COMPARATIVE STUDY OF ANTI-INFLAMMATORY ACTIVITY OF
WITHANIA SOMNIFERA WITH ASPIRIN IN EXPERIMENTAL ANIMALS (ALBINO RATS)"

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ABSTRACT
The roots of Withania somnifera consist primarily of compounds known as withanolides A & D, which are believed to account for its extraordinary medicinal properties. Objective of the study is to compare the anti-inflammatory effect of Withania somnifera & NSAIDs (Aspirin). Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1% freshly prepared solution. The groups consisted of complete Freund’s adjuvant (CFA)- 0.1ml injected rats challenged with doses of the test drug and standard drug administered orally 2 h before induction of arthritis. In our study we used 100mg/kg Aspirin as a standard drug for evaluation of anti-inflammatory activity. In present acute study, % inhibition of paw oedema was found to be 63.64% with aspirin as compare to 61.36% with Withania somnifera at the end 3hrs.

KEYWORDS: Withania somnifera, Anti-inflammatory activity, Aspirin.

INTRODUCTION
Herbal medicine is the use of plants and plants remedies in the treatment and prevention. Herbal medicines have been used for medicinal purpose since the existence of medicine and mankind. Herbal medicines are also known as phytomedicines and phototherapeutic agents.[1]
Humans have discovered the medicinal use of plants and their parts since the Stone Age. It can be claimed that many modern drugs are mere purified forms of the known herbal drugs originate from plants. Plants have been explored as a source of Novel compound.

*Withania somnifera*, also known as Ashwagandha, Indian ginseng and winter cherry, has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. Historically, the plant has been used as an aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat bronchitis, asthma, ulcers, emaciation, insomnia, and senile dementia. The roots of *Withania somnifera* consist primarily of compounds known as withanolides A & D, which are believed to account for its extraordinary medicinal properties.

Ayurveda claims that *Withania somnifera* is not only devoid of side effects but also have many beneficial effects. Plants base drugs and formulation are in use since ancient times, However pharmacological evaluation as well as comparison of the classical formulation is essential for the activities claimed in traditional Medicines.

Many modern drugs are available for the treatment of inflammation but they have many side effects. A drug effective for the treatment of inflammation and devoid of side effect is the need of time. Such medicine must be very useful for the treatment of chronic inflammation such as Rheumatoid arthritis. Pharmacological evaluation and comparison of the herbs and study of their active constituent and side effects play important role in new drug development.

**OBJECTIVES**

To compare the anti-inflammatory effect of Withania somnifera & NSAIDs (Aspirin).

**MATERIALS AND METHODS**

It was planned to conduct the study in experimental animal’s i.e. Rats (Albino Wistar ).

The details of the study are as under.

❖ *Locus of Study*

The present study was conducted in Department of pharmacology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha.
Duration of Study
The study was carried out for a period of 3 terms (18 month from 2011-2012).

Approval from Institutional Animal Ethical Committee
The research protocol was approved by the Institutional Animal Ethical Committee, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha.

Letter no. DMIMSUD; JNMC/IAEC/2009-10/75.

STUDY DESIGN / MATERIALS AND METHODS

MATERIALS

Collection of plant material – The plant material (Roots of Withania Somnifera) purchased from local herbarium “Shri Shail Herbarium, Nagpur”.

Authentication of of Plants – The plants were identified & authenticated with the standard sample preserved as Withania Somnifera by Botany Department, R. T. M. Nagpur University.

Purchase of chemicals – Drugs Aspirin was obtained from institutional medical store; Carrageenan from Himedia chemicals Mumbai; Complete Freund’s adjuvant from MM supplier Pune.

Instruments
- Digital Venire caliper.
- Feeding syringe & needle – For oral administration.
- Dispo van single use needle 26 no.
- Weighing machine.
- Soxhlet apparatus.

Animals
The study was conducted using 60 Wistar Albino Rats, of either sex weighing 150-200g from institutional animal house, Sawangi (Meghe), Wardha.

Inclusion criteria
- Sex : Male and Female rats.
- Weight : 150-250 gms. (6 rats in each groups).

