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SYNERGISTICALLY ANTI-INFAMMATORY EFFECT OF *ACHYRANTHES ASPERA, & CALOTROPIS GIGANTEAN* LEAF EXTRACTS IN RODENT MODES OF ACUTE AND CHRONIC INFLAMMATION: INVOMENT OF POSSIBLE MECHANISMS

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ABSTRACT

Ethanolic extracts from the leaves of *C. gigantean* and *A. aspera* were assessed at doses of 300 and 300 mg/kg and combination (*C. gigantean, A. aspera* dose of 300+150 mg/kg) for anti-inflammatory effects using both acute and chronic inflammatory models. It was found that the doses possessed inhibitory effects on the acute phase of inflammation as seen in carrageenan-induced paw edema as well as in a subacute study of cotton pellet-induced granuloma formation. The anti-inflammatory activity elicited by the both plants ethanolic leaves extracts may be due to the influence of the active constituents such as beta-sitosterol and stigmasterol. A possible mechanism may also be due to the inhibition of prostaglandin synthesis which is also evidenced by the delay in the formation of wet faeces. Further study is required to postulate the exact molecular mechanism involved in this process of inhibition of inflammation by these leaves extracts.

Keywords: ***Calotropis gigantean*, *Achyranthes aspera*, anti-inflammatory, cotton pellet, paw edema**

INTRODUCTION

The inflammation term is taken from the Latin word "inflammare" (to burn) (*de oliveira*). Inflammation is one of the most central processes required in defense of animal cells against certain injuries or microbial infections **[1-2]**. Nevertheless, inflammation regularly progresses to acute **[3]**. Chronic inflammation is caused due to a variety of diseases including neurodegenerative disorders, cancer, and cardiovascular diseases **[4]**.

Mechanism of inflammation represents a chain of organized, dynamic responses including both cellular and vascular events with specific humoral secretions. These pathways involve changing physical location of white blood cells (monocytes, basophils, eosinophils, and neutrophils), plasma, and fluids at inflamed site **[5]**. A group of secreted mediators and other signaling molecules (e.g., histamine, prostaglandins, leukotrienes, oxygen- and nitrogen-derived free radicals, and serotonin) are released by immune defense cells principally in the mechanism which can contribute in the event of inflammation **[6]**.

Whatever, the inflammatory response is triggered through two phases: (a) acute and (b) chronic, and each is apparently mediated by a different mechanism. These immune responses which involved in acute inflammation can be divided into vascular and cellular.

The responses which occur in microvasculature normally appear in few minutes following tissue injury or microbial infection in the presence of other inflammatory stimuli named vascular events **[7]**. The occurrence of these processes is rapid and eventually will lead to vasodilation and subsequently makes the vessels become more permeable. These processes will result in entry of inflammatory mediators and produces interstitial edema **[8]**.

Inflitration of white blood cells from circulatory system is essential during inflammatory responses **[9]**. A group of chemotactic agents such as microbial endotoxins holding amino terminal N-formyl methionyl groups, C5a complement fragment, and interleukins along with the secretions of basophils such as platelets activating factor, histamine, and leukotriene B can stimulate intense leukocytes infiltration within few minutes **[10-12]**. Among the leukocytes, neutrophils are the first inflammatory cells that are recruited at the acute inflammation site **[13]**. Infiltration of immune cells triggered via a complicated mechanism in which white blood cells work together with endothelium in postcapillary venules **[14]**.

Cellular events encompass the successive capture, trundling, and firming an adhesion to the microvascular endothelium **[15]**. These events in the mobilization pathway are arranged by cell adhesion molecules (CAMs). These CAMs include intracellular adhesion molecules (ICAM)-1, ICAM-2, integrins, and selectin. The selectin group of CAM contains three families; P-selectin and E-selectin produced by endothelial cells and L-selectin produced by white blood cells **[16]**.

The adhesion of high affinity presented on white blood cells in the endothelium is mediated by the interaction between integrins (CDII/CDI8), and adhesion molecules (CAM-l and CAM-2) expressed on white blood cells and endothelium cells, respectively **[17]**. Following a period of stationary adhesion, the white blood cells may leave the postcapillary venules extending pseudopodia between endothelial cells and reach into the subendothelial space. This complex event is often referred as white blood cell extravasations and transendothelial migration **[18]**.

