurvedic medicines include lead, mercury, chromium, iron, zinc, nickel, cadmium, arsenic and tungsten after application of specific physiochemical processes like sublimation, heating etc to detoxify the metals to avoid its toxicity. Inappropriate preparation and usage of poor quality raw materials lead to generate significant variations in Ayurvedic preparations.

Even though Bhasma plays a key role in Ayurveda, lack of well-established analytical protocols and safety monitoring systems plays a key barrier for the growth, development and application of Bhasma as a preventive and curative medicine due to inability to assess the quality of Bhasma in the system of traditional medicine.

Standardization of the procedures those are used to synthesis is essential to prevent the availability of poor quality products of Bhasma. In addition to that it will help in enhancing the quality, safety and efficacy of these metals based medicines to fine tune the application of modern and traditional systems of medicine for the creation of a "GoldenTriangle" between Modern Science & Technology, Modern Medicine and Ancient Medicine such as Ayurveda to improve the quality of life of the human kind.

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