



## A STUDY ON COMPARATIVE ANALYSIS OF WITHANIA SOMNIFERA (ASHWAGANDHA) WITH VALERIANA WALLICHI (TAGARA) IN RELATION TO THEIR ANTI-INFLAMMATORY ACTIVITY IN MALE ALBINO RATS

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### ABSTRACT

**Aim and objectives:** the present study was carried out 1) To evaluate the anti-inflammatory activity of Withania Somnifera, Valeriana wallichii in male albino rats. 2) To compare the anti-inflammatory activity of Withania Somnifera with Valeriana wallichii in male albino rats by using Plethysmograph. **Materials and methods:** Male albino wistar rats of 250 to 300gm weight were randomly allotted into three groups namely Control, Test-1 and Test-2. For control group rats 0.2 ml of normal saline, for test-1 group rats Withania Somnifera in a dose of 25.69 mg/kg, for test-2 group rats Valeriana wallichii in a dose of 120mg/kg, was administered as a single oral dose half an hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of hind paw and the paw edema of each rat was measured at 3hours **Results:** Both the test drugs have shown significant anti-inflammatory activity ( $p < 0.05$ ). However Withania somnifera in a dose of 25.69 mg/kg showed significantly higher anti-inflammatory effect than V.wallachi given in a dose of 120mg/kg. **Conclusion:** Withania somnifera and Valeriana wallachi have significant anti-inflammatory activity.

**KEYWORDS:** Anti-inflammatory, Withania Somnifera, Valeriana wallichii, Carrageenan, Plethysmograph.

### INTRODUCTION

Inflammation is primarily the protective response of body tissues to irritation or injury. Inflammation may be acute or chronic. Its cardinal signs are rubor, calor, tumor and dolor, often accompanied by loss of function. The process begins with a transitory vasoconstriction, and then is followed by a brief increase in vascular permeability.<sup>[1]</sup> The second stage is prolonged and consists of sustained increase in vascular permeability, exudation of fluids from the vessels, clustering of leukocytes along the vessel walls, phagocytosis of microorganisms, deposition of fibrin in the vessel, disposal of the accumulated debris by macrophages, and finally migration of fibroblasts to the area and development of new, normal cells. The severity, timing, and local character of any particular inflammatory response depend on the cause, the area affected, and the condition of the host. Histamines, kinins, and various other substances mediate the inflammatory process.<sup>[2]</sup> Phagocytic cells including neutrophils, eosinophils, macrophage and lymphocytes produce huge amount of

reactive oxygen species (ROS) which play an important role in the host defense mechanism. Besides their immune functions these excessively produced ROS deregulate the cellular functions causing cellular and tissue damage, which in turn augments the state of inflammation<sup>[3]</sup>. Acute inflammation can be defined as a short-term process taking place in response to tissue injury, usually appearing within minutes or hours and it is characterized by all the five cardinal signs of inflammation. It is nonspecific and may be induced by any injury. Acute inflammation is the first line of defense against injury and is characterized by changes in the microcirculation: exudation of fluid and emigration of leukocytes from blood vessels to the area of injury.<sup>[4]</sup> Chronic inflammation is long-term and occurs in "wear and tear" conditions, including osteoarthritis, autoimmune disorders like systemic lupus erythematosus disease, rheumatoid arthritis, allergies, asthma, hay fever, inflammatory bowel disease, multiple sclerosis and Crohn's disease etc.<sup>[5], [6]</sup> Other than these, habitual or environmental factors, such as excess weight, poor