The inflammations of chronic events are distinguished by mononuclear cell infiltration (e.g., monocyte and lymphocytes), fibroblasts proliferation, collagen fibers, and connective tissue formation, which ultimately result in 2-mm granuloma **[19]**. With chronic inflammation, the tissue degeneration is normally mediated by nitrogen species, proteases, and other reactive oxygen species released from infiltrated inflammatory cells **[20]**. Certainly, genomic alterations in p53 were approved as causes for many chronic inflammatory diseases (e.g., inflammatory bowel diseases and rheumatoid arthritis) in addition to cancers **[21-23]**.

MATERIAL AND METHODS

Collection of plant

The leaves of *A. aspera* & *C. gigantean* were collected from nearby from Lajwana" Jind district, Haryana, India

Preparation of extract

Leaves of plant *A. aspera & C. gigantean* were collected, reduced to small size after drying in shade for ten days and crushed to form coarse powder.

The both plants powdered drug (500 gm) was subjected to continuous hot extraction with the help of soxhlet apparatus using ethanol successively. Each time before extracting with the next solvent the plant material was dried in hot air oven at 50^0 C for an hour. After the effective extraction, the solvent were distilled off, the extracts were then concentrated on water bath to become dried. The obtained both plants extract with each solvent was weighed and stored in an air tight container.

Anti-inflammatory activity

Carrageennan induced paw edema [24-25]

The experiment model was based on various inflammatory mediator released by carragennen. Edema formation in the rat paw is a biphasic event. In the first phase is attributed to the release of histamine & serotonin while second phase is the release of prostaglandin, protease & lysosome. First phase begins after the injection (s.c) of carragennen and gets diminished in two hours while second phase continues up to five hours.

The experiment method was performing by **winter 1962.**

Procedure

Anti-inflammatory evaluation of *A. aspera, C. gigantean* ethanolic leaves extracts & their combination was used against carragennen induced paw edema model. Experimental animals were divided into following five groups. Each group contains six animals and weight approximately 180 to 200 gm.

Groups	Treatments
Group 1 (control)	Received vehicle
Group 2 (standard)	Received Diclofenac sodium dose of (50 mg/kg)
Group 3 (test-1)	Received *C. gigantean* leaf extract dose of (300 mg/kg)
Group 4 (test-2)	Received *A. aspera* leaf extract dose of (300 mg/kg)
Group 5 (test-3)	Received combination (*C. gigantean*, + *A. aspera* dose of 300+150 mg/kg)

All the groups were pretreated according to their treatments, 1 hour before the administration of 0.1 ml of 1% carragennen (suspended in sterile 0.9% normal sterilized saline) in subplanter region of right hind paw of rat. The initial paw volume (IPV) and final paw volume (FPV) was measured after 60,120,180,240 & 300 minutes of carragennen administration using plethysmometer. The difference initial and final paw volume was used to calculate the percentage inhibition using following equation:-

$$\text{Percentage inhibition} = \frac{(X-Y)}{X} \times 100$$

Where,

X= increase in paw volume of rats in the control group

Y= increase in paw volume of rat in the drug treated group

Cotton pellet induced granuloma [26-28]

The exudative & proliferative phase of inflammation was measured against cotton pellet induced granuloma method.

Procedure:

The experimental animals were divided in to five groups according to sex and weight approximately 180 to 200 gm.

Groups	Treatments
Group 1 (control)	Received vehicle
Group 2 (standard)	Received Diclofenac sodium dose of (50 mg/kg)
Group 3 (test-1)	Received *C. gigantean* leaf extract dose of (300 mg/kg)
Group 4 (test-2)	Received *A. aspera* leaf extract dose of (300 mg/kg)
Group 5 (test-3)	Received combination (*C. gigantean*, + *A aspera* dose of 300+150 mg/kg)

Experiment animal were fasted over night and feed was removed. Only tap water was provided. Animal were anaesthetized with ketamine (50 mg/kg; i.m.). The back skin was saved using 70% ethanol as disinfecting agent. An incision was made in the lumber region and subcutaneous tunnels were formed by a blunted forceps.

