Research Article

Study of General Properties of Abhrak Bhasma: A Nanomedicine

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ABSTRACT

There has been a constant surge in the demand for the traditional medicine like ayurvedic preparations. Abhrak bhasma is a type of ayurvedic preparation prepared from repeated incineration of mica mineral with decoction of various medicinal herbs. Traditionally, it has been used in the treatment of asthma, bronchitis, bleeding disorders, cough, cold, urinary disorders, diabetes, anemia, skin diseases, splenic disorders etc. It has also been considered to have anti-aging as well as anti-infertility properties and therefore used in various rejuvenating preparations. Despite their wide range of applications, these products are rarely validated at par with the modern medicines. There is also a paucity of literature that describes mode of action of these products at physiological and molecular level. In the current study, an attempt has been made to analyze the chemical modifications that abhrak bhasma possibly goes through in the GI tract. Acidic condition led to prominent changes in the absorbance pattern of the abhrak bhasma. The neutralization of acidic solution led to further modification in the acidified components and a shift in the absorbance pattern was observed. Further analysis revealed that these probable chemical modifications happen to iron related compounds. Additionally the antibacterial and antifungal properties of abhrak bhasma were also studied. No distinct antibacterial property was observed for abhrak bhasma though it inhibited the growth of yeast cells to certain extent. More intense studies, however, are needed for better understanding of the components of abhrak bhasma made available during such physiological action.

Keywords: Ayurvedic, abhrak bhasma, modifications and physiological action.

INTRODUCTION

Many traditional formulations in India have proved their efficacy in controlling several ailments. It supports a large and ever-growing industry that accounts for an annual turn over a billion dollar turnover a year[1]. Bhasma are a type of herbometallic ayurvedic preparations that involve repeated incineration of various metals/or their ores mixed with decoction of various herbal products in a puta (repeating cycles) system of incineration. These preparations are often considered nanomedicines, as the components of final product generally possess the size in the nanometer range[2]. Typically, any bhasma is prepared through Shodhana, Bhavana and Marana processes. These different levels of treatment ensure that bhasma are free from toxicity at therapeutic doses[2].

Abhrak bhasma (AB) is a type of bhasma prepared from repeated incineration of mineral mica with decoctions of about 72 herbs. The particle size of abhrak bhasma has been shown to be in the range of 29-88 nanometers and Fe, Ca, Se, Mg and K are found to be as major constituent[3].The quality of abhrak bhasma differs as per the number of puta performed. The sahastraputi abhrak bhasma that undergoes 1000 puta is considered to be of finest quality. Different grades of Abhrak bhasma are used in the treatment of a vast range of ailments and also as a constituent of many rejuvenating formulations.

Allopathic medicines involve rigorous research and clinical trials before they are allowed to be marketed and used. In contrast, traditional medicines rarely go through such procedures and are made freely available in the market without any stringent regulations. Though, various traditional literatures like Charak samhita, Susrutha samhita that describe the use, mode and method of feeding exist, these barely address to the mechanism of action at physiological and molecular levels[4,5].

Any substance consumed as dietary supplement passes through compartments of the GI tract with different pH[6]. One of the major chemical modifications of any consumed substance occurs in the stomach under acidic pH. This is followed by alkaline (in duodenum) to neutral pH in the remaining portion of the gut. These conditions affect the bioavailability of compounds consumed orally. There are no reports in literature addressing bioavailability of orally administered abhrak bhasma.

This study is therefore, focused on the possible chemical transformation of components of abhrak bhasma along its passage through the GI tract. The data obtained, thus, can be used for understanding the mode of action of abhrak bhasma in greater details. The efficacy of abhrak bhasma as an antibacterial and antifungal substance has also been evaluated to analyze whether it has the capacity to influence the flora and fauna of the GI tract.

MATERIALS AND METHODS

Abhrak bhasma

Sahastraputi abhrak bhasma was procured from Dhootapapeshwar Ltd, among the leading manufacturers of ayurvedic medicines (Batch no: P150300110).