diet, lack of exercise, stress, smoking, pollution, poor oral health and excessive alcohol consumption can also lead to chronic inflammation. Though traditional herbal therapy is an unwritten science, it is well developed and is being practiced in some countries and has become a way of life in almost 80% of population in rural areas. A chronic anti-inflammatory disease is one of the greatest health concerns of the world's population E.g. Rheumatoid arthritis. At present, although synthetic drugs are dominating the market, the element of toxicity these drugs entail, cannot be ruled out. The prolonged usage of these synthetic drugs lead to severe adverse effects and the most common are gastrointestinal bleeding and peptic ulcers<sup>[7],[8]</sup>. Non steroidal anti-inflammatory drugs (NSAIDs) represent one of the most common classes of medications used worldwide with an estimated usage of >30 million per day for inflammation and related disorders. Unfortunately, besides the excellent anti-inflammatory potential of the NSAIDs, the severe side effects such as gastrointestinal (GI) ulceration, perforation and bleeding has limited the therapeutic usage of NSAIDs. In the recent years, the use of plant based traditional medicine has again received considerable interest. Hence many research studies were planned and conducted and some are in progress to invent new anti-inflammatory agents with minimum side effects. Search for safe and effective anti-inflammatory agents have been given priority in scientific research in herbal system of medicine. Some of the herbal plants produce secondary metabolites like glycosides, flavonoids, alkaloids, terpenoids and steroids which have a wide range of pharmacological activities like analgesic, anti-inflammatory and anti-pyretic activities etc.<sup>[9]</sup>

The perennial plant *Valeriana wallichii*, commonly called Tagara belonging to the family *Valerianaceae* was selected in the present research work on the basis of ethanobotanical information which reveals that the plant is widely distributed throughout the world specifically in India, Nepal and China.<sup>[9]</sup> *Valeriana* spp. is now listed in the European and USA pharmacopeias. It is also sold as a diet supplement in the USA and is one of the highest selling natural medicines in Europe and the USA.<sup>[10]</sup> The plant extracts are widely used in the ayurveda as anti-inflammatory compound and for the treatment of arthritis pains, pain in abdomen, paralysis, convulsions, hysteria, wounds, cough, asthma, skin diseases, fever and cases of animal bites.

*Withania Somnifera* is a small, woody shrub in the Solanaceae family. It is commonly called as Ashwaganda and is most commonly utilized herbs in Ayurveda grasping an importance similar to that of ginseng in China. Ayurvedic practitioners treated the plant like 'rasayana' herb or potent tonic to promote greater vitality and longevity. Ashwaganda is believed to balance 'vata' and 'kapha' in Ayurvedic medicine. Traditionally it is used to treat a myriad conditions, Type-II diabetes, cancer, osteoarthritis, anxiety,

antioxidant, boost stamina and relieve fatigue, promote recovery after illness etc.<sup>[11]</sup>

The present study was designed and conducted to assess and compare the anti-inflammatory effect of two different herbal extracts *Withania somnifera* and *Valeriana wallichii* in male albino rats using plethysmograph.

## MATERIALS AND METHODS

The present investigation is a randomized controlled trial conducted on Inbred pathogen free healthy albino wistar rats of male sex weighing between 250-300g. Animals were maintained under standard conditions in the animal house of Dr. Pinnamenani siddhartha institute of medical sciences & Research foundation. The herbal preparation used for this study is taken from Himalaya Herbal Healthcare, in which extracts of this herbal capsules are available.

**Model of Carrageenan-induced Edema in Rat:** The male albino rats were grouped in polyacrylic cages and categorized into following groups: group-1 (Control), group-2 (Test-1) and group-3 (Test-2). Each group contains 6 rats. All the rats were marked at the ankle joint (tibio-tarsal joint). Initial paw edema of each rat is measured before giving drug by using plethysmograph. For Control group rats, 0.2 ml of normal saline is administered orally half an hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of the hind paw and the paw edema of each rat is measured at 3hours. For Test-1 group rats, *Withania somnifera* in a dose of 25.69 mg/kg is administered as single oral dose half an hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of hind paw and the paw edema of each rat is measured at 3hours. For Test-2 group rats, *Valeriana wallichii* in a dose of 120mg/kg is administered as single oral dose half an hour before injecting 0.1ml of 1% carrageenan to the sub-plantar region of hind paw and the paw edema of each rat is measured at 3hours. Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan suspended in normal saline into sub-plantar tissues of the left hind paw of each rat. Paw volumes were determined using a mercury plethysmograph. The paw volume was measured before injecting the carrageenan and after at 0 hr and at 3 hrs using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the control group.<sup>[12,13,14]</sup>

**Data Analysis:** The results were expressed in terms of percentage of inhibition in rat paw edema. The data is expressed as mean  $\pm$  Standard Deviation (SD). Results were analyzed using one-way ANOVA at 5% significance level. Differences were considered as statistically significant at  $P < 0.05$ , when compared with control.

