Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(9):194-197

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In vitro anti-inflammatory activity of Raupya (Silver) Bhasma

Shyam Baboo Prasad[1], Yashwant[1] and Vidhu Aeri*[2]

[1]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara (Punjab)
[2]Faculty of Pharmacy, Jamia Hamdard, New Delhi

ABSTRACT

Formulation of silver nitrate and silver sulphadiazine is choice of drug in topical treatment of burns and related inflammation. Ancient silver based nanomedicine of Ayurveda is raupya bhasma (RB) used in treatment of different ailments but RB is still not explored to their anti-inflammatory activity. To consider this an attempt has been made to evaluate in-vitro anti-inflammatory activity of RB against denaturation of protein. Denaturation of tissue proteins is one of the well-documented causes of inflammation. Different concentration of RB was incubated with egg albumin in specified experimental conditions and subjected to determination of absorbance and viscosity to assess the in-vitro anti-inflammatory property using diclofenac sodium as standard against denaturation of protein. RB inhibited protein denaturation in dose dependent manner. The effect of RB as anti-inflammatory agent was found to be better than standard (diclofenac sodium) as the IC50 value of RB and diclofenac sodium are 43.2 µg/mL 46.1µg/mL respectively. Inhibition of denaturation of protein was further confirmed by change in viscosity. On the basis of present research it is concluded that RB possessed marked anti-inflammatory activity against the denaturation of protein.

Keywords: Raupya bhasma, Silver nanoparticle, Anti-inflammatory activity, In vitro anti-inflammatory activity, Inflammation.

INTRODUCTION

Inflammation is a process of body defence mechanism, which is associated with pain and involves the increase of vascular permeability, increase of protein denaturation and membrane alteration. Inflammation may be due to chemical agent, physical agents and microbes. It is characterized by swelling, redness, pain, heat, and loss of function of injured area[1]. Injury of cells may leads to release of kinins, prostroglandins and histamine. The release of these mediators causes vasodilation, increase in permeability of the capillaries which may lead to increased blood flow to injured site. Inflammation can be classified as both acute andchronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues [1,2]. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation is known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Current drugs available for treatment of inflammation such as opioids and non-steroidal anti-inflammatory drugs (NSAIDS) are not useful in all cases of inflammatory disorders, because of their severe side effects. As a result, a search for other alternatives seems necessary and beneficial [1,2,3].From the history of civilization traditional medicines were used to cure human aliments in every possible condition. In modern era we have the option to use them over the synthetic molecules because traditional drugs have lesser side effects [4]. Modern era is of nanomedicine owing to their various therapeutic applications with more efficacies and lesser side effects. The popularity is due to their potential for achieving specific process and selectivity in pharmacological action[5]. Bhasma the ancient concept of nanomedicine is used for treatment of various chronic

ailments since 7th century BC [6,7,8]. *Bhasma* literally means anything inorganic or organic burnt into its ash. However metal based preparation of Ayurvedic system of medicine is known as bhasma. According to literature from all the ancient civilizations, it is clear that metals were used in disease treatment since time immemorial [9]. The metals used in Ayurveda system of medicine include silver, gold, copper, mercury, iron, lead and zinc etc. [10]. The antibacterial activity of silver has been known from thousands of years with the ancient Greeks cooking from silver pot. The antimicrobial activity of silver was utilized as early as 1000 BC to keep water safe[5]. One of silver based nanomedicine used in Ayurvedic system is RB. Raupya means silver and bhasma means fine powder produced by calcination. As RB is a product of calcination so final product may be oxide of silver. The size of RB was found to be 16 nm [11]. RB is therapeutically used in neuropsychological disorder [10]. It strengthens brain, liver and heart[11]. It also possesses analgesic, aphrodisiac, nervine tonic and general tonic activity[11,12, 13].Silver sulphadiazine is used in treatment of burns and related inflammation[14].Silver based nano medicine of Ayurveda (RB) is still not explored to their anti-inflammatory activity. To consider this an attempt has been made to evaluate in-vitro anti-inflammatory activity of RB.

EXPERIMENTAL SECTION

Preparation of RB

It is prepared by method described in Rasendrasara Samagraha: an Ayurvedic text[15]. Pure silver leaves were mixed with equal quantity of sulphur by weight and half quantity of arsenic sulphide and then soaked in lemon juice and subjected to calcination process in sealed earthen container. The material was scrapped after cooling, triturated with lemon juice, pulverized and calcined again. The procedure was repeated 14 times to obtain the ash of silver.

Evaluation of RB

The quality of bhasma was evaluated by traditional method for evaluation like Nishchandratvam, Rekhapurnata, Varitara test and Unama test [15].

