EVALUATION OF ANALGESIC ACTIVITY OF WITHANIA SOMNIFERA IN ALBINO RATS: AN EXPERIMENTAL STUDY

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Abstract: Pain is an ill-defined unpleasant sensation usually evoked by an external or internal noxious stimulus. The problems associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs) and opiates as analgesics underline the urgent need to screen and identify plant material used as pain relievers in traditional medicine. The purpose of present study was undertaken to assess the analgesic effect of Withania somnifera in albino rats and mice. The analgesic action was studied by acetic acid induced writhing, tail flick and hot plate method. In each experiment animals were divided in 5 groups of 6 animals each. 1st group was given normal saline in dose of 5ml/kg, 2nd standard analgesic drug and 3rd, 4th and 5th group W. somnifera in doses of 400, 800 & 1600 mg/kg respectively. Normal saline group serves as a control. In all experiments drugs were used orally. W. somnifera showed significant analgesic activity (p<0.05) in all three models used to evaluate analgesic activity as compared to saline treated group.

Key words: Analgesic activity, Non-steroidal anti-inflammatory drugs, Withania somnifera.

INTRODUCTION

Pain is an ill-defined unpleasant sensation usually evoked by an external or internal noxious stimulus. It is a warning signal and primarily protective in nature, but causes discomfort. Pain is the most important symptom which brings the patient to the physician. Analgesics are the drugs that selectively relieve pain by acting on the central nervous system (CNS) or on peripheral pain mechanisms, without significantly altering consciousness. Though considerable progress has been achieved in medical science in recent time, management of pain still remains a challenge for medical community. Use of the currently available analgesic drugs such as Non steroidal anti inflammatory drugs (NSAIDs) and opiates are respectively associated with gastro intestinal side effect and tolerance as well as dependence. Therefore, analgesic drugs lacking these side effects are being searched all over the world as alternatives to NSAIDs and opiates. Medicinal plants synthesize a large number of chemical substances, some of which produce important pharmacological effects on various physiological systems of body. As a result, more and more people are turning to herbal medicines as the alternative treatment of pain since these herbal drugs are virtually free from side effects.

Withania somnifera commonly known as Ashwagandha is member of solanaceae family. It grows as a stout shrub that overly reaches a height of five feet. W. somnifera bears yellow flowers and red fruit which are berry like in the size. The roots are fleshy and cylindrical. W. somnifera is found throughout the drier parts of India, in West Asia and Northern Africa. W. somnifera contains steroidal compounds of great interest to researchers, including ergosteriole type steroidal lactones, including withanolides A-Y, dehydrowithanolide-R, withasomniferin-A, withasomniferon, withashanosiferol, withanone, and others. Other constituents include the phytochemicals sitoindosides VII-X and β-sitosterol, as well as alkaloids (e.g. ashwagandhine, cuscohygrine, tropine, pseudotropine, isopeltierine and anaferine), a variety of amino acids including tryptophan, and high amounts of iron. Traditionally it is used to enhance the vitality and vigour and helps in building greater endurance.

METHODOLOGY

The study was conducted on healthy albino mice (25-30 gm) and albino wistar rats (100 – 150 gm) of either sex, maintained at an ambient temperature of 25 – 35°C with food and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and was executed according to the
guidelines of the committee for the purpose of control and supervision of the experiments on animals (CPCSEA), India.

Dried root of the plant were pulverized in an electric blender to form a powder. 100 g of the prepared powder weighing was macerated and soaked in 500 ml of distilled water for 24 h. It was then filtered through a 1mm mesh sieve and the filtrate was concentrated to a dark green residue by heating at 40 °C, till complete evaporation of water was achieved. Finally, the aqueous extract was kept in desiccators for 2 weeks to remove the excessive moisture and was used for further studies.

In each experiment animals were divided in 5 groups of 6 animals each. 1st group was given normal saline in dose of 5ml/kg, 2nd standard analgesic drug and 3rd, 4th and 5th group W. somnifera in doses of 400, 800 & 1600 mg/kg respectively. Normal saline group serves as a control. In all experiments drugs were given orally.

(1) Acetic acid induced writhing method6:

The Acetic acid induced writhing method was assessed on mice. For inducing writhing animals of each group were challenged with intra peritoneal (i.p.) injection of acetic acid solution in dose of 10 ml/kg of 0.6% in normal saline. Counting of writhing movements were started after 5 minutes of induction through acetic acid and counted over a period of 10 minutes. All the drugs were given once orally and standard analgesic drug used was Aspirin in dose of 100mg/kg.

(2) Tail Flick method7:

The tail flick method was assessed on rats using analgesiometer. The instrument has a nichrome wire, which would be heated to the required temperature and maintained by means of heat regulators. The strength of the current passing through the naked nichrome wire was kept constant at 4 Amps. The rat was kept in a rat holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The environmental and stray heating effects were kept to a minimum by circulating water at room temperature around the rat’s tail. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting. A cut-off reaction time was fixed at 10 second to avoid tissue damage. Those rats were included in the study which showed the reaction within the range of 4-6 seconds. The procedure was repeated and reaction time is noted before and after 30 minutes, 60 minutes and 90 minutes. All the drugs were given once orally and standard analgesic drug used was Tramadol in dose of 5mg/kg.

(3) Hot plate method8:

The hot plate method was assessed on mice. Animals of each group were challenged with noxious stimuli by placing them into the Perspex cylinder on the heated surface; the temperature of which is maintained at 55.0 ± 0.2° C and a basal latency to a discomfort reaction (licking hind paws or jumping) time was noted. Those mice were included in the study which showed the reaction within 6-8 seconds. A cut-off reaction time was fixed at 15 second to avoid tissue damage. The procedure was repeated and reaction time is noted before and after 30 minutes, 60 minutes and 90 minutes. All the drugs were given once orally and standard analgesic drug used was Tramadol in dose of 5mg/kg.