## RESULTS

The acute and sub-acute phases of inflammation in male albino rats was evaluated by carrageenan-induced hind paw edema. Different test compounds and control which include control (normal saline), Test-1 compound (*Withania somnifera*) and Test-2 compound (*V.wallichii*) were injected into three different groups individually at half an hour before eliciting paw edema. In order to stimulate edema, injection of 0.1 ml of 1% carrageenan suspension with normal saline was injected into the rat's right plantar region. Rats' paw volumes were measured by digital Plethysmograph. Measurement was done immediately at 0 hrs and at 3hrs. From the Table-1 and Table-2 it was found that, the paw edema volume was increased in all the three different groups; control, test-1 and test-2 after 3hrs of carrageenan administration and it was highly significant. The results also showed that *Withania somnifera* was more effective anti-inflammatory agent than *V.wallichii* because the paw

edema with *Withania somnifera* was less than *Valeriana wallichii* after 3 hrs of administration of inflammatory compound. However the paw volume was same with both test samples at 0 hrs. Table-3 showed the percentage inhibition of paw edema volume in rats treated with control and two test compounds. The percentage inhibition of edema with *Withania somnifera* was 52% while comparing with normal saline (Figure-1). The percentage inhibition of paw edema in rats treated with *V.wallichii* was 36% in comparison with normal saline. By comparing the percentage inhibition of paw edema with both drugs it was 25% in rats. Hence it was concluded that *Withania somnifera* is more effective anti-inflammatory agent than *V.wallichii*. The current research had proved that *Withania somnifera* was one of the best anti-inflammatory herbal drugs, but still some more clinical research trials are required to ascertain its use.

**Table 1: Comparison of mean increase in paw volume in three different groups; Control (normal saline), Test-1 (*Withania somnifera*) and Test-2 (*Valeriana wallichii*).**

Group	Drug	Dose	Mean increase in paw volume in ml at hrs		Paired t-value	p-value	Inference
			At 0 Hrs Mean $\pm$ SD	At 3 Hrs Mean $\pm$ SD			
Control (Normal saline)	Normal saline	0.2 ml	4.11 $\pm$ 0.04	4.37 $\pm$ 0.08	7.32	<0.01	HS
Test-1(W. <i>somnifera</i> )	<i>Withania somnifera</i>	25.69mg/kg	4.12 $\pm$ 0.04	4.23 $\pm$ 0.05	7.0	<0.01	HS
Test-2 ( <i>V.wallichii</i> )	<i>Valeriana wallichii</i>	120 mg/kg	4.12 $\pm$ 0.04	4.28 $\pm$ 0.05	8.66	<0.01	HS

SD: Standard deviation; p-value: probability value, t-value: tukey's value

**Table 2: Increase in mean paw edema volume after 3 hrs.**

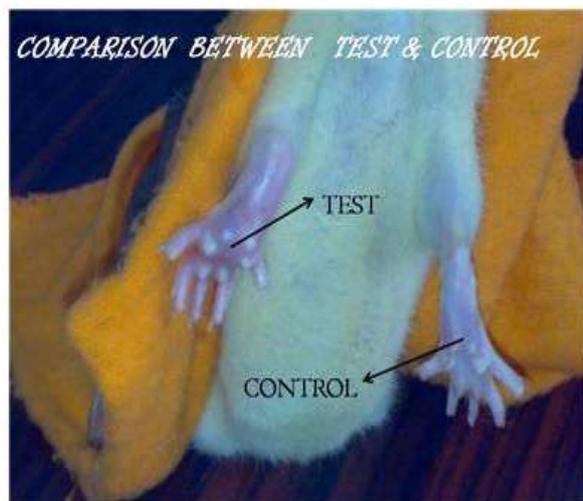
Groups	Mean	SD
Control	0.25	0.08
Test-1	0.12	0.04
Test-2	0.16	0.04

