**Potential of Analgesic and Anti inflammatory Activity of *Withania somnifera* Linn. Leaves**

**Abstract**

Medicinal plants to prevent and treat illnesses have been a part of herbal medicine for thousands of years. This study aimed to test the pain-relieving and inflammation-reducing effects of *Withania somnifera* (genus: Withania, family: Solanaceae) leaf extract using well-known rat models. Research on *Withania somnifera* has demonstrated that its active ingredients confer analgesic and anti-inflammatory properties. Withanolide, one of the plant's primary active chemical ingredients, drives its vast spectrum of biological activities. Numerous formulations for various illnesses, including cancer, neurological diseases, cardiovascular diseases, asthma, arthritis, dementia, hypertension, anxiety, and more, contain withanoides as a component. Significant polyphenolic chemicals found in the plant helped lessen the paw edema caused by carrageenan. The anti-inflammatory effect was assessed using the carrageenan-induced paw edema model.On the other hand, we determined the analgesic efficacy using the tail-flick method and the acetic acid-induced writhing test. The 600 mg/kg and 900 mg/kg dosages had statistically significant anti-inflammatory effects (p < 0.05). In pain relief studies, a dose of 900 mg/kg showed a significant reduction in pain (p<0.05) based on the writhing test, meaning it reduced pain by 10.66%. However, the analgesic effects of the 900 mg/kg dosage were statistically significant (p<0.05). The primary bioactive chemicals found in the plant are withanolides A, withaferin A, withasomniferin A, withasomnidienone, withasomnierose A-C, and withanone, among others. The results support the traditional use of *Withania somnifera* in pain and inflammation treatment and are consistent with previous studies.

**Keywords:** *Withania somnifera;* withanoide; anti-inflammatory; analgesic; carrageenan; traditional medicine; rat method; tail flick test; writhing test**.**

**Introduction**

The International Association for the Study of Pain (IASP) now defines pain as "an unpleasant sensory and emotional experience linked to actual or potential tissue damage, or articulated about such damage." The IASP Council approved this definition in 1979 after it was put forth by the Subcommittee on Taxonomy [1]. Pain is a component of human suffering that causes discomfort. The underlying cause is inflammation and the following inflammatory response, regardless of the type of pain—acute or chronic, peripheral or central, nociceptive or neuropathic. Since there are currently no safe, targeted, or effective treatments for chronic pain, which affects 20–30% of people worldwide, it is the leading cause of suffering for people [2]. Five common symptoms of inflammation are redness, swelling, heat, pain, and loss of function. Inflammation is linked to discomfort as well as several illness outcomes. Based on the development of the pathology beneath the wounded tissue, inflammation can be classified into three types: Subacute inflammation is a transitional phase between acute and chronic stages that lasts between two and six weeks; chronic inflammation is the result of acute inflammation not going away, which can persist for months or even years; and acute inflammation happens soon after injury and lasts for a few days [3]. Among the medications commonly used as analgesics or anti-inflammatories are opioids, diclofenac, paracetamol, and ketorolac. Aspirin, codeine, and morphine are a basic analgesic combination; however, each has various side effects, such as effects on the stomach, heart, kidneys, brain, and immune system [4]. Therefore, the development of highly effective anti-hyperlipidemic medications with negligible side effects is essential.

In addition to serving as a valuable and abundant source of naturally occurring chemicals for medicinal reasons, plants play a crucial role in discovering and synthesizing novel medications [5]. According to experts, certain chemical compounds obtained from medicinal plants may have therapeutic properties. To effectively treat various illnesses, researchers are constantly looking for novel herbal cures and other plant-derived medications [6]. In many nations worldwide, traditional medicines have long been used as remedies made from plants, dietary supplements, and complementary therapies. The use of traditional medicine has increased significantly, and many people across the country now use it as their primary source of healthcare [7]. Numerous chemical components in medicinal plants enable them to provide a broad range of pharmacological and therapeutic effects. These compounds include tannins, glycosides, alkaloids, saponins, polysaccharides, essential oils, terpenoids, resins, and plant lipids [8-9]. Genetically modified plants can produce the desired therapeutic effect by precisely controlling chemical concentrations. Enhancing the production of secondary metabolites, such as alkaloids, is one of the many possible uses for reverse genetics [10]. Exploration of plant species' therapeutic qualities has increased due to advancements in scientific research worldwide [11].

