IJPSR (2015), Vol. 6, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 May, 2015; received in revised form, 23 July, 2015; accepted, 30 September, 2015; published 01 December, 2015

ANTI-INFLAMMATORY ACTIVITY OF TRANS-CINNAMALDEHYDE AND PIPERINE COMBINATION AGAINST CARRAGEENAN INDUCED PAW EDEMA

Swapnil A. Jadhav * and Kedar Prabhavalkar

SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Vile Parle(West), Mumbai-400056, Maharashtra, India.

Keywords:

Inflammation, trans-cinnamaldehyde, piperine, carrageenan, indomethacin

Correspondence to Author:
Swapnil A. Jadhav

Rm. no. 04, 1st floor, A5 Building, P-&-T Colony, MMM Road, Mulund (West), Mumbai-400080, India.

Email: niel1904@gmail.com

ABSTRACT: Inflammation is one of the unique mechanism that help body to protect itself against burn, infection, allergens, toxic chemicals, or other noxious stimuli. It is a defense reaction of the body in order to eliminate or reduce the spread of injurious agent. However, over reaction of the body may be harmful or undesirable. This has resulted in extensive development of anti-inflammatory drugs. Despite the benefits that the drugs hold, it also carries the side effects such as gastric lesions, allergy reactions, tolerance and dependence, as well as resistance. Hence, researchers are working towards ideal medicines of anti-inflammatory with highest efficacy, best potency and lowest or none side effects. This research study evaluates the anti-inflammatory activity of trans-cinnamaldehyde and piperine combination. Preclinical testing of cinnamaldehyde and piperine was done earlier individually on inflammatory models but the combination was not validated for the treatment of inflammation. Therefore, the present study was carried out to evaluate the anti-inflammatory potential of the trans-cinnamaldehyde and piperine combination using carrageenan-induced paw edema model. The combination of trans-cinnamaldehyde and piperine exhibited significant anti-inflammatory activity which was comparable with the standard drug, indomethacin.

INTRODUCTION: Inflammation is an important process in the body's defence system. It acts to remove and repair damaged tissue or neutralize harmful agents. The cascade includes; elevated permeability in micro-vessels, attachment of circulating cells to the blood vessels in the vicinity of injury site, migration of several cell types, growth of new tissue and blood vessels. Inflammation may release or generate a diverse population of pro-inflammatory mediators like bradykinins, serotonin, histamines, prostaglandins and nitric oxide.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(12).5188-92

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(12).5188-92

Inflammation is one of the major condition associated with various diseases [1]. Drugs used for the management of pain and inflammation are either narcotics (e.g.Opiods) or non-narcotics(e.g. Salicylates, corticosteroids). All these drugs are well known for side effects such as intestinal tract ulcers and erosions of the stomach linings [2]. As a result, the interest has been focused on finding out safer and potent anti-inflammatory drug. The natural products today represent safety in contrast to the synthetic drugs that are regarded as unsafe to humans and environment. So, people are returning to the natural products with the hope of safety and security [3]. Hence the present study focuses on evaluating the anti-inflammatory activity of trans-cinnamaldehyde and piperine combination. Trans-cinnamaldehyde has been reported to,

- Inhibit nitric oxide(NO), tumor necrosis factor(TNF-α), and prostaglandin E2(PGE2) [4, 5],

- Prevent protein expression of inducible nitricoxide synthase (iNOS), cyclooxygenase - 2(COX-2), nuclear transcription factor kappa B(NF-κB), and IκBα [6],

- Escalate the activities of catalase(CAT), superoxide dismutase(SOD), and glutathionse peroxidase (GPx) in the edema paw after Carr injection [7], and

- Reduce malondialdehyde(MDA) level and myeloperoxidase(MPO) activity in the paw tissue [7, 8].

Whereas, piperine has been reported to,

- Inhibit prostaglandin(PGs) release [9, 10], and

- Reduce the expression of (interleukin) IL6 and (matrix metalloproteinase) MMP13 in synoviocytes [11].

Thus, the advent of trans-cinnamaldehyde and piperine with anti-inflammatory action would be unique and highly beneficial to treat signs of acute and sub-acute inflammation in carrageenan induced paw edema [12].

MATERIALS AND METHODS:

Drugs and reagents:

Trans-cinnamaldehyde(Sigma Aldrich Chemicals Pvt. Ltd.), Piperine(Sigma Aldrich Chemicals Pvt. Ltd.), Indomethacin(Sigma Aldrich Chemicals Pvt. Ltd.), Carrageenan(Sigma Aldrich Chemicals Pvt. Ltd.), Carboxy methyl cellulose(S D Fine-Chem Ltd.), and Tween 20(S D Fine-Chem Ltd.) were used in the study. Trans-cinnamaldehyde (for dosage of 5mg/kg and 3mg/kg) was prepared as an emulsion in Tween 20:distilled water(3:1) for i.p. administration whereas, indomethacin(for dosage of 3mg/kg) and piperine(for dosage of 15mg/kg and 8mg/kg) were prepared as suspension with 0.5%w/v of carboxy methyl cellulose.