Spectrophotometric analysis of Abhrak bhasma

Abhrak bhasma at 3mg/ml or 8mg/ml concentration was suspended in distilled water, varying concentration of HCl (0.01N, 0.1N, 0.5N and 1N) and 1N NaOH. These solutions were thoroughly mixed by vigorous shaking and kept at room temperature for 30 minutes. The mixtures were centrifuged at 3000 rpm for 5 minutes. The clear supernatants were then transferred into fresh vials using a micropipette. These solutions were subjected to spectrophotometric analysis using BioTek spectrophotometer (Model: EPOCH-Gen5).

IR study of Abhrak bhasma:

The infrared spectroscopy was performed using ATR technique by applying direct powder of abhrak bhasma on ZnSe crystal in BRUKER instrument. The percent transmission was recorded from 500-4000 wavenumber/cm.

Antibacterial study of abhrak bhasma

Bacteria culture plates were prepared using LB-Agar medium under sterile conditions. These plates were kept overnight in incubator at 37°C for ruling out any possible contamination. Four bacterial strains namely *Escherichia coli, Staphylococcus sp., Micrococcus sp.* and *Bacillus subtilis* were spread on separate sterile culture plates. The sterile filter paper strips of 6 mm diameter were then placed over the solid media at various locations. The test solutions, 10 µl each, were aseptically loaded onto these strips in duplicates. Plates were finally incubated overnight at 37°C. The solutions used in the preparation of test compound were also loaded as negative control whereas; Ampicillin at 100mg/ml concentration was used as positive control.

Antifungal study of Abhrak bhasma

Using yeast as a model system, the efficacy of test solutions against fungi was evaluated. Baker's yeast pellet was suspended in yeast growth medium (0.1% yeast extract + 1% dextrose) to make stock suspension of 10 mg/ml. The separate test sets were prepared using 1mg of Abhrak bhasma powder, 50 µl of acid extracted AB solution and Acid solution with yeast growth medium and equal concentration of yeast cells in each set. The control set was prepared without the test compounds in the above mixture. After thorough mixing of contents, a fixed volume from the mixture was loaded into the microtitre plate and absorbance at 570 nm was recorded. Another set of readings was taken at the end of 20 hour incubation at room temperature and percent growth of yeast cells was calculated.

RESULTS AND DISCUSSION

Acidic modification of abhrak bhasma

Abhrak bhasma after mixing with 1N HCl and centrifugation, absorption pattern of the clear supernatant was recorded in Biotek spectrophotometer. Under acidic condition, the absorption of solution increased in the range of wavelengths 220-280 nm and 300-400 nm (**figure 1**). A distinct peak of absorption was encountered at the wavelength of 330 nm. The size of this peak was found to be directly proportional to the concentration of abhrak bhasma used and also with the strength of HCl acid (**figure 2**).

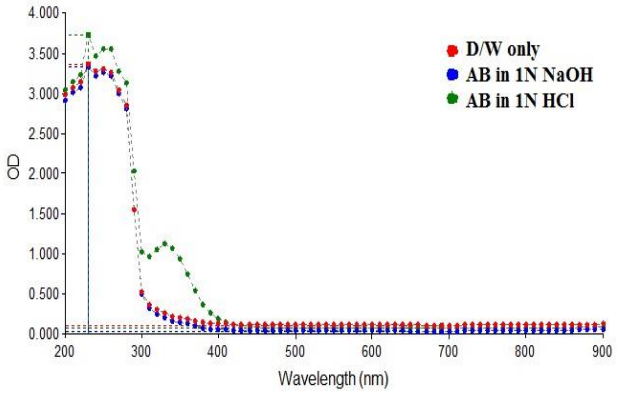

Figure 1: Spectrophotometric analysis of acidified Abhrak bhasma. The abhrak bhasma (AB) dissolved at 3mg/ml concentration in 1N HCl show distinct peak at 330nm and various peaks in the range of 220-280nm. (D/W: distilled water)

When neutralized with 1N NaOH, the absorption pattern shifted and distributed to the left side of the peak absorption at 330 nm. There was, however, no major shift in the absorbance in the range of 220-280 nm wavelengths (**figure 3**).

