



# EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ETHANOL EXTRACTS OF STEM AND LEAF OF ANDROGRAPHIS ALATA (VAUL) NEES. IN MALE WISTAR RATS

Ruth Sylvia A.C<sup>\*1</sup>, Sumathi P<sup>1</sup>, Vimal priya<sup>1</sup> and C.M. Ganesan<sup>2</sup>

PG & Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore <sup>1</sup>, Govt. Arts College, Udumalpet, Tirupur dt<sup>2</sup>. Tamil Nadu, India

## ABSTRACT

This study investigated the general acute anti-inflammatory effects of ethanol extracts of stem and leaf of *Andrographis alata* with dose of 500mg/kg body weight in male wistar rats using carrageenin induced rat paw oedema. Continuous observation was made with one hour interval up to 6 hrs. results were analyzed and listed in Mean  $\pm$  SEM. The significant inhibition of 93.33% and 89.48% of ethanolic extracts of stem and leaf was observed at 5<sup>th</sup> and 4<sup>th</sup> hour respectively. The study clearly states that the stem has more anti-inflammatory activity rather than leaf extract.

**Key Words:** Anti-inflammatory, Wistar rat, Carrageenin, *Andrographis alata*

## I. INTRODUCTION

Inflammation is a biological response of vascular tissues of the body to foreign infections (or) invaders such as microbial infection. It's cardinal signs are redness, heat, pain and swelling with a homeostatic process (Ebert, 1965; Bonta, 1977). Whenever a disease is developed, the body's immune system has triggered the inflammation and caused damage to the cells. There are acute and chronic inflammations. The acute one is short lived, subsides and showed the signs of edema, erythema and loss of functions. It is also triggered by the inflammation of tissues/cells by serum and white blood corpuscles, leucocytes. The chronic inflammation is resulted in a progressive shift in the type of cells at the site of inflammation. It is thus characterized by destruction and healing of injured tissue of inflammation. The mediators are histamine, kinin system and prostaglandins (Arrigoni-Martelli.). The first phase of carrageenan inflammation is adopted by histamine and kinin systems and later one is maintained by prostaglandins (Di Rosa et al., 1971). Whenever a tissue got injured with any agents' leucocytes are migrated to the injured site and solved the problems or neutralized it. During this process, all the lysosomes are damaged and released the hydrolytic enzymes which caused severe damage to tissues. The major mechanism/process to find out anti-inflammatory constituents are through inhibition of prostaglandin synthesis at the site of infection, lysosomal membrane stabilization and inhibition of tissue metabolism. To overcome this allopathic medicine are generally employed, it may be non-steroidal anti-inflammatory (or) steroidal drugs. But these may cause several side-effects such as gastric irritation, stomach ulcer etc. Opioids drugs are acted in both phases, but indomethacin inhibited in the late phase only. The phytoconstituents are interfered with inflammatory mediators like prostaglandins, bradykinins, serotonin and released histamine due to tissue damage (Prabhu et al., 2012). In recent times, herbal medicines are involved in the process of anti-inflammatory action that inhibited cyclooxygenase and arachidonic acid metabolism, lipo-oxygenase, pro inflammatory cytokines, transcription activation factor (NF- $\kappa$ B) and uncoupling of oxidative phosphorylation (Nwory et al., 2015; Zeibrou et al., 2016). The western medicinal plants (*Matricaria chamomilla* and *Aruica montana*, *Salix alba* and *Glycyrrhiza*) and Asian plants (*Curcuma domestica*, *C. longa*, *Zingiber officinale* and *Momordica charantia*) are employed to treat anti-inflammatory plants (Shah et al., 2011). *Andrographis alata* of the family Acanthaceae is an endemic medicinal plant and distributed in the southern western Ghats of Tamil Nadu state, India. Therefore, it is taken to study its potential efficiency.

## II. MATERIALS AND METHODS

In this experimental study, adult wistar male rats weighing between 160-200g were obtained from the animal house of, biogen laboratory animal facility, Bangalore, India. They were housed in polyacrylic cages (38 x 23 x 10 cm<sup>3</sup>) with not more than six animals per cage and maintained under standard laboratory conditions with a temperature of 25 $\pm$ 2 $^{\circ}$  C with a dark and light cycle (14/10hrs). they were allowed free access standard dry pellet diet (Saidurga animal feed, Bangalore, India) and water ad libitum.