For a long time, people have employed *Withania somnifera* (L.) Dunal (Solanaceae) as a Rasayana plant [12, 13]. *Withania somnifera*, also referred to as Indian ginseng or ashwagandha, is an important medicinal plant that has been used for more than 3,000 years in Ayurvedic and traditional medicine [14]. Because of their considerable therapeutic and nutraceutical potential, plants in the genus Withania, which belongs to the Solanaceae family, are well acknowledged for their primary medical significance [15]. It is an upright, greyish, evergreen shrub that grows to a height of 1.25 m. It has long, tuberous roots, short stems, oblong, stalked leaves, and greenish, bisexual flowers in the axils [16]. It is common in the desert regions of North Africa, the Canary Islands in Europe [17–21], and parts of Asia and Africa, including India, Baluchistan, Pakistan, Afghanistan, Sri Lanka, the Congo, South Africa, Egypt, Morocco, and Jordan [22]. *W. somnifera* contains active chemicals like alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins with an extra acyl group (sitoindoside VII and VIII), and withanolides that have glucose at carbon 27 (sitoindoside XI and X) [23]. According to Gupta and Singh (2014) and Paval et al. (2009), withaferin-A inhibits the production of inflammatory mediators like prostaglandins, histamine, interleukins, and cytokines [24]. The plant *W. somnifera* has various important chemical parts, including alkaloids, anthocyanins, glycosides, carotenoids, flavonoids, lignins, steroids, phytosterols, tannins, amino acids, reducing sugars, and starch, which contribute to its health benefits. The main known alkaloids in this plant are withanolides, which include withanolide-A, withaferin-A, and sitoinoside (IX, X) [25]. Because of its anti-inflammatory, anti-hypoxic, antiischemic, neuroprotective, immunomodulatory, hepatoprotective, cardioprotective, anti-diabetic, adaptogenic, anti-arthritic, anti-stress, and antibacterial qualities, it has a wide range of therapeutic indications [26]. According to traditional medicine, it has been used as an anti-stress agent, narcotic, diuretic, anemia treatment, aphrodisiac, constipation remedy, anti-parasitic, liver disease, leprosy, anti-inflammatory, cardiovascular, joint pain, antibacterial, nervous system disorders, and arthritis, among other uses [27]. *W. somnifera* was used to help with inflammation because it blocked inflammation-related substances and reduced signs of inflammatory activity [28].

The present study aims to investigate the analgesic and anti-inflammatory properties of *Withania somnifera* in Rats.

**Materials and Methods**

**Drugs, Chemicals, and Instruments**

Sigma Aldrich (Germany) provided acetic acid, carrageenan, ethanol, and alloxan. Healthcare Pharmaceutical Limited (UK) gave away ibuprofen and aspirin as free samples. An analgesia meter and a plethysmometer measured the analgesic and anti-inflammatory effects.

**Plant Collection and Extract Preparation**

After being confirmed and taxonomically recognized, the leaf of *Withania somnifera* was removed from the University of Dhaka's Faculty of Pharmacy's medicinal plant garden. The plant specimens were kept according to the Bangladesh National Herbarium storage guidelines. The herbarium authorities assigned accession number 47380 to the leaf, which had been shade-dried for 7–10 days and then roughly crushed for subsequent use on 11-2-2019. The powdered leaves were steeped in 70% ethanol and vigorously shaken over 96 hours. The extract was filtered after soaking, and the liquid was put away. A rotary evaporator was then used to filter the concentrated extract. After drying, the concentrated extract was stored for further use.