Rats were obtained from the animal house of J. N. Medical College, Sawangi (M), Wardha.
**Exclusion criteria**
1. Pregnant female rats
2. Old rats
3. Unhealthy / Diseased rats

**METHODS**

- **Preparation of plant material in powdered form.**
  Roots were already in dried form. Dried Withania somnifera was coarsely powered.

- **Preparation of extract**
  **Ethanolic extract Withania somnifera:** The powdered plant material of *Withania somnifera* roots was extracted with ethanol in a Soxhlet apparatus for 48 hrs. The extracts were filtered through Whatmann filter paper (No.1) and concentrated by vacuum evaporation. The yield of extract as per solvent used was 3.25% w/w. The dried extracts were suspended in 2% gum acacia and used for experiments.

- **Phytochemical screening**
  The preliminary phytochemical studies was done at Laboratory of Department of Pharmacognosy, R.T.M.Nagpur University and Nagpur. It shows the following results.
  Ashwagandha contains withanolides as its major active ingredients.
  Extracts were tested for preliminary phytochemical studies using standard procedure.

- **Acclimatization**
  Animals were acclimatized for 8 days in the laboratory before experiment. Animals were kept on standard nutritional & environmental condition in separate cages. They were housed under standard condition of light, temperature & humidity. They were fed with standard laboratory chow & provided with water ad libitum.

- **Grouping of animals** - Animals were divided into 4 groups of six animals each.
  **Group 1:** Treated with Normal Saline (Control).
  **Group 2:** Treated with ethanolic roots extract of *Withania somnifera* (12mg/kg p.o).
  **Group 3:** Treated with ethanolic roots extract of *Withania somnifera* (25 mg/kg p.o).
  **Group 4:** Treated with Aspirin (100 mg/kg p.o.).
**Determination of doses**
- Ethanolic root extract of *Withania somnifera*: 12 mg/kg p.o.
- Ethanolic root extract of *Withania somnifera*: 25 mg/kg p.o.
- Aspirin: 100mg/kg p.o.

**Preparation of working solution**
The required amount of powered extract of *Withania somnifera* measured as per the dose/bodyweight (12mg/kg & 25 mg/kg) of rats daily & fresh solution used to make with honey & water (2ml).

**Anti-inflammatory activity**

*Carrageenan-induced paw oedema*
Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1% freshly prepared Carrageenan in normal saline in right hind paw of rats. Control groups were treated with normal saline, Test groups with Ethanolic root extract of *Withania somnifera*, 12mg/kg p.o & 25 mg/kg p.o and Standard groups with Aspirin (100mg/kg p.o.) one hour before Carrageenan injection. The paw volume was measured at an interval of 1, 2, 3, 4, 6 hrs. after Carrageenan injection by using vernier caliper.[5,6]

The difference in paw thickness after and before induction of inflammation was calculated and presented as mean increase in paw thickness (mm). The ability of anti-inflammatory drug to suppress paw inflammation was expressed as a percentage of inhibition of paw oedema and this percentage can be calculated according to the following equation.

**Percentage of inhibition (%) = 100 \times (1 - X / Y)**

Where X= mean increase in paw volume, thickness of treated rats
Y= mean increase in paw volume, thickness or weight of control rats.

**Freund’s Adjuvant Induced Arthritis**
Rats were divided into four groups of 6 animals each. Adjuvant arthritis was induced by subcutaneous injection of complete Freund’s adjuvant (CFA - 0.1ml of 0.5% w/v suspension of heat killed *Mycobacterium tuberculosis* cells in liquid paraffin) into the sub plantar tissue of the right hind paw of each rats.
Rats are divided into 4 groups

**Group 1** - Normal Saline. (Non Inflamed Control)

**Group 2** - Complete Freund’s adjuvant (0.1ml injected in sub planter Region of hind paw. (Inflamed Control).

**Group 3** - Test drug ethanolic extract of Withania Somnifera (25mg/kg p.o.)

**Group 4** - Standard drug Aspirin (100 mg /kg p.o.)

The groups consisted of complete Freund’s adjuvant (CFA) - 0.1ml injected rats challenged with doses of the test drug and standard drug administered orally 2 h before induction of arthritis. The drug administration were continued daily at the same time of the day for 12 more days.

