Research Article

In vitro anti-inflammatory activity of unpurified and purified Manahshila

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Abstract

Objective: Realgar (arsenic sulphide), popularly known as Manahshila (in India), used for the treatment of various diseases like eye, skin, respiratory, psychological disorders and also including pain related ailments. This study is aimed to evaluate the anti-inflammatory efficacy of Ashodhita Manahshila (AM) and Shodhita Manahshila (SM) using *in vitro* models of inflammation. **Material and methods:** Shodhana (Purification) of Manahshila was carried out by seven Bhavana (levigation) with ginger juice. Five concentration levels (20, 40, 60, 80 and 100 µg/mL) of the AM, SM were evaluated by using heat induced protein denaturation and heat induced hemolysis of the erythrocyte and subjected to determination of absorbance to assess the anti-inflammatory activity. Indomethacin was used as the positive control at same concentration levels. **Results:** The present findings exhibited a concentration dependent inhibition of protein denaturation and hemolysis of the erythrocyte by the SM. The effect of AM was found to be less when compared with the SM and indomethacin. From the present study it can be concluded that SM marked *in vitro* anti-inflammatory effect as compared to AM against the denaturation of protein and hemolysis of the erythrocyte. **Conclusion:** By this study it can be concluded that after Shodhana, drug not only transformed into physical and chemical changes but also safely digestible as well as improve therapeutic efficacy.

Keywords: indomethacin, Manahshila, protein denaturation, Shodhana

Introduction

Inflammation is the body's response toward attacking pathogens, which is typically distinguished by redness, swelling, pain, including heat. It can also be observed as a compact system an associated with homeostatic disturbances which started from within the body (like the autoimmune disease) or from the outside of the body (like in infections). Basically, whole acute and chronic diseases are either induced or modulated by inflammation. Earlier reports have given evidence that inflammation is associated with the pathogenesis of many diseases, including aging (Finch, 2005), cancer (Caruso et al., 2004), atherosclerosis (Paoletti et al., 2004), cardiovascular disease (Rus and Niculescu, 1997), arthritis (Firestein, 2006), neurodegenerative disease (Klegeris et al., 2007), diabetes mellitus (Libby and Plutzky, 2007), obesity (Yan et al., 2008), and other life-threatening and debilitating diseases (Tsirpanlis, 2005). There is an extended perception that the role of inflammation is more and more relevant in a wide spectrum of diseases (Nathan, 2002). Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, diclofenac, are directed global for the management of pain, inflammation, and fever, as well as cardiovascular protection. However, their uses were confined because of the major fact with gastrointestinal destruction (Wolfe et al., 1999; Scheiman, 2001), in spite of NSAIDs have given adequate management of pain and inflammation extremely. Similarly, kidney damage, increase in blood pressure and some other cardiovascular obstacles have been found with NSAIDs (Burke et al., 2006). Now it is a growing concern all over for the development of new safe and potent, less toxic anti-inflammatory drugs. Therefore, there is a need to investigate for more naturally accessible alternatives for the treatment of inflammation. Hence, during the past decades, several researchers have directed on medicinal plants but very less research has focused on mineral drugs for patients to exhibit anti-inflammatory drugs.

Manahshila (Mineral arsenicals) have long been used in Indian and Chinese system traditional medicines for various