**Experimental Animal Handling**

A 12:12 light:dark cycle and a constant temperature of 25 degrees Celsius were maintained in male Wistar rats weighing between 125 and 200 g, which were acquired from the Zoology Department of Jahangirnagar University in Bangladesh and kept at the Institute of Nutrition and Food Science at the University of Dhaka. Standard pellet food and fresh water were provided daily to the rats, who were kept there to acclimate before the trial started. Every experiment using rats complied with the Institutional Animal Ethics Committee's (IEAC) guidelines. The Swiss Academy of Sciences (SCNAT) and the Swiss Academy of Medical Sciences (SAMS) established guidelines for the treatment and use of animals in scientific investigations.

**Experimental Guidelines**

All investigations used the ethical principles outlined in the 2013 Declaration of Helsinki.

**Experimental Design**

Rats were weighed individually to determine their body weight, and then they were split up into groups (Table 1), with five rats in each group evenly distributed according to body weight.

**Evaluation of Analgesic Activity**

We examined the analgesic and anti-inflammatory properties of a reference drug and the extract of *Withania somnifera* by inducing inflammation in rats using carrageenan.

**Table 1**: Group specification for Analgesic activity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group Number | Group Specification | Treatment species | Dose Treatment species (mg/kg) | Abbreviation of Groups |
| 1 | Carrageenan Control | N/A | N/A | Car |
| 2 | Carrageenan + Ibuprofen | Ibuprofen | 10 | Car+Ib10 |
| 3 | Carrageenan + *Withania somnifera* | *Withania somnifera* | 300 | Car+WS300 |
| 4 | Carrageenan + *Withania somnifera* | *Withania somnifera* | 600 | Car+WS600 |
| 5 | Carrageenan + *Withania somnifera* | *Withania somnifera* | 900 | Car+WS900 |

**Carrageenan-Induced Acute Inflammatory Model**

The conventional technique for evaluating the effectiveness of analgesic and anti-inflammatory drugs is the carrageenan-induced test for rat paw edema. We evaluated the analgesic and anti-inflammatory using a plethysmometer and other specialist tools. The next step was to measure the size of each rodent's paw. To cause edema, researchers applied 0.1 mL of a 1% carrageenan solution per 100 g of body weight to the subplanar tissue of the rat's left hind paw. After that, an hour was allotted. Rats were given different amounts of the test medication and extracts. We used a plethysmometer to measure the paw volume between 0 and 6 hours after the carrageenan infusion. The rate of edema obstruction was calculated using the following formula.

Percentage Inhibition =

Here,

VPC = volume of animals' paws in Positive Control rat

V0=volume of animals' paws in Treatment Group

**Assessment of anti-inflammatory activity:** The acetic acid-induced writhing test and the tail-flick method subjected the mouse to discomfort.

**Table 2**: Group specification for anti-inflammatory activity by acetic acid writhing method

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group Number | Group Specification | Treatment species | Dose Treatment species (mg/kg) | Abbreviation of Groups |
| 1 | Acetic Acid Control | Physiological Saline | 10ml/kg | Ace |
| 2 | Aspirin + Acetic Acid | Aspirin | 100 | As100+Acetic Acid |
| 3 | *Withania somnifera + Acetic acid* | *Withania somnifera* | 300 | WS300+Acetic Acid |
| 4 | *Withania somnifera + Acetic acid* | *Withania somnifera* | 600 | WS600+Acetic Acid |
| 5 | *Withania somnifera + Acetic acid* | *Withania somnifera* | 900 | WS900+Acetic Acid |

**Acetic acid-induced writhing test**

Peripheral analgesic and anti-inflammatory effects were assessed using the acetic acid-induced writhing method. Several test samples were given half an hour before the intraperitoneal acetic acid injection. We administered an intraperitoneal injection of 0.9% acetic acid (10 ml/kg) to the rats as they responded to unpleasant stimuli. The frequency of muscle contractions, or writhes, was measured for 20 minutes after the acetic acid injection. The percentage of writhing inhibition was determined by counting the occurrences of abdominal muscle contractions, hind limb retractions towards the abdominal walls, hind limb extensions, and intermittent back arching over twenty minutes. The equation calculates the percentage of writhing suggestive of analgesic and anti-inflammatory effects.