All wistar rats were acclimatized to local laboratory conditions for one week before commencement of experiment. All the experiment were carried out as per the ethical guidelines for the investigation of experimental pain in conscious animals and guidelines of the Institutional Animal Ethical Committee (IEAC)

### II.1 Acute oral toxicity study

Adult male wistar rats (160 -200 g) were used in the study. Acute oral toxicity study was carried out as per OECD – 423 guide lines (Ecobichon, 1997). All the rats were fasted overnight and provided only water and then extract was administered to all groups orally at the dose level of 5mg/kg<sup>-1</sup> body weight by gastric incubation and observed for two weeks for mortality if any. This protocol was repeated for higher doses. The toxicity of the *A. alata* extracts (stem and leaf) was determined based on mortality rate Viz. 2/3 (or) 1/3 rats become diseased, and some dose was repeated. If there was no mortality the same procedure was repeated for higher doses like 500,1000,1500 and 2000 mg/kg to observe the responses by birds. Here, acute inflammation was induced by sub-planar injection of 0.1ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats. One hour later the administration of the extract, the diameter of paw was measured by digital calipers after the carrageenan injection. All the rats were pre medicated with diclofenac, 10mg/kg orally as a standard drug. Here, the extracts were subjected to acute toxicity studies and 1/10<sup>th</sup> of LD<sub>50</sub> dose was used for pharmacological activity. The difference between initial and subsequent was provide the acute edema volume. The paw diameter was measured by using digital calliper at the intervals of 0, 1, 2, 3, 4, 5 and 6h after the Carrageenan injection. The anti-inflammatory activity was calculated as percentage inhibition of carrageenan induced paw edema using the following formula after Winter et al., (1962) and Poster (1957).

$$\text{Percent inhibition} = 1 - \frac{dt}{dc} \times 100 / dc$$

Where: dt = paw diameter in treated; dc = paw diameter in control

In every experiment, the rats were divided in five groups, each group consist of six rats as follows.

#### Experimental Design

: Served as a negative control, rats injected with 1% carrageenan solution (5ml/kg, p.o.)

**Group II** : Served as standard drug, diclofenac 10mg/kg (body weight) treated rats

**Group III** : Served as test group, the rat received carrageenan + Leaf Extract 500mg/kg (p.o.)

**Group IV** : Served as test group, the rat received carrageenan + Stem Extract 500mg/kg (p.o.)

### III. RESULTS AND DISCUSSION

The anti-inflammatory activity of stem and leaf parts of ethanolic extract of *Andrographis alata* was evaluated by carrageenan induced paw oedema method and presented in Table -1. Both the extracts were tested at 500mg kg<sup>-1</sup> dose levels at '0' minutes to 6 hours with one hour interval between each. The average paw oedema volumes were taken for the study. The ethanolic stem extract was significantly (P<0.05) reduced carrageenan induced paw oedema volume as compared to the standard drug, diclofenac. Similar trend was also found to be exhibited in the leaf extract of the plant. In both parts of the extracts, these terms were found to be declined in 5<sup>th</sup> and 6<sup>th</sup> hours respectively (Table- 1). These results are outcomes of acute inflammation that resulted in successful resolution at repair of tissue damage. In the first phase, histamine (or) serotonin is released and reached the site of inflammation. In the second phase, oedema developed because of release of prostaglandin (Brito and Antonio, 1998; Saha and Masud, 2007). It also led to control the biosynthesis of prostaglandin and become very low as uninflamed tissues and if not, prostaglandins could have increased prior to recruitment of leucocytes and infiltration of immune cells. In stem extract of *Andrographis alata* the maximum percent of inhibition (93.33%) activity was showed in the 5<sup>th</sup> hour after the carrageenan induced oedema in rats and standard drug diclofenac with 97.29% of inhibition of oedema in late phase II. *A. alata* extract has exhibited anti-inflammatory activity in the phase I in the histamine phase. The leaf extract showed 89.48% of the inhibition of carrageenan induced paw oedema rats at the 4<sup>th</sup> hour and it was declined in the later hours.

The control rats (Group I) exhibited 68.78 percent of inhibition which indicated lowest rate of inhibition. Besides, paw oedema volume was decreased significantly in 500mg kg<sup>-1</sup> of *A. alata* stem extract treated rats from 0 min to 6 hrs. showed 3.575±0.197 and 6.902±0.150 at 0 and 4<sup>th</sup> hrs. and later declined. Where as in leaf, it was 3.665±0.296 and 6.94 at 0 and 4<sup>th</sup> hour respectively.

It was suggested that a NSAID's like aspirin offered a relief from inflammatory pain by inhibiting pain substances in the peripheral tissues; prostaglandin and bradykinin were involved in pain process. Prostaglandin elicit pain by direct stimulation of sensory nerve ending to other provoking stimuli (Kanodia and Das, 2008). Therefore, it is understood that ethanol extract of *A. alata* also could able to suppress the formation of prostaglandins and bradykinin or it may be antagonized the action if these substances and these extracted anti-inflammatory activity. *A. alata* stem ethanolic extract showed more anti-inflammatory activity compared to leaf extracts. The present results were found to be statistically significance (P< 0.05) relative to control and it is comparable to standard drug, diclofenac. It is suggested that the presently obtained results were showed that ethanolic extracts of stem and leaf samples of *A. alata* have exhibited anti-inflammatory activity on an acute inflammatory process like that of carrageenan induced paw oedema in rat's paw. It is known that leucocytes migrated to the injured tissues in an important process of the inflammatory process, both of the histamine and serotonin are responsible for the immediate inflammatory response, whereas, kinins and prostaglandins mediated the prolonged response (Perez et al., 2005). The carrageenan induced the acute local paw inflammation which is the appropriate criteria for the evaluation of anti-inflammatory agents (Winter et al., 1962). The time courses of oedema development in carrageenan induced model in rats is represented by a biphasic one (Vinegar et al., 1969).