Then after previously sterilized pre-weighed cotton pellets of (10 mg) was placed on both side in the scapular region. Each animal was kept in single cage and drug treatment was started two hours after cotton pellet implantation & continued to 7 consecutive days. On the 8th day, animal were sacrificed and granulomas were removed. They were dried 24 hours in an oven at 60^{0}C until the weight was stabilized. The weight of dry cotton pellet was determined. The weight of granulomas tissue formed were calculated by subtracting initial weight from the final dry weight of cotton pellet and percentage protection by the drug was calculated using following formula.

$$\text{Percentage Inhibition} = \frac{(X-Y)}{X} \times 100$$

Where,

X = increase in weight of cotton pellet in the control group

Y = increase in weight of cotton pellet of rat in drug treated group

RESULT AND DISCUSSION

Carragennan induced paw oedema

The ethanolic extracts of leaves of *C. gigantean* & *A. aspera* were evaluated for anti-inflammatory activities using the carragennen induced paw oedema model. At the dose levels of *C. gigantean* (300 mg/kg) & *A.aspera* (300 mg/kg) combination (300 + 150 mg/kg) body weight where as diclofenac sodium (50 mg/kg) was used as positive reference standard and the result were as shown below in **Table 1**.

Effect on cotton pellet induced granuloma

Ethanolic extracts of leaves of *A. Aspera* (300mg/kg) & *C. gigantean* (300 mg/kg) and there combination (300 + 150 mg/kg) were evaluated by comparing granuloma dry weight cotton & percentage granuloma inhibition in different groups of animals as show in **Table 2** and **Figure 1, 2**.

Table 1: Effects of ethanolic extract of leaves of *A. aspera* & *C. gigantean* and there combination on carragennen induced paw volume (in cm)

Paw oedema volume (cm) measured						
Mean± SEM						
Groups	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
Control	**1.205±.004**	**1.816±.026**	**2.231±.010**	**2.210±.007**	**2.423±.010**	**2.453±.016**
Standard 50 mg/kg	**1.055±.014**	**1.196±.014*****	**1.320±.024*****	**1.40± .022*****	**1.34±.023*****	**1.28±.020*****
Test1 *C.giganten* 300mg/kg	**1.153±.003***	**1.391±.011***	**1.476±.016***	**1.883±.044***	**1.861±.039***	**1.595±.037***
Test 2 *A.Aspera* 300mg/kg	**1.121±.009***	**1.420±.022***	**1.548±.035***	**1.945±.029***	**1.906±.020***	**1.748±.022***
Test 3 C.G.& A.A. (300+150) mg/kg	**1.116±.010****	**1.416±.011****	**1.545±.019****	**1.871±.020****	**1.651±.027****	**1.383±.026****

All valves were shown as mean ± SEM n=6 by one way ANOVA, **P<0.01, ***P<0.001 vs control.

Table 2: Mean of weight cotton pellet induced granuloma

Treatment	Dose	Granuloma weight in mg	
		Weight of first cotton pellet	Weight of second cotton pellet
Control		**107.83±.708**	**92.16±.654**
Standard	**50mg/kg**	**51.66±1.475*****	**62.83±.792*****
Test 1 (C.G.)	**300mg/kg**	**61.50±1.231***	**74.83±1.077***
Test 2 (A.A.)	**300mg/kg**	**65.50±1.565***	**78.66±1.782***
Test 3 (C.G+A.A)	**300+150 mg/kg**	**52.50±.428****	**65.33±1.452****

All valves were shown as mean ± sem n=6 by one way ANOVA, *P<0.05, **P<0.01, ***P<0.001 vs control