Percent inhibition=

Where = the mean number of writhing of each test group

= The mean number of acetic acid control group writhing.

After a seven-day break, the extract's ability to reduce pain and inflammation is assessed using the "Tail Flick Method" on the same experimental rat model. The acetic acid injection's activity had stopped by this point.

**Table 3**: Group specification for analgesic and anti-inflammatory activity by tail flick method

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group Number | Group Specification | Treatment species | Dose Treatment species (mg/kg) | Abbreviation of Groups |
| 1 | Tail Flick Stress (control) | Physiological Saline | 10ml/kg | TFS |
| 2 | Aspirin + Tail Flick Stress | Aspirin | 100 | As100+TFS |
| 5 | *Withania somnifera* + Tail Flick Stress | *Withania somnifera* | 300 | WS300+TFS |
| 6 | *Withania somnifera* + Tail Flick Stress | *Withania somnifera* | 600 | WS600+TFS |
| 7 | *Withania somnifera* + Tail Flick Stress | *Withania somnifera* | 900 | WS900+TFS |

**Tail flick method**

The tail-flick experiment, a nociceptive test first reported by Love and Smith in 1941, evaluates an animal's behavioral response to painful stimuli. A tail-flick analgesia meter (UGO BASILE®, Germany) calibrated with radiant heat was used to quantify the time interval between the onset of the avoidance reaction and stimulus exposure. With the help of the heat controls, the exposed nichrome was continuously supplied with a current of 4 amps to reach the proper temperature. It may cause pain if radiant heat is applied to the middle part of the rats' tails. The time it took to exhibit a tail-flick reaction was recorded for untreated and treated rats. We conducted trials at 0, 15, 30, 45, and 60 minutes after administering test drugs to the animals.

**Statistical analysis**

We used Microsoft Excel to document and evaluate our results (raw data), which were then grouped into several categories, covering a wide range of study parameters. The data was subjected to descriptive statistics; the mean and standard deviation (SD) were displayed as results. SPSS 1600's "One-Way ANOVA Test" tool assessed the statistical significance of the observed change across groups. The occurrence is considered statistically significant when the p-value is less than 0.05 (p < 0.05).

**Results and discussion:**

**Table 4:** Anti-inflammatory activity of different doses of *Withania somnifera* extract and Ibuprofen through paw edema test in a rat model.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group** | **Time µL** | | | | |
| 0 Minutes before carrageenan injection) | 1 hour (just before treatment) | 2 Hours | 3 Hours | 4 Hours |
| **Car** | 108.45±6.45 | 117.25±8.54 | 128.25±7.39 | 137.25±7.02 | 142.56±8.26 |
| **Car+Ib10** | 112.25±5.76 | 117.49±6.23 | 122.58±6.40 | 127.89±5.59 | 130.57±5.78 |
| **Car+WS300** | 110.45±6.28 | 114.25±6.28 | 126.45±7.58 | 131.65±6.62 | 137.90±7.28 |
| **Car+WS600** | 111.25±6.82 | 114.56±6.79 | 120.45±7.42\* | 125.56±5.55\* | 132.52±6.24\* |
| **Car+WS900** | 110.25±5.99 | 115.59±7.04 | 121.24±6.32\* | 126.42±6.24\* | 128.90±6.29\* |

Note: The results were expressed in Mean±SEM (standard mean error) \*p< 0.05, \*\*p< 0.01, and \*\*\*p< 0.001 were considered statistically significant. The statistical analysis was followed by a one-way analysis of variance (Dunnett’s test) compared to the control.