The first phase between 0 to 2hrs after injection of the phlogiston agent which is attributed to the phlogistic agent which is attributed to the release of histamine (or) serotonin (Crunkhorn and Meacock, 1971). The oedema volume was reached its maximum approximately 4<sup>th</sup> post treatment and then begins to decline. The second phase of inflammatory reaction which is measured at 3<sup>rd</sup> hr. and caused the release of bradykinin, protease, prostaglandins and lysosomes (Crunkhorn and Meacock, 1971; Di Rosa and Willoughby, 1971). NSAID such as indomethacin and diclofenac are to inhibit cyclooxygenase (COX -1) I and II (COX -2) are implicated in the production of inflammation- mediating agent PGE2 from arachidonic acid (Dhara et al., 2001; Moody et al., 2006). The stabilization of human red blood cells membrane by hypo-toxicity induced membrane lysis could be considered as an invitro measurement of anti-inflammatory activity of the drug (or) enhance the efflux of the intracellular components (Vadivu and Lakshmi, 2008). The results of this study exhibited that *A. alata* extracts possess anti-inflammatory activity. *Andrographolide* a derivative of *A. paniculata* also known as 'King of Bitter' have anti-inflammatory effects in experimental model (Thangasekaran et al., 2013).

**IV. CONCLUSION**

The outcome of the present study that *A. alata* stem and leaf ethanolic extracts endeavour an excellent anti-inflammatory effect in the *in-vivo* (Male Wistar rats) study.

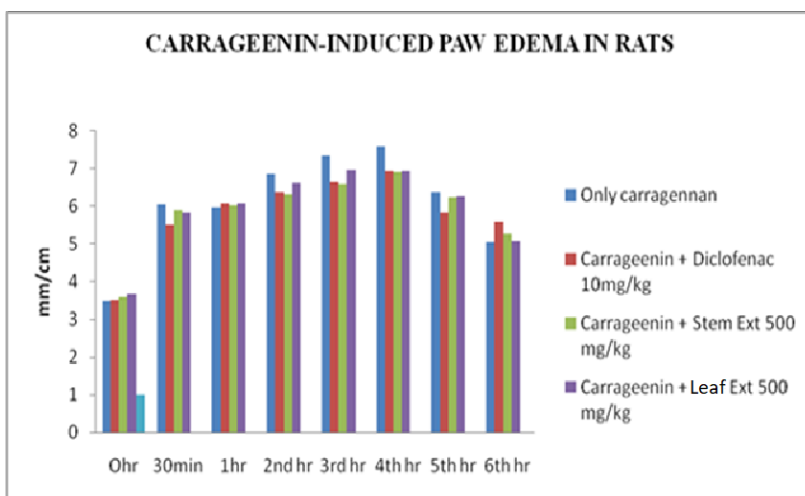
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**Table – 1 Anti-inflammatory activity of Ethanol extracts of *Andrographis alata* stem and leaf on carrageenan induced oedema**

Group	Mean paw volume before carrageenan injection	Increase in paw volume (ml) after carrageenan injection (mean ± SEM)			(% )Percent inhibition of oedema		
	0 min	30 min	1h	2h	30 min	1h	2h
Control	3.47±0.256	6.025±0.339	6.235±0.386	6.842±0.057	73.21	79.68	97.11
Standard	3.515±0.244	5.5±0.403	6.05±0.226	6.362±0.194	56.69	72.36	80.99
Carrageenin + Stem Ext 500mg/kg	3.575±0.192	5.862±0.284	5.997±0.279	6.292±0.250	64.14	67.78	76.12
Carrageenin + Leaf Ext 500 mg/kg	3.665±0.286	5.812±0.291	6.062±0.288	6.595±0.303	58.74	65.57	80.05

Group	Mean paw volume before carrageenan injection	Increase in paw volume (ml) after Carrageenan injection (mean ± SEM)				(% )Percent inhibition of edema			
	3h	4h	5h	6h	3h	4h	5h	6h	
Control	7.325±0.104	7.56±0.112	6.345±0.097	5.045±0.262	52.62	68.79	82.85	45.38	
Standard	6.635±0.150*	6.925±0.212*	5.805±0.226	5.587±0.325	89.03	97.29	65.38	59.17	
Carrageenin + Stem Ext 500mg/kg	6.565±0.177*	6.902±0.150*	6.235±0.141	5.257±0.155	83.89	93.33	74.64	47.25	
Carrageenin + Leaf Ext 500 mg/kg	6.94±0.202 <sup>ns</sup>	6.93±0.171*	6.245±0.048	5.072±0.246	89.61	89.48	70.62	38.57	



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