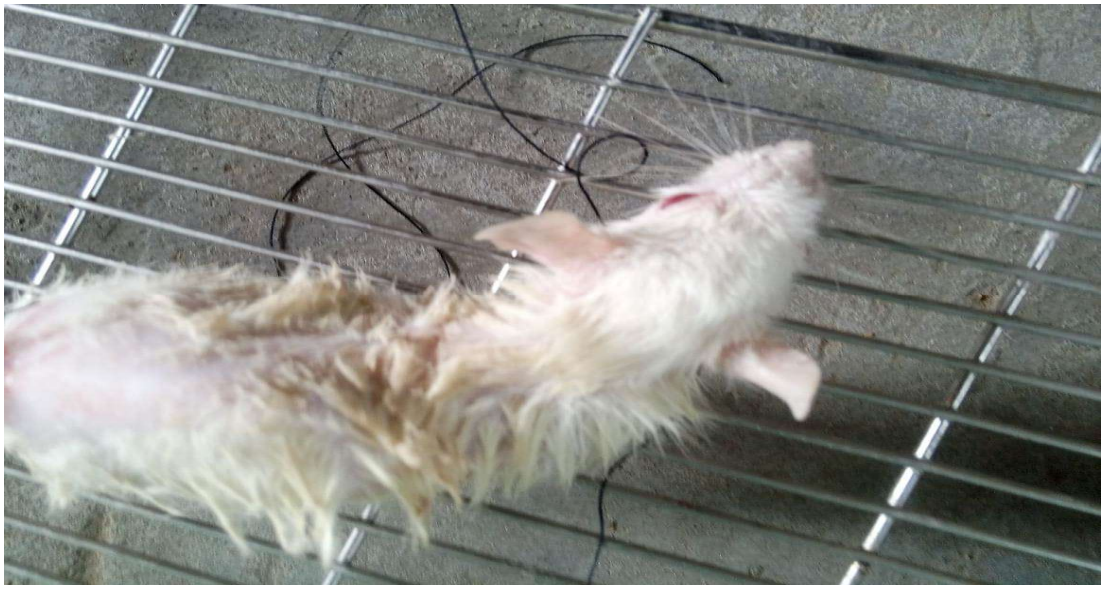

Figure 1: Initial stage of before cotton pellet induced animal

Figure 2: cotton pellet induced ANIMAL

Cotton pellet granuloma is well established test for the assessment of chronic anti-inflammatory process. Chronic condition predominantly consists of a transudative and proliferative test the dry weight of the implanted cotton pellet correlates with the amount of granulomatous tissue formation. The mean dry weight of cotton pellet were as presented in table no. 4.18 the standard drug diclofenac sodium showed significant anti-inflammatory activity by redusing dry weight of the cotton pellet when compared with normal control. The ethanolic leaves extract combination of *C.gigantean & A.aspera* at dose level of 300 + 150 mg/kg showed significant decrease in drug weight of cotton pellets & inhibition was noted comparable to the control group. Hence decrease in the weight of granuloma indicated that the proliferative phase was less suppressed by the combination of *C. gigantean & A.aspera.*

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AUTHOR CONTRIBUTION STATEMENT

Mr. Jitender Kumar conceptualized and gathered the data with regard to this work. Dr. Bhagwati Devi analyzed these data and necessary inputs were given towards the designing of the manuscript. Both authors discussed the methodology and results and contributed to the final manuscript.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCE

[1] Isailovic N, Daigo K, Mantovani A, Selmi C. Interleukin-17 and innate immunity in infections and chronic inflammation. *J. Autoimmun.* 2015; 60:1–11.

[2] Todd I, Spickett G, Fairclough L, editors. *Lecture Notes: Immunology.* New York: John Wiley & Sons; 2015.

[3] Serhan C.N, Dalli J, Colas R.A, Winkler J.W, Chiang N. Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. *Biochim Biophys. Acta (BBA) Mol. Cell Biol. Lipids.* 2015; 1851:397–413

[4] Uttara B, Singh A.V, Zamboni P, Mahajan R.T. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.* 2009; 7:65–74

[5] Huether S.E, McCance K.L. *Understanding Pathophysiology.* Förlag: Elsevier Health Sciences; 2015.

[6] Anwikar S, Bhitre M. Study of the synergistic anti-inflammatory activity of *Solanum xanthocarpum* Schrader and Wendl and *Cassia fistula* Linn. *Int. J. Ayurveda Res.* 2010; 1(3):167.

[7] Nguyen T.T. *Systems Biology Approaches to Corticosteroid Pharmacogenomics and Systemic Inflammation (Doctoral dissertation, Rutgers University-Graduate School-New Brunswick)* 2012

[8] Porter S. *Tidy's Physiotherapy.* Amsterdam: Elsevier Health Sciences; 2013.

[9] Goljan E.F. *Rapid Review Pathology: With Student Consult Online Access.* Philadelphia, PA: Elsevier Health Sciences; 2014.

[10] Kumar V, Abbas A.K, Aster J.C. *Robbins Basic Pathology.* Philadephia, United States: Elsevier Health Sciences, </AQ28>; 2013.