Development of adjuvant induced swelling in the paws of both the injected and non injected paws of each rat were monitored daily as the percentage increase in paw oedema.

<table>
<thead>
<tr>
<th>Site</th>
<th>Degree of inflammation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear</td>
<td>absence of nodules and redness</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>presence of nodules and redness</td>
<td>1</td>
</tr>
<tr>
<td>Nose</td>
<td>no swelling of connective tissue</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>intense swelling of connective tissue</td>
<td>1</td>
</tr>
<tr>
<td>Tail</td>
<td>absence of nodules</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>presence of nodules</td>
<td>1</td>
</tr>
<tr>
<td>Fore paws</td>
<td>absence of inflammation</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>inflammation of at least 1 joint</td>
<td>1</td>
</tr>
<tr>
<td>Hind paws</td>
<td>absence of inflammation</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Slight inflammation</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Moderate inflammation</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Marked inflammation</td>
<td>3</td>
</tr>
</tbody>
</table>

The percentage inhibition of paw oedema compared with that of the inflamed control was taken as anti-arthritic activity. Paw oedema of both hind limbs and body weights were recorded daily from the day of injection.

Purposely, from day 13 to 21, the animals are not dosed with the test compound or the standard. As daily, on day 21st, the body weight is determined again and the severity of the secondary lesions is evaluated visually and graded according the following scheme.\[^{7,8,9}\]
a) For primary lesions: The percent inhibition of paw volume of the injected left paw over vehicle control is measured at day 5.

b) For secondary lesions: The percentage inhibition of paw volume of the non-injected right paw over controls is measured at day 21.

c) An arthritic index is calculated as the sum of the scores as indicated above for each animal. The average of the treated animals is compared with the control group.\[5,6\]

d) The total percentage change is calculated as follows by addition of: percent inhibition of the injected paw on day 5 + percent inhibition of the non-injected paw on day 21 + percent change of the arthritic index.

From Day 21 to 28 again test drug and standard drug were given again. Percentage change in paw edema and body weight changes, arthritic index were recorded.

**Statistics**

The data was subjected to statistical evaluation by applying the tests of significance as under

Paired t-test, One way ANOVA, Dunnett’s multiple comparison test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Dose (mg/kg, p.o)</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>6hr</th>
<th>12hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.51±0.02</td>
<td>0.42±0.007</td>
<td>0.44±0.02</td>
<td>0.34±0.004</td>
<td>0.29±0.004</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td>2</td>
<td>WS (25 mg/kg p.o.)</td>
<td>0.36±0.006*</td>
<td>0.18±0.007*</td>
<td>0.17±0.01*</td>
<td>0.14±0.01*</td>
<td>0.13±0.007*</td>
<td>0.15±0.06*</td>
</tr>
<tr>
<td></td>
<td>(29.41%)</td>
<td>(57.14%)</td>
<td>(61.36%)</td>
<td>(58.82%)</td>
<td>(55.17%)</td>
<td>(42.31%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>WS (12 mg/kg p.o.)</td>
<td>0.39±0.006*</td>
<td>0.28±0.004*</td>
<td>0.28±0.04*</td>
<td>0.22±0.004*</td>
<td>0.19±0.005*</td>
<td>0.17±0.005*</td>
</tr>
<tr>
<td></td>
<td>(23.53%)</td>
<td>(33.33%)</td>
<td>(36.36%)</td>
<td>(35.29%)</td>
<td>(34.48%)</td>
<td>(34.62%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Aspirin (100 mg/kg p.o.)</td>
<td>0.28±0.008*</td>
<td>0.18±0.007*</td>
<td>0.16±0.007*</td>
<td>0.13±0.01*</td>
<td>0.13±0.004*</td>
<td>0.14±0.007*</td>
</tr>
<tr>
<td></td>
<td>(45.10%)</td>
<td>(57.14%)</td>
<td>(63.64%)</td>
<td>(61.76%)</td>
<td>(55.17%)</td>
<td>(46.15%)</td>
<td></td>
</tr>
</tbody>
</table>
RESULT AND DISCUSSION