**Analgesic Activity of *Withania somnifera:***

**Table 5:** Analgesic effect of different doses of *Withania somnifera* extract and Aspirin by acetic acid writhing test (\* presents the significance level of the result).

|  |  |  |  |
| --- | --- | --- | --- |
| **Group specification** | **Dose** | **Number of writing** | **% Inhibition** |
| **Ace** | N/A | 98.27±8.74 | - |
| **As100+Acetic Acid** | 100 | 64.28±5.43 | - |
| **WS300+Acetic Acid** | 300 | 93.45±6.97 | 4.90% |
| **WS600+Acetic Acid** | 600 | 91.46±6.24 | 6.93% |
| **WS900+Acetic Acid** | 900 | 87.79±5.59\* | 10.66% |

Note: The results were expressed in Mean±SEM (standard mean error) \*p< 0.05, \*\*p< 0.01, and \*\*\*p< 0.001 were considered statistically significant. The statistical analysis was followed by a one-way analysis of variance (Dunnett’s test) compared to the control.

**Table 6:** Analgesic activity of *Withania somnifera* and Aspirin by the tail-flick test method.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Group No** | **Group Specification** | **Basal Reaction** | **Reaction time in the second** | | | |
| After 30 minutes | After 1 Hour | After 2 Hour | After 4 Hour |
| 1 | TFS | 3.54±0.77 | 4.40±0.84 | 5.53±0.73 | 6.12±0.53 | 6.99±0.93 |
| 2 | As100+TFS | 3.57±0.96 | 5.18±0.87 | 6.63±0.74 | 7.18±0.88 | 8.21±0.77 |
| 3 | WS300+TFS | 3.78±0.69 | 5.03±0.97 | 5.45±0.83 | 6.16±0.71 | 6.77±0.97 |
| 4 | WS600+TFS | 3.80±0.82 | 4.69±0.97 | 5.67±0.94 | 6.32±0.87 | 7.18±0.77 |
| 5 | WS900+TFS | 3.84±0.86 | 4.82±0.88 | 5.87±0.74 | 6.80±0.97\* | 762±0.92\* |

Note: The results were expressed in Mean±SEM (standard mean error) \*p< 0.05, \*\*p< 0.01, and \*\*\*p< 0.001 were considered statistically significant. The statistical analysis was followed by a one-way analysis of variance (Dunnett’s test) compared to the control.

Indigenous communities have used traditional herbal remedies to cure various illnesses for thousands of years, demonstrating the long-standing recognition of the therapeutic benefits of plants. This study investigates the analgesic and anti-inflammatory properties of leaves of *Withania somnifera* to evaluate their potential as a natural therapeutic agent for managing pain and inflammation and to scientifically validate their traditional use [29]. Withaferin-A has shown it can reduce inflammation by stopping the activation of NF-kB, which prevents the phosphorylation of IkB and the activation of IkB kinase [30]. Using a rat model's paw edema test, the anti-inflammatory effects of 600 mg/kg and 900 mg/kg dosages were statistically significant (p < 0.05) at 2-, 3-, and 4-hour intervals. However, the absence of an additional dosage did not result in any statistically significant differences. Traditional medical systems employ numerous natural items to alleviate pain symptoms [31]. More studies are needed on phytochemicals to understand their roles better, as some, like tannins and glycosides, might also help reduce pain [32], as seen in rat tests. The writhing test showed statistical significance (p < 0.05) at the dosage of 900 mg/kg, which could indicate an inhibition of 10.66%. At 2-hour and 4-hour intervals, however, the 900 mg/kg dosage was statistically significant (p<0.05) according to the tail-flick test. This study provides specific in vivo data and scientific backing for traditional uses. It enhances our knowledge of *Withania somnifera's* anti-inflammatory and pain-relieving properties and emphasizes the need for more thorough phytochemical research to fully comprehend its therapeutic potential. More research is needed to pinpoint the chemical responsible for the analgesic and anti-inflammatory effects.

**Conclusion**

This study revealed, utilizing a rat model and different dosages of ethanolic extract and reference drugs, that *Withania somnifera* leaves display considerable analgesic and anti-inflammatory properties, especially at 900 mg/kg. Including withanolides, flavonoids, condensed tannins, glycosides, and free amino acids likely enhance these bioactivities. Despite several results lacking statistical significance, the findings are consistent with previous studies, reinforcing the plant's therapeutic promise and necessitating further exploration to optimize dosage and efficacy.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

Disclaimer (Artificial intelligence)

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