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diseases like skin, respiratory and digestive systems, eye disorders etc (Sharma et al., 2016). Yet inorganic Manahshila is highly toxic and carcinogenic (Liu et al., 2007; IARC, 2004). However, Ayurveda has emphasized that a strong poison may be converted into a safe and potent therapeutic drug by applying specific pharmaceutical processes as described in the Ayurvedic literature (e.g. Shodhana, Marana, etc.) (Agnivesha, 2011). Shodhana is not only a process of chemical purification but it is a specific process of addition and separation which causes physical, chemical and biological changes in the metals/minerals. These changes depend on the structure, constituents/constitution, impurity and properties of the particular substance. To reduce toxicity simple digests and absorbable to make them proper for metabolic changes and digestible by the tissue cells and to make them therapeutically effective numerous methods for Shodhana of minerals have been prescribed. Depending upon the nature of the minerals and the disease for which they are meant to be used, the specific process for purification vary from one mineral to the another and the process is repeated for several times (Belge and Belge, 2012). Manahshila has been studied in the last several years and has been reported to possess potent anticancer activity (Sharma et al., 2016). However, information on the anti-inflammatory property of Manahshila is lacking. The present study was carried out to evaluate the anti-inflammatory activity of unpurified and purified Manahshila, *in vitro* models of inflammation to ascertain pharmacological claims. Moreover, this study also provides the first evidence for it's *in vitro* anti-inflammatory activity.

Material and methods

Procurement of raw materials

Realgar (95% w/v purity) was purchased from Sigma Aldrich Co. (519111-25G; St. Louis, MO, USA). The plant ingredients *Zinger officinale* Ros were collected from the herbal garden of Rajiv Gandhi South Campus, Barchakha, Banaras Hindu University, Varanasi, India and authenticated by Dr. S.K. Maurya, Department of Dravyaguna, Banaras Hindu University, Varanasi, India. The voucher specimen (No.: APRIL/HERB/15-16/03) of the plant has been deposited in Department of Ayurvedic Pharmacy Research Laboratory, Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur (U.P), India for future reference.

Chemical reagent and instrument

The standard drug indomethacin was procured from IPCA Pharmaceuticals, Mumbai, India. All the other chemicals and reagents were of analytical grade and were purchased from S.D. fine Chemicals Pvt. Ltd., Mumbai, India. BOD incubator (Navyug, India Ltd, All the spectrophotometric analysis was carried on Varian UV-spectrophotometer with Carry-100 software.

Purification of Manahshila

Purification of Manahshila was carried out by seven Bhavana (levigation) with ginger juice. Ashodhita (unpurified) Manahshila was taken into a dried clean mortar and powdered by using a pestle. Powdered Manahshila was levigated with ginger juice. After completion of one Bhavana same procedure was repeated for next six times. In this way, a total of seven impregnations of Manahshila with different juice will be carried out (Vagbhatta, 2010). Prepared dried Shodhita Manahshila was packed into polyethylene packet.

Assessment of *in vitro* anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of Ashodhita (unpurified) and Shodhita (purified) was studied by using inhibition of albumin denaturation technique (Mizushima & Kobayashi, 1968; Sakat et al., 2010). The reaction mixture was consists of AM, SM and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N-HCl. The sample was incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660 nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

Preparation of Red Blood cells (RBCs) suspension

The blood was collected from and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted with 10% v/v suspension with normal saline.

Heat-induced hemolysis

The reaction mixture (2mL) consisted of 1 ml test sample of different concentrations (20, 40, 60, 80, 100 µg/mL) and 1 ml of 10% RBCs suspension, instead of test sample the only saline was added to the control test tube. Indomethacin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 660 nm. The experiment was performed in triplicates for all the test samples (prakash, 2011).

Table 1. Effects of Standard drug, and Test drug (Ashodhita & Shodhita Manahshila) on heat induced protein denaturation

S.N	Treatment of drug	Concentration (µg/mL)	Absorbance (660 nm)	% Inhibition
1	Control	-	1.65±0.028	-
2	Standard	20	1.25±0.052	24.24
		40	0.98±0.047	40.60
		60	0.62±0.072	62.42
		80	0.48±0.034	70.90
		100	0.19±0.039	88.48
3	Ashodhita Manahshila	20	1.58±0.008	4.2
		40	1.35±0.082	18.19
		60	1.08±0.045	34.54
		80	0.85±0.004	48.48
		100	0.68±0.027	58.78
4	Shodhita Manahshila	20	1.38±0.014	16.36
		40	1.01±0.052	38.78
		60	0.82±0.064	50.30
		80	0.55±0.007	66.66
		100	0.41±0.015	75.15

