Evaluation of Anti-inflammatory Effect of Ashwagandha: A Preliminary Study in vitro

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ABSTRACT

Introduction: Ashwagandha (*Withania somnifera*) is an important medicinal plant in Indian traditional system of medicine and traditionally has been used for several medicinal purposes in India. The present study was conducted to evaluate the anti-inflammatory effect of hydroalcoholic extract of ashwagandha against denaturation of protein in vitro.

Methods: The test extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug.

Results: The present results exhibited a concentration dependent inhibition of protein (albumin) denaturation by the ashwagandha extract. The effect of diclofenac sodium was found to be less when compared with the test extract.

Conclusion: From the present findings it can be concluded that ashwagandha possessed marked anti-inflammatory effect against denaturation of protein in vitro. The effect was plausibly due to the alkaloid and withanolide contents of ashwagandha.

Key words: *Withania somnifera*, anti-inflammatory, protein denaturation, viscosity.

INTRODUCTION

Inflammation is a bodily response to injury, infection or destruction characterised by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells.[1] The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers.[2,3] Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

Ashwagandha, also known as Indian ginseng and winter cherry, consists of dried roots of *Withania somnifera* (L.) Dunal. (Family: Solanaceae). It is a perennial plant indigenous to India, grown and cultivated throughout subtropical India. It has been recognized as an important herb in the Ayurveda, the traditional system of Indian medicine for more than 3000 years. Traditionally it has been used for several important medicinal purposes in the Indian subcontinent. Recently there has been renewed interest on ashwagandha for its effectiveness in several disease conditions, adaptogenic, immunomodulator and other health benefits.[4,5] Previous researchers have reported several pharmacological properties of ashwagandha on animals and humans.[6-10] The present study was conducted to evaluate the anti-inflammatory effect of ashwagandha extract against the denaturation of protein in vitro.
RESULTS AND DISCUSSION

There are certain problems associated with animal use in experimental pharmacological research such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in vitro assessment of anti-inflammatory property of hydroalcoholic extract of ashwagandha (HAWS). Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of proteins in vivo.\(^{[11,12]}\) Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

In the present investigation, the anti-inflammatory effect of HAWS was evaluated against the denaturation of egg albumin in vitro. The results are summarized in Table 1. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by HAWS throughout the concentration range of 31.25 to 1000 µg/ml. Diclofenac sodium (at the concentration range of 78 to 2500 µg/ml) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 2); however, the effect of diclofenac sodium was found to be less as compared with HAWS. This was further confirmed by comparing their IC\(_{50}\) values (Table 3).

The increments in absorbances of test sample with respect to control indicate stabilization of protein i.e. inhibition of protein (albumin) denaturation by HAWS and reference drug. The extract/drug concentration for 50% inhibition (IC\(_{50}\)) was determined by plotting percentage inhibition with respect to control against treatment concentration.

MATERIALS AND METHODS

Plant material
The dried roots of Ashwagandha (\textit{Withania somnifera} (L.) Dunal. family: Solanaceae) were procured in the month of July, 2011 from Kangalicharan & Sons., Kolkata, West Bengal, India and identified at the Botanical Survey of India, Howrah, West Bengal, India. Just after procurement, the roots were ground mechanically into a coarse powder and kept into an air-tight container for use in the study.

Drugs and chemicals
Diclofenac sodium was obtained from Organic Chemical Industries Pvt. Ltd., Kolkata 70001, West Bengal, India. Double distilled water from all-glass still was used throughout the study.

Preparation of extract
The powdered plant material (50 g) was extracted with 50% aqueous ethanol (400 ml) by boiling under reflux for 30 minutes. The extract was filtered and evaporated to dryness to yield the dry extract (HAWS, yield: 37.44%). The dry extract was kept in a refrigerator until use. Different concentrations of HAWS for anti-inflammatory assay were prepared freshly from the dry extract by dissolving in double-distilled water immediately prior to use.

Evaluation of anti-inflammatory effect in vitro
The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen’s egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of HAWS so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000 µg/ml. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37 ± 2°C in a BOD incubator (Labline Technologies) for 15 mins and then heated at 70°C for 5 mins. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2500 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:

\[
% \text{ inhibition} = 100 \times \left[ \frac{V_t}{V_c} - 1 \right]
\]

Where, \(V_t\) = absorbance of test sample, \(V_c\) = absorbance of control.

The extract/drug concentration for 50% inhibition (IC\(_{50}\)) was determined by plotting percentage inhibition with respect to control against treatment concentration.

### Table 1: Effect of HAWS against protein denaturation

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
<th>Viscosity (cp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>1.45</td>
</tr>
<tr>
<td>31.25</td>
<td>20</td>
<td>0.79</td>
</tr>
<tr>
<td>62.5</td>
<td>40</td>
<td>0.83</td>
</tr>
<tr>
<td>125</td>
<td>300</td>
<td>0.83</td>
</tr>
<tr>
<td>250</td>
<td>500</td>
<td>0.90</td>
</tr>
<tr>
<td>500</td>
<td>600</td>
<td>0.94</td>
</tr>
<tr>
<td>1000</td>
<td>1100</td>
<td>1.03</td>
</tr>
</tbody>
</table>

### Table 2: Effect of diclofenac sodium against protein denaturation

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
<th>Viscosity (cp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>1.45</td>
</tr>
<tr>
<td>78.125</td>
<td>12.5</td>
<td>0.80</td>
</tr>
<tr>
<td>156.25</td>
<td>12.5</td>
<td>0.86</td>
</tr>
<tr>
<td>312.5</td>
<td>25</td>
<td>1.0</td>
</tr>
<tr>
<td>625</td>
<td>50</td>
<td>1.13</td>
</tr>
<tr>
<td>1250</td>
<td>212.5</td>
<td>1.15</td>
</tr>
<tr>
<td>2500</td>
<td>812.5</td>
<td>1.26</td>
</tr>
</tbody>
</table>
drug diclofenac sodium.\[^{[13]}\] 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.\[^{[14]}\] In the present study, the relatively high viscosity of control dispersion substantiated this fact. Presence of HAWS prevented this, implying inhibition of protein denaturation. Here, viscosities decreased with respect to control where no test extract/drug was added. However, the viscosities were found to decrease with concanomitant decrease in concentration of test extract and reference drug as well. Although, the viscosities of the test samples (extract/drug), of all concentrations were always less than that of control. The observed decrease in viscosities may be due to decrease in concentration of test extract/drug, or other uncertain physicochemical factors. Nevertheless, the viscosity data indicated inhibition of protein (albumin) denaturation. The effect of concentration of test agent on viscosity behaviour of denatured protein dispersion requires further studies.

The major constituents of ashwagandha are several alkaloids and steroidal lactones commonly called withanolides which are responsible for its wide ranging biological effects.\[^{[15,16]}\] In the present study, anti-inflammatory effect of ashwagandha can be attributed to its alkaloid and withanolide contents. The effect may be due to synergistic effect rather than single constituent.

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 (pH: 6.2 – 6.5).\[^{[17]}\] Therefore, form the results of the present preliminary study it can be concluded that ashwagandha possessed marked anti-inflammatory effect against the denaturation of protein in vitro. Previous workers have reported anti-inflammatory activity of ashwagandha in experimental animal models.\[^{[18]}\] The present findings corroborated this property of ashwagandha in vitro. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions both in vivo and in vitro.

### Table 3: IC\(_{50}\) values of HAWS and diclofenac sodium against protein denaturation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IC(_{50}) values (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAWS</td>
<td>65</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>625</td>
</tr>
</tbody>
</table>

### ACKNOWLEDGEMENT

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### REFERENCES