Animals:

Male Sprague-Dawley rats of weight 150-200 grams and of 3-4 months of age were used for research purpose. Animals were housed under standard laboratory conditions at 25 ± 2°C with 12h light and 12h dark cycle in groups of three with access to standard pellet diet and water *ad libitum* under strict hygienic conditions. They were acclimatized to the laboratory conditions for a period of 6 days before the study. The study was carried out in the Department of Pharmacology after approval of the protocol by the Institutional Animal Ethics Committee.

Anti-Inflammatory Study:

Carrageenan induced Paw edema in rats:

This invivo model tested the ability of anti-inflammatory drugs to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent [13]. Phlogistic agent(irritant) used was sulfated polysaccharides like carrageenan [14]. The volume of the injected paw was measured before and after application of the irritant and the paw volume of the treated animals is compared to the control animals [15].

Procedure:

Male Sprague-Dawley rats with a body weight between 150 and 200 g were used. The animals were starved overnight. Five groups of male rats were used during the study. Group I (Control) received the vehicle (distilled water-2ml/kg), group II (Standard) received standard drug indomethacin (3mg/kg), group III received piperine (15mg/kg), group IV received trans-cinnamaldehyde (5mg/kg) and group V received [Piperine (8mg/kg) + Trans-cinnamaldehyde (3mg/kg)] combination [16]. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to that mark. The paw volume was measured plethysmographically immediately after injection, 0mins, then after 60mins, 120mins, 180 mins and 4th hour.

Evaluation:

The inflammation was measured in terms of ml i.e. replacement of water by edematous paw in plethysmometer immediately after carrageenan injection(0 mins) and then 1st, 2nd, 3rd and 4th hour after carrageenan injection. The percent inhibition of edema was calculated for each group with respect to its vehicle control (Group I). The anti-inflammatory activity was calculated using relation

$\{(V_c-V_t/V_c)X\ 100\}$, where V_c and V_t denote mean increase in paw volume of control and treatment animals respectively. Effectively treated animals show much less edema [17].

Statistical Analysis: Values for anti-inflammatory activity are expressed as "Mean increase in paw volume ± SEM". Significance of difference between means is determined by One-way ANOVA followed by Dunett's multiple comparisons test where values of P<0.05 are considered significant.

RESULTS:

TABLE 1: *IN VIVO* ANTI-INFLAMMATORY ACTIVITY OF TRANS-CINNAMALDEHYDE AND PIPERINE COMBINATION MEASURING PAW VOLUME (ML)

Groups	Mean paw volume(ml) ± SEM				
	0 minutes	1st hour	2nd hour	3rd hour	4th hour
Group I (Control- 2ml/kg distilled water)	0.355 ± 0.005	0.715 ± 0.006455	1.073 ± 0.0025	1.20 ± 0.004082	1.288 ± 0.004787
Group II (Standard- 3mg/kg indomethacin p.o.)	0.355 ± 0.002887	0.4475 ± 0.004787**	0.50 ± 0.004082***	0.41 ± 0.004082***	0.38 ± 0.004082***
Group III (15mg/kg piperine p.o.)	0.3625 ± 0.0025	0.6125 ± 0.008539*	0.8750 ± 0.006455*	0.8125 ± 0.008539*	0.7150 ± 0.00119*
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	0.36 ± 0.004082	0.5975 ± 0.004787*	0.8575 ± 0.006292*	0.7825 ± 0.004787*	0.6475 ± 0.004787*
Group V (8mg/kg piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	0.3575 ± 0.004787	0.5475 ± 0.004787*	0.8075 ± 0.008539*	0.705 ± 0.006455**	0.54 ± 0.009129**

All values are expressed as mean ± SEM, N=4 *P<0.05, **P<0.01, ***P<0.001 compared with control (Group I) by Dunnett's multiple comparisons test.