Table 2. Effects of Standard drug and Test drug (Ashodhita & Shodhita Manahshila) on heat-induced hemolysis of erythrocyte

S.N	Treatment groups	Concentration (µg/mL)	Absorbance (660 nm)	% Inhibition
1	Control	-	0.69±0.012	-
2	Standard	20	0.53±0.040	23.18
		40	0.48±0.016	30.43
		60	0.36±0.042	47.82
		80	0.29±0.024	57.97
		100	0.18±0.019	73.91
3	Ashodhita Manahshila	20	0.62±0.008	10.14
		40	0.57±0.082	17.39
		60	0.49±0.045	28.98
		80	0.41±0.004	40.57
		100	0.38±0.027	44.92
4	Shodhita Manahshila	20	0.55±0.014	20.28
		40	0.50±0.052	27.53
		60	0.42±0.064	39.13
		80	0.32±0.007	53.62
		100	0.22±0.015	68.11

The percentage inhibition of hemolysis was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

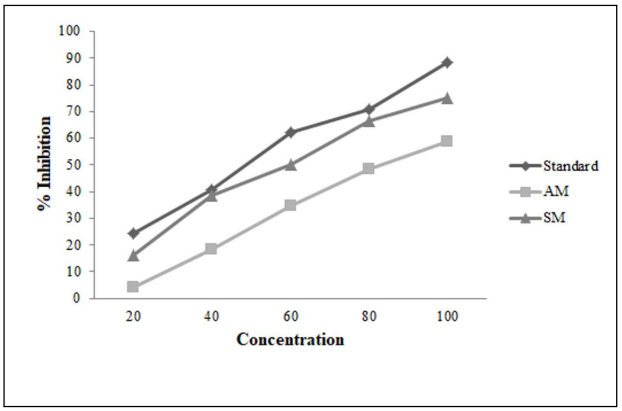

Figure 1. Percentage inhibition of Standard drug, and Test drug (Ashodhita & Shodhita Manahshila) on heat-induced protein denaturation

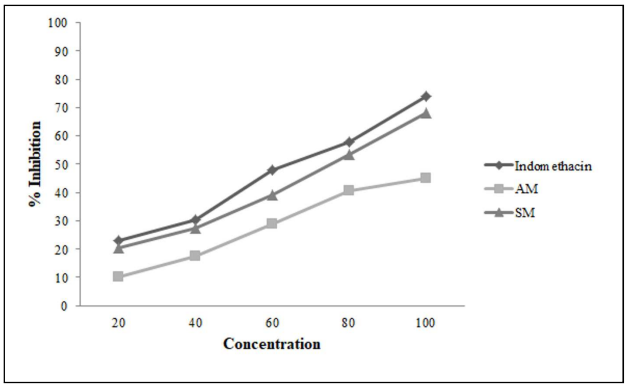

Figure 2. Percentage inhibition of Standard drug, and Test drug (Ashodhita & Shodhita Manahshila) on heat-induced hemolysis of erythrocyte

Statistical analysis

Results are expressed as Mean ± SD. Statistical analysis was done using Microsoft Excel and SPSS 15.0 version.

Results

In the present investigation, the *in vitro* anti-inflammatory effect of AM and SM was evaluated against denaturation of egg albumin and hemolysis of the erythrocyte. The results are summarized in table and figure 1 & 2. The present findings exhibited a concentration-dependent inhibition of protein (albumin) denaturation and hemolysis of erythrocyte by AM and SM throughout the concentration range of 20, 40, 60, 80 and 100. Indomethacin (at the same concentration range) was used as a standard drug which also exhibited concentration-dependent inhibition of protein denaturation and hemolysis of erythrocyte (Table 1 & 2).

