**Original Research Article**

**An Investigation of Analgesic and Anti-inflammatory Activity of *Withania somnifera* Linn. Leaves**

**Abstract**

The use of medicinal plants to prevent and treat illnesses has 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 shown that its active ingredients give it analgesic and anti-inflammatory qualities. Withanolide, one of the plant's main 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. Rats were given three different doses of the *Withania somnifera* extract: 300, 600, and 900 mg/kg. The 600 mg/kg and 900 mg/kg dosages had statistically significant anti-inflammatory effects (p < 0.05) in the anti-inflammatory trial, but the other dosages had no discernible impact. 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). These results back up the traditional use of *Withania somnifera* for easing pain and swelling, likely because the plant has many flavonoids, alkaloids, condensed tannins, and glycosides. The results support the traditional use of *Withania somnifera* in pain and inflammation treatment and are consistent with previous studies. The results show this herb's considerable therapeutic potential, even though it depends on timing and dose.

**Key words:** *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 of Taxonomy [1]. Pain is a component of human suffering that causes discomfort. The underlying cause is inflammation and the inflammatory response that follows, regardless of the type of pain—acute or chronic, peripheral or central, nociceptive or neuropathic. White blood cells and their secretions play a physiological role in inflammation, which protects the body from viral and bacterial invaders. In some conditions, including arthritis, the immune system causes inflammation even when there are no infections to fight. As long as the damage is not severe enough to jeopardize the tissue's structure and health at the same time, inflammation has recently been defined as a sequence of alterations that take place in living tissue after damage [2]. 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 [3]. Therefore, the development of extremely 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 the discovery and synthesis of novel medications [4]. Certain chemical compounds obtained from medicinal plants may have therapeutic properties, according to experts. To effectively treat a variety of illnesses, researchers are constantly looking for novel herbal cures and other plant-derived medications [5]. In many nations around the world, 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 main source of healthcare [6]. Numerous chemical components found in medicinal plants enable them to provide a broad range of pharmacological and therapeutic effects. Tannins, glycosides, alkaloids, saponins, polysaccharides, essential oils, terpenoids, resins, and plant lipids are some examples of these compounds [7-9]. By precisely controlling chemical concentrations, genetically modified plants can eventually produce the desired therapeutic effect. Enhancing the production of secondary metabolites, such as alkaloids, is one of the many possible uses for reverse genetics [10]. Exploration of the therapeutic qualities of plant species has increased as a result of advancements in scientific research worldwide [11].

For a long time, people have employed *Withania somnifera* (L.) Dunal (Solanaceae) as a Rasayana plant [12]. *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 [13]. Because of their considerable therapeutic and nutraceutical potential, plants in the genus Withania, which belong to the Solanaceae family, are well acknowledged for their major medical significance [14]. It is an upright, greyish, evergreen shrub that grows to a height of 0.5 to 2 m. It has long, tuberous roots, short stems, oblong, stalked leaves, and greenish, bisexual flowers in the axils. The fruits are globose berries that are 6 mm in diameter, turn orange-red when ripe, and are covered in an inflated, persistent, membrane-like calyx [15]. It is common in the desert regions of North Africa, the Canary Islands, southern Europe [16–20], and parts of Asia and Africa including India, Baluchistan, Pakistan, Afghanistan, Sri Lanka, Congo, South Africa, Egypt, Morocco, and Jordan [21]. *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) [22]. 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 [23]. 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) [24]. Because of its anti-inflammatory, antihypoxic, antiischemic, neuroprotective, immunomodulatory, hepatoprotective, cardioprotective, anti-diabetic, adaptogenic, anti-arthritic, anti-stress, and antibacterial qualities, it has a wide range of therapeutic indications [25]. 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 [26]. *W. somnifera* was used as a medicine to help with inflammation because it blocked substances related to inflammation and reduced signs of inflammatory activity [27].

The objective of the present study is to investigate the analgesic and antiinflammatory properties of *Withania somnifera* in Rat.

**Materials and Methods**

**Drugs, Chemicals and Instruments**

Acetic acid, carrageenan, ethanol, and alloxan were provided by Sigma Aldrich (Germany). Ibuprofen and aspirin were given away as free samples by Healthcare Pharmaceutical Limited (UK). An analgesia meter and a plethysmometer were used to measure the analgesic and anti-inflammatory effects, respectively.

**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 storage guidelines of the Bangladesh National Herbarium. 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 the whole 96-hour duration. The extract was filtered after soaking, and the resultant liquid was then 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 was carried out in compliance 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 conducted the analgesic and anti-inflammatory evaluation 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 sub planar 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' paw in Positive Control rat

V0=volume of animals' paw 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. In the half hour before the intraperitoneal acetic acid injection, several test samples were given. 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 starting right after the acetic acid injection. By counting the occurrences of abdominal muscle contractions, hind limb retractions towards the abdominal walls, hind limb extensions, and intermittent back arching over a twenty-minute period, the percentage of writhing inhibition was determined. Equation calculated the percentage of writhing suggestive of analgesic and anti-inflammatory effects.

Where = the mean number of the writhing of each test group

= The mean number of the writhing of acetic acid control group.

The extract's ability to reduce pain and inflammation is then assessed using the "Tail Flick Method" on the same experimental rat model after a seven-day break. 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. For both untreated and treated rats, the amount of time it took to exhibit a tail-flick reaction was recorded. 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 a number of different categories, covering a wide range of study parameters. The data was subjected to descriptive statistics; the mean and standard deviation (SD) were the results that were displayed. SPSS 1600's "One-Way ANOVA Test" tool was used to assess the observed change across groups' statistical significance. When the p-value is less than 0.05 (p < 0.05), the occurrence is considered statistically significant.

**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 Minute (Just 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 as statistically significant. The statistical analysis followed by 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 level of significance of result).

|  |  |  |  |
| --- | --- | --- | --- |
| **Group specification** | **Dose** | **Number of writhing** | **% 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 as statistically significant. The statistical analysis followed by 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 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 as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett’s test) compared to the control.

Indigenous communities have used traditional herbal remedies to cure a variety of 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* in an effort to evaluate their potential as a natural therapeutic agent for the management of pain and inflammation and to scientifically validate their traditional use [28]. 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 [29,30]. Additionally, it suppressed PGE2 synthesis and COX-2 expression in BV-2 cells and primary microglia stimulated by lipopolysaccharide (LPS). These effects are mediated, at least in part, by decreased STAT1 phosphorylation and nuclear translocation [31]. 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. Numerous clinical conditions, including vascular diseases, cancer, and arthritis, are linked to pain and inflammation (Weitzmann, S.A., 1990). Different traditional medical systems employ numerous natural items to alleviate pain symptoms [32]. More studies are needed on phytochemicals to understand their roles better, as some, like tannins and glycosides, might also help reduce pain [33], 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. It will need more research to pinpoint the exact 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. The inclusion of withanolides flavonoids, condensed tannins, glycosides, the 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.

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