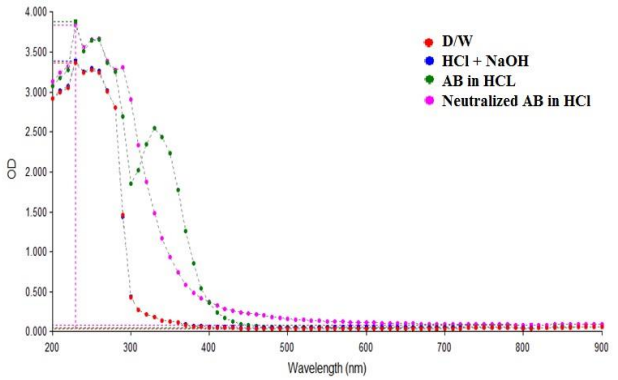

Figure 2: Neutralization of Acidified Abhrak bhasma. The acidic solution of abhrak bhasma (AB) when neutralized with 1N NaOH show shift in the absorption peaks towards left side of the spectrum i.e. towards lower wavelengths. (D/W: distilled water)

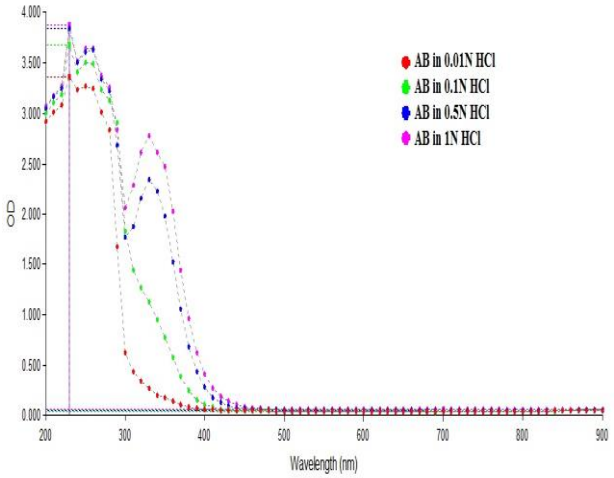

Figure 3: Increase in 330nm peak with increase in acid strength. The acidic extraction of abhrak bhasma (AB) at 8mg/ml concentration in 0.01N, 0.1N, 0.5N and 1N HCl exhibit prominent increase in the 330nm wavelength.

In order to find the possible compound responsible for this shift we looked into the major components of the abhrak bhasma as provided by Bhatia *et al.*(2013) [3]. An attempt was made to ascertain the precise component among the chemical constituents of AB that undergoes modification responsible for the shift in absorbance. The salt form of Ca, Si, K, P and Fe were dissolved in the 1N HCl and processed in a manner similar to abhrak bhasma. Only iron salt exhibited an absorption peak in the range of absorption observed for abhrak bhasma (**figure 4**). But, additional peaks were also observed in the range of wavelengths 400-500 nm, which were not obtained for acidic solution of abhrak bhasma. The acidic solution of Ca, Si, K and P did not produce peaks in the range of wavelengths between 220 and 280 nm as well as 300 and 400nm.

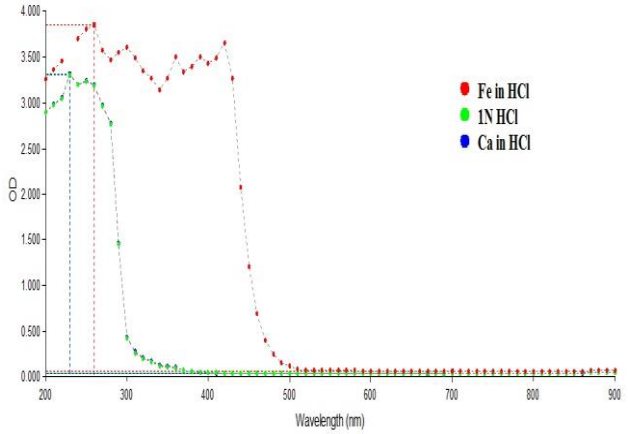

Figure 4: Absorption spectrums of Iron salt in 1N HCl. Acidic solution of Iron show various peaks in the range of 200-500nm wavelength.

High metal composition

The Infra-red spectroscopy revealed that there were no traces of moisture or organic compounds since there were no peaks in the region between 4000 and 1200 wave number per cm. It also indicated a mix of several metal ions in the composition though no peak could be related to specific metal moiety (**figure 5**). Distinct peaks of absorption were noticed at 968.82 and 683.78 wavenumber cm^{-1} but the exact characterization of these peaks was not possible using the current technique.