Preparation of test solution

Test solution of raupya bhasma of varying concentration (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) was prepared in phosphate buffer of pH 7.4.

Preparation of standard solution

Standard solution of declofenac sodium of varying concentration (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) was prepared in phosphate buffer of pH 7.4.

Evaluation of in vitro anti-inflammatory activity

Invitro anti-inflammatory activity of RB against denaturation of protein was carried out as per method described by Mizushima and Kobayashi [16].The 5ml of reaction mixture consisted of 0.2 mL of egg albumin obtained from fresh hen's egg, 2.8 ml of phosphate buffered saline (PBS, pH 7.4) and 2 ml of different concentrations of RB so as to obtain the final concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml. Equal volume of triple-distilled water served as control. After that the mixtures were incubated at (37±2) °c in a BOD incubator (Navyug, India Ltd) for 30 min and heated at 70°c for 15 min. After cooling, the absorbance was measured at 660 nm (Shimadzu 1800, Japan) by using vehicle as blank and the viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml)was used as reference drug and treated similarly for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = (Vt / Vc - 1) x 100

Where, Vt = absorbance of test sample, Vc = absorbance of control. The extract/drug concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS AND DISCUSSION

The prepared bhasma as per ancient Ayurvedic literature passed all test of evaluation indicating that the prepared bhasma is of good quality. In this investigation, in vitro anti-inflammatory effect of RB was evaluated against denaturation of egg albumin. The results obtained for inhibition of protein and change in viscosity are summarized in Table 1. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by RB throughout the concentration range of 20 to 100µg/ml. Diclofenac sodium (at the concentration range of 20 to 100µg/ml) was used as reference which also exhibited concentration dependent inhibition of protein denaturation

(Table 2); however, the effect of diclofenac sodium was found to be less when compared with RB. This was further confirmed by comparing their IC50 values. RB possessed IC50 value 43.2 µg/mL whereas that of diclofenac sodium was found to be 46.1 µg/ml.

Table (1) Effect of RB on protein denaturation

Concentration (µg/ml)	% inhibition	Viscosity(cp)
Control	-	-
20	28.9196±0.0852	0.628±0.027
40	47.5154±0.152	0.785±0.039
60	60.5934±0.106	0.815±0.016
80	71.0142±0.0569	0.880±0.023
100	73.6988±0.126	0.970±0.015

Data is presented as Mean±SEM (n=5)

Table(2) Effect of diclofenac sodium on protein denaturation

Concentration(µg/ml)	% inhibition	Viscosity
Control	-	-
20	25.4704±0.121	0.657 ±0.012
40	44.4752±0.0730	0.740±0.015
60	56.2944±0.0632	0.823±0.012
80	66.38361±0.0494	0.86±0.021
100	71.4242±0.0628	0.97±0.012

Data is presented as Mean±SEM (n=5)

The prepared bhasma was found to be of black colour and as it was filled in between furrows of finger indicating that size of bhasma was very fine. The prepared bhasma was evaluated for in vitro anti-inflammatory activity as there are certain problems in using animals in for biological activity of new chemical entity (NCE), such as ethical issues and the lack of rationale for their use when invitro methods are available or could be investigated. To consider this, in the present study the protein denaturation bioassay was selected for in vitro evaluation of anti- inflammatory activity of RB. Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of tissue proteins is one of the well-documented causes of inflammation[17,18]. Agents that can prevent protein denaturation would be worthwhile for anti-inflammatory drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by RB and reference drug diclofenac sodium[19]. From the IC50 values it becomes evident that RB was more active than diclofenac sodium, being effective in lower concentrations. This anti- denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation [20]. In the contemporary study, the comparatively high viscosity of control dispersion validated this fact. Presence of RB prevented this, implying inhibition of protein denaturation. Here, the viscosities reduced when compared with control where no RB was added. However, the viscosities were found to reduce with associated decrease in concentration of test drug and reference drug as well. Although, the viscosities of the test samples, of all concentrations were always less than that of control. This decrease in viscosities may be due to decrease in concentration of test /drug in reaction mixture, which resulted in decreased viscosity; and/or other uncertain physico-chemical factors. However, the viscosity data indicated inhibition of protein (albumin) denaturation [20]. The effect of concentration of test agent on viscosity behaviour of denatured protein dispersion requires further studies. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH[21]. Therefore, form the results of the present preliminary study it can be decided that RB possessed marked in vitro anti-inflammatory effect against the denaturation of protein. Further conclusive studies are necessary to determine the mechanisms behind its anti-inflammatory actions. As the inflammation is associated with arthritis and other inflammatory disease so RB can be evaluated in that condition also [22].

Acknowledgements
The authors are thankful to management of Lovely Professional University for providing necessary facilities for the present study.

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