[11] Kumar V, Abbas A.K, Aster J.C, Robbins S.L. *Inflammation and repair. Robbins Basic Pathology.* Philadelphia, London: Saunders; 2012. pp. 29–74.

[12] Bitencourt C.S, Bessi V.L, Huynh D.N, Ménard L, Lefebvre J.S, Lévesque T, Marleau S. Cooperative role of endogenous leucotrienes and platelet-activating factor in

ischaemia–reperfusion-mediated tissue injury. *J. Cell Mol. Med.* 2013; 17:1554–1565.

[13] Curcic S, Holzer M, Frei R, Pasterk L, effector responses are suppressed by secretory phospholipase A 2 modified HDL. *Biochim. Biophys. Acta (BBA) Mol. Cell Biol. Lipids.* 2015; 1851:184–193.

[14] McDonald B, Kubes P. *Leukocyte Trafficking. Inflammatory Diseases of Blood Vessels.* Canada: Department of Physiology and Pharmacology, Faculty of Medicine, University of Calgary; 2012. p.28.

[15] Nourshargh S, Hordijk P.L, Sixt M. Breaching multiple barriers:Leukocyte motility through venular walls and the interstitium. *Nat. Rev. Mol. Cell Biol.* 2010; 11:366–378

[16] Springer T.A, Anderson D.C, Rosenthal A.S, Rothlein R, editors. Leukocyte Adhesion Molecules: Proceedings of the First International Conference on: Structure, Function and Regulation of Molecules Involved in Leukocyte Adhesion, Held in Titisee, West Germany, September 28-October 2, 1988. *Springer Science and Business Media.* 2012

[17] Ogra P.L, Mestecky J, Lamm M.E, Strober W, McGhee J.R, Bienenstock J. *Handbook of Mucosal Immunology.* San Diego: Academic Press; 2012.

[18] Sies H, editor. *Oxidative Stress.* London: Elsevier; 2013.

[19] Gleeson M, Bishop N.C, Stensel D.J, Lindley M.R, Mastana S.S, Nimmo M.A. The anti-inflammatory effects of exercise:Mechanisms and implications for the prevention and treatment of disease. *Nat. Rev. Immunol.* 2011; 11:607

[20] Murakami M. *The Molecular Mechanisms of Chronic Inflammation Development.* Tokyo: Frontiers E-Books; 2012.

[21] Ogrunc M, Di Micco R, Liontos M, Bombardelli L, di Fagagna F.D.A. Oncogene-induced reactive oxygen species fuel hyperproliferation and DNA damage response activation. *Cell Death Differ.* 2014; 21:998–1012.

[22] Kong A.N.T. *Inflammation, Oxidative Stress, and Cancer:Dietary Approaches for*

Cancer Prevention. London, New York: CRC Press; 2013.

[23] Niederhuber J.E. *Abeloff's Clinical Oncology.* Philadelphia, PA: Churchill Livingstone Elsevier; 2014.

[24] Vetriselvan S., Subasini U., Velmurugan C., Muthuramu T., Shankar Jothi., Revathy (2020) *"anti-inflammatory activity of cucumis sativus seed in carrageenan and xylene induced edema model using albino wistar rats"* International Journal of Biopharmaceutics, vol: 4(1); pp:- 34-37.

[25] Neha Mohan P.V., Suganthi. V., Gowri. S. (2020), *"Evaluation of anti-inflammatory activity in ethanolic extract of Coriandrum sativum L. Using carrageenan induced paw oedema in albino rats"* Scholars Research Library, vol: 5(2); pp:-139-143

[26] Jian-Yu Su, Qing-Chan Li, Liang Zhu (2011) *"evaluation of the in vivo anti-inflammatory activity of a flavone glycoside from cancrinia discoidea (ledeb.) Poljak"* excli Journal, vol:10; pp:-110-116

[27] T. Dimo, Agathe L. Fotio T.B. Nguelefack, E.A. Asongalem, P. Kamtchouing (2005) *"antiinflammatory activity of leaf extracts of Kalanchoe crenata andr"* Indian J Pharmacol, Vol: 38(2): pp:-115-119

[28] R. Gupta1, M. Lohani, S. Arora (2010) *"anti-inflammatory activity of the leaf extracts/fractions of bryophyllum pinnatum saliv.syn"* International Journal of Pharmaceutical Sciences Review and Research, vol: 3(1); pp:-16-18