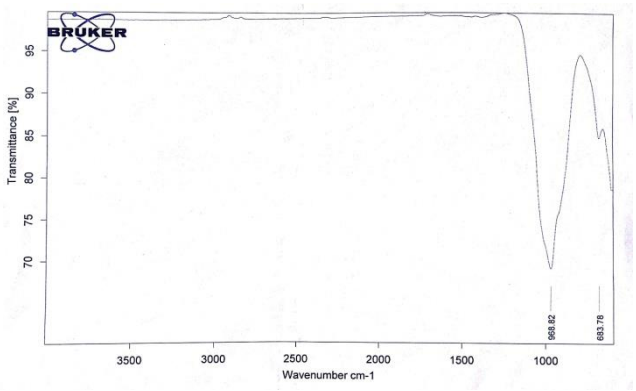

Figure 5: Infrared spectroscopy of Abhrak bhasma powder. The result indicates low moisture and organic content in the powder. Presence of high amount of metals was also deduced from the absorption pattern.

No antibacterial action

To find the efficacy of abhrak bhasma as antibacterial agent, it was tested against four different types of bacteria (**figure 6**). The direct application of abhrak bhasma dissolved in distilled water to bacteria culture did not have any antibacterial effect against any of the bacterial strains tested. Abhrak bhasma processed in acid as per the protocol used in spectrophotometric analysis was also tested for antibacterial property. This solution inhibited the bacterial growth but HCl solution alone also exhibited similar inhibitory effect so that it was difficult to ascribe antibacterial property to AB.

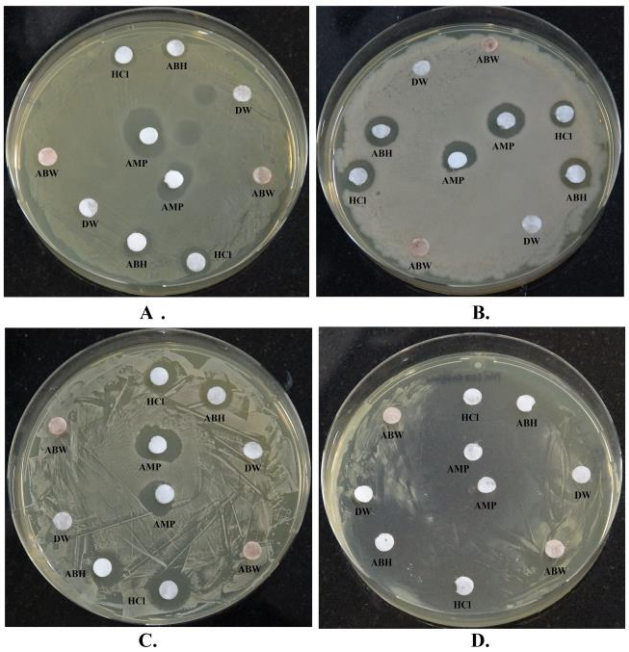

Figure 6: Antibacterial effect of abhrak bhasma (AB) on A. *Escherichia coli,* B. *Bacillus subtilis* C. *Staphylococcus sp.,*

D. *Micrococcus sp.* (HCl: Hydrochloric acid, ABH: AB in HCl, DW: Distilled water, ABW: AB in DW, AMP: Ampicillin) The clear zones indicates inhibition of bacterial growth.

Abhrak bhasma inhibits yeast cell growth

To evaluate the effect of abhrak bhasma on fungal cell growth, yeast model system was selected. Abhrak bhasma (powder) as well as acid extract of abhrak bhasma was added to the yeast cell suspension. Over 20 hours, the density of yeast cells increased by more than 200% in control. The addition of direct abhrak bhasma to the yeast culture limited the growth only by 56 % of the initial density. When the extract of Abhrak bhasma in acid solution was added to the culture, the increase in density was merely by 4.9% of the initial density while in presence of the acid control solution, the density of yeast cells was found to grow by 10% (**figure 7**).