Graph 1: Effect of ethanolic root extract of *Withania somnifera* & Aspirin against Complete Freund’s Adjuvant Induced arthritis in rats. (Right injected paw)

Graph 2: Effect of ethanolic roots extract of *Withania somnifera* & Aspirin against Complete Freund’s Adjuvant Induced arthritis in rats. (Left Non Injected Paw)
Tabel 2: Changes in Arthritic Index after treatment with ethanolic root extract of Withania somnifera & Aspirin in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>5 day (mg/kg, p.o)</th>
<th>15 day (mg/kg, p.o)</th>
<th>21 day (mg/kg, p.o)</th>
<th>24 day (mg/kg, p.o)</th>
<th>28 day (mg/kg, p.o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inflamed Control</td>
<td>4.51±0.58</td>
<td>6.21±0.14</td>
<td>6.33±0.18</td>
<td>6.45±0.12</td>
<td>6.45±0.12</td>
</tr>
<tr>
<td>2</td>
<td>Aspirin (100 mg/kg p.o.)</td>
<td>3.63±0.09* (19.51%)</td>
<td>3.21±0.10* (48.31%)</td>
<td>3.46±0.04* (45.34%)</td>
<td>3.43±0.06* (46.82%)</td>
<td>3.21±0.08* (50.23%)</td>
</tr>
<tr>
<td>3</td>
<td>WS (25 mg/kg p.o.)</td>
<td>3.81±0.03* (15.52%)</td>
<td>3.95±0.09* (36.39%)</td>
<td>3.51±0.03* (44.55%)</td>
<td>3.48±0.04* (46.05%)</td>
<td>3.21±0.05* (50.23%)</td>
</tr>
</tbody>
</table>

DISCUSSION

Carrageenan-induced oedema is mediated through the release prostaglandin and slow reacting substances which peak at 3 hrs. It shows a biphasic response The first phase is due to release of histamine and serotonin and second accelerating swelling is due to release of PG. Significant inhibition of paw edema in the early hours of study by Withania somnifera could be attributed to the inhibition of histamine and serotonin. The decrease in paw edema inhibition at +6h attributed to the termination of test drug action. The Withania somnifera produced dose-dependent reduction of paw edema in rats. In arthritis model, the doses of 25 mg/kg of the ethanol extract of Withania somnifera produced 48.70% after 28th days when compared with that of the standard drug i.e. 52.52% with Aspirin respectively. Ethanolic extract of Withania somnifera exhibit significant anti-inflammatory and anti-arthritic activities.

In present study significant increase in arthritic index is seen in inflamed control group on day 28th i.e. 6.16±0.40 while non significant rise seen in all the 3 groups, standard drug arthritic index in Aspirin 1.50±0.54 as compare to 1.50±0.50 of Withania somnifera. Withania somnifera shows significant inhibition of secondary lesion, decrease the severity of spread of lesion, the results are comparable with Hydrocortisone and Aspirin indicates that Withania somnifera is also useful in chronic inflammatory conditions. A study by A Chakraborty et al. used 100 mg/kg Aspirin as a standard drug for preliminary study on anti-inflammatory and analgesic activities of spilanthes acmella. Similarly in a study by S. Balian 150mg/kg oral dose of Aspirin used as a standard drug for evaluation of anti-inflammatory activity of leaf and leaf callus of Silybum marianum(L) Gaetn.
In our study we used 100mg/kg Aspirin as a standard drug for evaluation of anti-inflammatory activity. In present acute study, % inhibition of paw edema was found to be 63.64% with aspirin as compare to 61.36% with Withania somnifera at the end 3hrs.

CONCLUSION
1. Ethanolic extract of Withania somnifera elicited significant dose dependant acute and chronic anti-inflammatory activity in carrageenan induced paw edema comparable to aspirin.
2. A single dose of Withania somnifera has a good duration of action as it could effectively suppress the inflammation after 6 hr of its administration.
3. Withania somnifera possess dose dependent anti-inflammatory activity.
4. This justifies the use of Withania Somnifera in our indigenous medicine, as a potent anti-inflammatory drug without any side effects.

REFERENCE