SD: Standard deviation

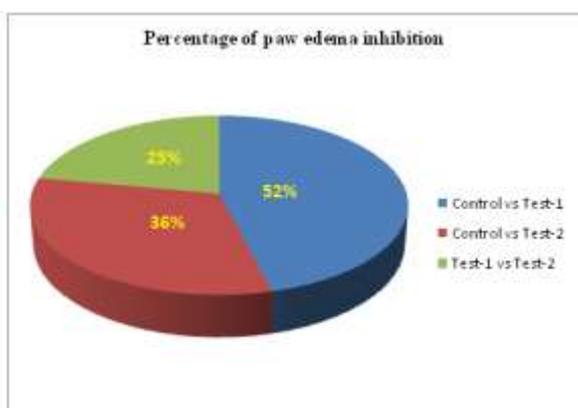
**Table 3: Comparison between the groups regarding paw edema volume inhibition.**

Comparison	Percentage inhibition of paw volume (%)	Independent t-value	p-value	Inference
Control vs Test-1	52	3.56	0.005	HS
Control vs Test-2	36	2.47	0.03	S
Test-1 vs Test-2	25	1.73	0.11	NS

t-value: tukey's value; p-value: probability value



**Figure 1: Showing Rat paw edema in comparison between Test and Control**



**Figure 2: Percentage of paw edema inhibition.**

## DISCUSSION

Certain herbal plants in Ayurveda system of healing are claimed to provide relief of pain and inflammation. An in-vivo model carrageenan induced paw edema of inflammation has been used commonly to evaluate the anti-edematous effect of the contribution of mediators like serotonin, histamines, heparin etc. involved in vascular changes during acute inflammation. This carrageenan paw model is a biphasic response. During the early stage of hyperemia characterized by 0-2hrs after injection of 1% carrageenan, some of the inflammatory mediators like histamine, serotonin and bradykinin are released and lead to vascular permeability. The edema volume is generally greater at the first 1 hr after administration of inflammatory compound and later started to decline. The second phase i.e. late phase of the inflammatory response is due to the potentiating effect of released bradykinins, prostaglandins which produce edema after mobilization of the leukocytes.<sup>[15]</sup> Nitric oxide (NO) is a potent vasodilator and is also involved in carrageenan-induced edema, which may be related to its ability to increase vascular permeability and edema through changes in local blood flow. In the present study the effect of two

different herbal drugs *W.somnifera* and *V. wallichii* on inflammation were studied using carrageenan-induced rat paw edema in male albino rats at two different hours 0 hr and 3 hrs and the results were compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume. The values obtained from each group were expressed as Mean  $\pm$  Standard deviation. t-test was done to compare the statistical significant changes between control, Carrageenan induced paw odema with two different test compounds. The experimental results showed that the test compounds exhibited statistically significant anti-inflammatory activity ( $p < 0.05$ ). Similar study was conducted by *Chandra et al.* (2012)<sup>[16]</sup> to evaluate the anti-inflammatory activity of herbal drug *Withania somnifera* by using different concentrations of test extract incubated with egg albumin and determined the absorbance and viscosity and found that diclofenac sodium a reference drug was less effective than *Withania somnifera*. Their findings suggested that *W.somnifera* possessed marked anti-inflammatory effect against denaturation of protein *in vitro* due to effect of alkaloid and withanolide contents of ashwagandha. In contrast to the above study Anbalagan and Sadique (1981)<sup>[17]</sup> found that *Withania somnifera* was less effective than diclofenac sodium. The results of the current study also correlates with the studies of Sangeetha Pikhwal Sah *et al.* (2010)<sup>[18]</sup> and Sah *et al.* (2010)<sup>[19]</sup> who found that *Valeriana wallichii* extract has effective anti-inflammatory activity by carrageenan induced inflammation model in albino rats. As the plants possess various chemical constituents like alkaloids, glycosides, flavonoids, steroids etc. the observed anti-inflammatory activity may be attributed to any of these phytoconstituents.

## CONCLUSION

This study has shown that *Withania somnifera* and *Valeriana wallichii* possess good anti-inflammatory activity. However *Withania somnifera* showed more anti-inflammatory activity than *V.wallichii* on paw oedema induced by carrageenan. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation, the results of this study are an indication that *W.somnifera* can be effective in acute inflammatory disorders.

“The study declared no conflict of interest”

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