Discussion

In traditional period metals and minerals were impregnated with decoctions, juices of different types of herbal drugs and after that reduced to a state of the fine particle by trituration process. Untreated metals or minerals will not be

digested, metabolized, absorbed, and assimilated to the tissue cells of the body. Therefore, they will be therapeutically ineffective. On the other hand, these heterogeneous drugs are likely to produce a serious toxic effect in the body. To reduce toxicity simple digests and absorbable to make them proper for metabolic changes and digestible by the tissue cells and to make them therapeutically effective numerous methods for Shodhana of minerals have been prescribed. Depending upon the nature of the minerals and the disease for which they are meant to be used, the specific process for purification vary from one mineral to the another and the process is repeated for several times. Natural Manahshila is found as the mixture of arsenic trioxide and arsenic sulfide. Arsenic trioxide is more toxic in nature than arsenic disulfide. So Shodhana with the juice of ginger rhizome juice is implied to reduce the toxicity of Manahshila which is in the form of arsenic trioxide. In the previously reported by Sharma et al., that zingiberene which is act as a nuclear factor kappa B (NF-kB) directly the particular of the inflammation zingiberene not on the particular anti-inflammatory effect and also chelates the particular free arsenic from the Manahshila. Denaturation of tissue proteins is one of the well-documented problems of inflammatory conditions. Reproduction of auto-antigens in several inflammatory conditions may be due to denaturation of proteins *in vivo*. Agents that can inhibit protein denaturation, since, would be beneficial for anti-inflammatory drug development. The increases in absorbance of test samples with respect to control showed stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by AM, SM and Indomethacin. From the percentage of inhibition, it becomes evident that Shodhita Manahshila was found more active than Ashodhita Manahshila, being effective in lower concentrations. In the present study, the *in vitro* anti-inflammatory activity of Shodhita Manahshila can be attributed to its zingiberine (as chelating agent) content. The Shodhana effect may be due to the synergistic effect of zingiberine rather than Ashodhita Manahshila. The SM significantly and dose dependently reduced the heat induced protein denaturation and heat induced hemolysis of the erythrocyte. The percentage inhibitions of denaturation of protein for AM were found to be 4.2, 18.18, 34.54, 48.48, 58.78 for the concentration of 20, 40, 60, 80 and 100 µg/mL respectively while for SM the percentage inhibitions of denaturation of protein was found to be 16.36, 38.78, 50.30, 66.67, 75.15 for the concentration of 20, 40, 60, 80 and 100 µg/mL respectively. While the inhibitions of the hemolysis of the erythrocyte 16.36, 38.78, 50.30, 66.67, 75.15 for the concentration of 20, 40, 60, 80 and 100 µg/mL respectively. The percentage inhibitions of hemolysis of the erythrocyte for AM were found to be 10.14, 17.39, 28.98, 40.57, 44.92 for the concentration of 20, 40, 60, 80 and 100 µg/mL respectively while for SM the percentage inhibitions of hemolysis of the erythrocyte was found to be 20.28, 27.53, 39.13, 53.62, 68.11 for the concentration of 20, 40, 60, 80 and 100 µg/mL respectively. AM and SM at concentrations of 20-100µg/mL protected the human erythrocyte membrane against heat-induced hemolysis of erythrocyte. While inflammation, there are lyses of lysosomes which release their component enzymes that provide a variety of ailments. Non-steroidal anti-inflammatory drugs (NSAIDs) use their useful effects by both inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membranes. Vulnerability to red blood cells to damaging substance like heat results in the lysis of the membranes, characterized by hemolysis and oxidation of hemoglobin. Because human red blood cell membranes are alike to lysosomal membrane components, Membrane stabilization directs to the inhibition of leakage of serum protein and fluids into the tissues through a period of increased permeability produced by inflammatory mediators. The test drug (Manahshila) possibly stabilized the red blood cell membrane by inhibiting the release of lytic enzymes including active mediators of inflammation.

Conclusion

Hence, form the results of the existing study it can be assumed that Shodhita Manahshila possessed marked *in vitro* anti-inflammatory effect against the denaturation of the protein and heat-induced hemolysis of the erythrocyte. Moreover, comprehensive studies are essential to determine the mechanisms and constituents following its anti-inflammatory actions.

Conflicts of interest: No

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