Statistical Analysis

Results were calculated by one way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparisons. Results were expressed as mean ± SD. P values were calculated referring to the appropriate tables. Values of P<0.05 were considered as statistically significant.

RESULTS

(i) Acetic acid induced writhing: (Table I)

The analgesic activity was expressed as percent reduction in mean number of writhing movement comparing with control as 100% writhing movement. Writhing response is suppressed significantly (p<0.05) by standard drug as well as by W. somnifera at the dose of 400, 800, 1600 mg/kg. At the dose of 400 mg/kg W. somnifera showed 64.18 % reduction in acetic acid induced writhing response, while at dose of 800 mg/kg and 1600 mg/kg % reduction is 79.24% and 84.95% respectively. Standard drug Aspirin showed 86.82% suppression. W. somnifera was found to increase the basal reaction time in a dose-dependent manner.
Table- I Effect of *W. somnifera* and aspirin on acetic acid induced writhing movement in albino mice (n=6)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose(mg/kg, oral)</th>
<th>Number of writhing movement ± SD</th>
<th>% inhibition in writhing movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>5 ml</td>
<td>17.67 ± 1.53</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>100</td>
<td>2.33 ± 0.57*</td>
<td>86.82</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>400</td>
<td>6.33 ± 0.56*</td>
<td>64.18</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>800</td>
<td>3.67 ± 0.57*</td>
<td>79.24</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>1600</td>
<td>2.66 ± 0.57*</td>
<td>84.95</td>
</tr>
</tbody>
</table>

*P < 0.05 (as compared to saline treated group)

(ii) Tail flick method: (Table II)

In this method latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick. In the present study, *W. somnifera* showed a significant (p<0.05) analgesic effect compared to that of control group at all the three doses (400, 800, 1600 mg/kg) used.

### Table- II Effect of *W. somnifera* and tramadol on algesia induced by tail flick method in albino rats (n=6)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose(mg/kg, oral)</th>
<th>Reaction time(Seconds)±SD Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Saline</td>
<td>5 ml</td>
<td>3.03±0.09</td>
<td>3.12±0.19</td>
</tr>
<tr>
<td>Tramadol</td>
<td>5</td>
<td>3.55±0.14</td>
<td>4.06±0.16*</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>400</td>
<td>4.01±0.13</td>
<td>4.04±0.25*</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>800</td>
<td>3.12±0.07</td>
<td>3.38±0.18*</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>1600</td>
<td>3.75±0.11</td>
<td>4.12±0.24*</td>
</tr>
</tbody>
</table>

*P<0.05 (as compared to saline treated group)

(iii) Hot plate method: (Table III)

The analgesic activity was expressed as percent elevation in mean reaction time in *W. somnifera* treated group taking control as 100% reaction time. *W. somnifera* was found to increase the basal reaction time in a dose-dependent manner. *W. somnifera* at the dose of 400, 800, 1600 mg/kg showed statistically significant analgesic activity (p<0.05) compared to saline treated group.

### Table - III Effect of *W. somnifera* and tramadol on algesia induced by hot plate method in albino mice (n=6)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose(mg/kg, oral)</th>
<th>Reaction time(Seconds)±SD Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Saline</td>
<td>5 ml</td>
<td>5.84±0.20</td>
<td>5.81±0.25</td>
</tr>
<tr>
<td>Tramadol</td>
<td>5</td>
<td>5.90±0.40</td>
<td>8.89±0.25*</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>400</td>
<td>5.85±0.25</td>
<td>6.25±0.30*</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>800</td>
<td>5.91±0.36</td>
<td>6.88±0.28*</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>1600</td>
<td>5.83±0.20</td>
<td>8.01±0.60*</td>
</tr>
</tbody>
</table>

*P<0.05 (as compared to saline treated group)

**DISCUSSION**

Pain sensation are received at different levels like peripheral, spinal and at supra spinal level. In our study, three of the most common phasic nociceptive tests have been investigated: writhing response, tail-flick, hot-plate method. In these tests, a brief noxious stimulus of short duration is applied, detected by free nerve endings...
and conducted through conducting neuronal pathways. Writhing response of the animals to an intra-peritoneal injection of noxious chemical such as acetic acid and thermal stimuli in tail flick and hot plate method were used to screen both peripheral and central analgesic activity, respectively. The results of the present study show significant anti nociceptive effect of *W. somnifera* in writhing response (Table I) tail flick method (Table II) and in hot plate method (Table III) at all three doses used (400, 800, 1600 mg/kg).

Acetic acid induced writhing is used to evaluate drugs acting on pain produced by inflammation and local irritation which involves release of mediators of inflammation like prostaglandins, histamine, serotonin, substance P etc. i.e. Drugs acting at the peripheral level by exciting the pain nerve endings. Inhibition of cyclo-oxygenase in the peripheral tissues, results in interference with the mechanism of transduction in primary afferent nociceptors because it is principal enzyme responsible for production of inflammatory mediators. Some steroidal lactones (Withanolides, Withaferin etc) are constituents of *W. somnifera*. Cyclo-oxygenase enzyme inhibiting activity of these steroidal lactones may be responsible for inhibition of peripheral pain mechanism.

Tail Flick method is predominantly a spinal response and Hot Plate method is predominantly supraspinal response. The mechanism responsible for the central analgesic activity of *W. somnifera* is probably mediated via opioid receptors.

**CONCLUSION**

It can be concluded from this study that aquousk extracts of *W. somnifera* possess significant central and peripheral analgesic activities. Information of this study may help the future researchers to explore analgesic activity of the plant.

**References**

3. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd ed. Asiatic society of Bangladesh, Dhaka, 2003; 69.