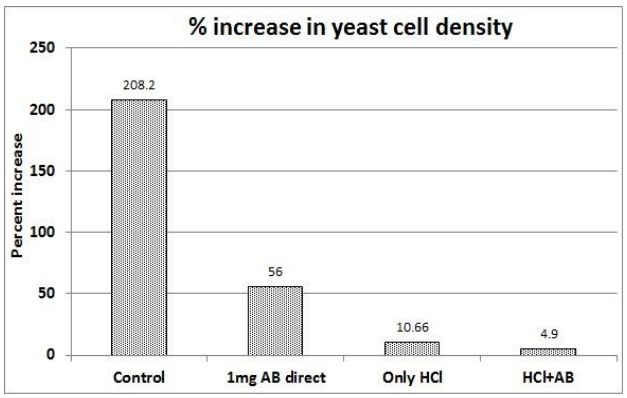

Figure 7: Effect of abhrak bhasma (AB) on yeast cell growth.

A steady increase in the consumption of traditional medicines has been noticed by several researchers[1,3]. This popularization is due to the belief that traditional medicines have minimum side effects as compared to the allopathic counterparts. It is the necessity of the time to establish validity/efficacy and safety of such preparations. Abhrak bhasma is one such traditional- ayurvedic preparation being used in treatment of several ailments like respiratory disorders, diabetes, anemia, general debility and impotency[7]. There is a paucity of reports on the efficacy of abhrak bhasma at physiological and molecular level.

The modifications in the GI tract are crucial in determining the bioavailability and subsequently the efficacy of abhrak bhasma. The spectral analysis of abhrak at acidic pH that mimics the conditions in the stomach of an organism indicates chemical transformation of various constituents of abhrak bhasma. In most of the animals, the acidic condition is neutralized, in post gastric portion, by various substances secreted by the GI tract and associated glands. This, in turn, is crucial for the action of enzymes, which require neutral or alkaline pH to function. The components of the abhrak bhasma also exhibit modifications under alkaline condition as noticed through the shift in absorbance pattern after neutralization of acid extracted solution of abhrak bhasma. Examination of the spectral pattern of major constituent of abhrak bhasma suggests that these modified components might be oxides of Iron. But, the exact components that undergo chemical changes and also the nature of modification could not be deduced through present investigation. Additionally, the infrared spectroscopy also revealed high metal contents but was incapable of providing the resolution at molecular level for the components undergoing chemical modifications. The IR spectrum obtained indicated no moisture and organic content in the abhrak bhasma powder. Distinct peak at 500-1000 regions, however, could not be assigned to any specific molecules in present investigation. Similar IR spectrums were also obtained by other researchers working on other type of bhasma but showed presence of moisture and organic components[8,9]. Dave and Chopda (2014) have demonstrated the role of Fe based nanoparticles in quenching heavy metals[10]. This supports an assumption that iron on abhrak bhasma quenches heavy metals that are otherwise deleterious to health. Pal *et al.* (2014) have endorsed this and have claimed that this might be a significant contribution to the action of abhrak bhasma[7].

Local environment of the GI tract is also modified by gut micro biota in the respective regions[11,12] and this is crucial for maintaining proper functioning of the GI tract. These environments are altered in various diseases and after the consumption of toxic substances[12]. It has been established by this study that though abhrak bhasma does not have any antibacterial effect, by itself, the acidic environment in stomach endows this property to its constituent/s. Since abhrak bhasma inhibits the growth of yeast cells it might as well be effective in checking the growth of other fungal organisms in GI tract, thus preventing putrefaction and fermentation of food. Species specific tests, however, are needed to establish this.

Due to the nano-size of the constituents, abhrak bhasma and more specifically the acid modified moieties of it can be readily cross the lining of GI tract to ensure its bioavailability. The ability of these components to cross blood-tissue barriers has already been established[13] thus affecting its efficacy.

CONCLUSION

The study has established the probable role of iron based constituents in efficacy of abhrak bhasma through the chemical modifications in the compartments of GI tract. The abhrak bhasma, in turn, influences the micro flora to ensure a smooth functioning of the compartments of GI tract. This in turn improves various physiological and behavioral responses in the organism as confirmed by our earlier report[14]. The identification of other active ingredients of the abhrak bhasma will allow individual metal oxides to be formed artificially. This may open new avenues in traditional medicines and use of specific components may help in development of better treatment for various diseases.

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