TABLE 2: *IN VIVO* ANTI-INFLAMMATORY ACTIVITY OF TREATMENT GROUPS (GROUPS II, III, IV, AND V) MEASURING PERCENT INHIBITION AT VARIOUS TIME INTERVALS

Treatment Groups	Percent Inhibition(%) at various time intervals			
	1st hour	2nd hour	3rd hour	4th hour
Group II (Standard- 3mg/kg indomethacin p.o.)	37.41	53.37	65.83	70.48
Group III (15mg/kg piperine p.o.)	14.33	18.41	32.29	44.48
Group IV (5mg/kg trans-cinnamaldehyde i.p.)	16.43	20.04	34.79	49.70
Group V (8mg/kg piperine p.o. + 3mg/kg trans-cinnamaldehyde i.p.)	23.42	24.70	41.25	58.06

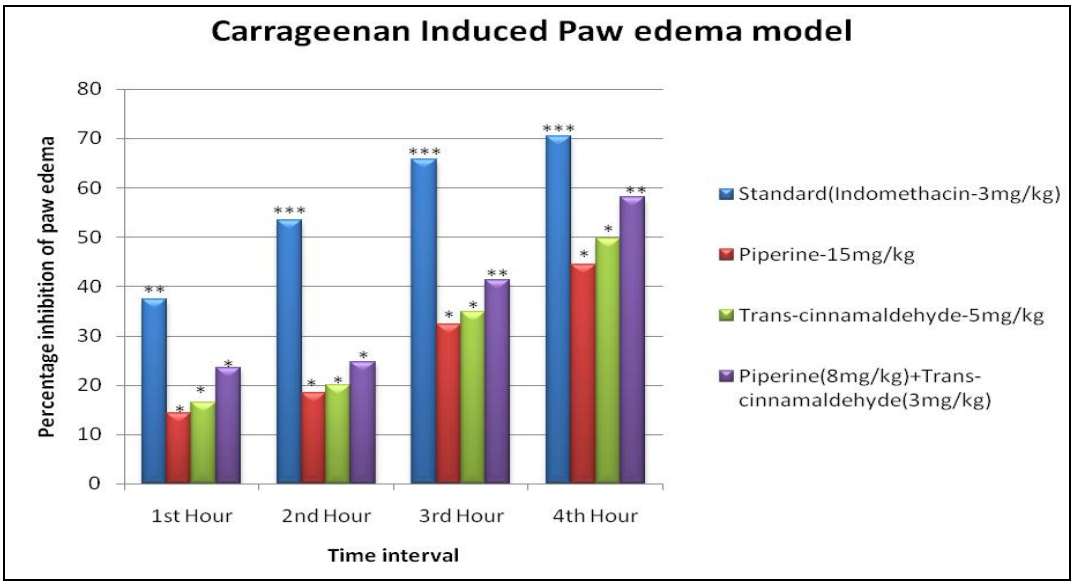

FIG.1: GRAPH OF TIME INTERVAL VS. PERCENTAGE INHIBITION FOR ANTI-INFLAMMATORY ACTIVITY OF TRANS-CINNAMALDEHYDE AND PIPERINE COMBINATION.

The anti-inflammatory activity was compared for standard drug indomethacin (3mg/kg), trans-cinnamaldehyde (5mg/kg), piperine (15mg/kg), and {trans-cinnamaldehyde (3mg/kg) and piperine (8mg/kg)} combination with control Group I. From the results it can be observed that the treatment groups (Group II-Group V) showed significant anti-inflammatory activity from 1st hour by decreasing paw volume as compared with the control group (Group I). The {trans-cinnamaldehyde and piperine} combination decreased the edema significantly (**P<0.01) at 3rd and 4th hour after administration. When compared to the control group(Group I), the anti-inflammatory effect of the combination treatment was almost comparable with standard drug indomethacin(Group II) at 4th hour after administration.

DISCUSSION: In the current investigation, the phytoconstituents trans-cinnamaldehyde and piperine were evaluated in combination as anti-inflammatory agents for its activity by *In Vivo* (Carrageenan induced paw edema) method. This method measures the ability of a compound to reduce edema volume induced in the rat paw after injection of phlogistic agent. Carrageenan induced paw edema model has been commonly used as an experimental model for acute and sub-acute inflammation and is believed to be biphasic. The early phase (1st - 2nd hour) of paw edema model is mediated by histamine, serotonin and increased synthesis of prostaglandins at the injured tissue site. The late phase(2nd - 4th hour) is uninterrupted by prostaglandin release and mediated by bradykinin, polymorphonuclear cells, leukotrienes and prostaglandins produced tissue macrophages.

The anti-inflammatory activity was expressed as "Mean increase in paw volume ± SEM" in terms of ml and percentage inhibition in paw volume by different doses of phytoconstituents. The results of the present study revealed that the combination of trans-cinnamaldehyde (3mg/kg) and piperine (8mg/kg) showed inhibitory effect on "mean increse in paw volume(ml)" induced by carrageenan injection in sub-plantar region of rat's paw. The combination fashioned dose-dependent and significant inhibition of carrageenan induced paw edema. The groups treated with indomethacin (3mg/kg), piperine (15mg/kg), trans-cinnamaldehyde (5mg/kg) and {piperine (8mg/kg) + trans-cinnamaldehyde (3mg/kg)}, i.e. Groups II, III, IV and V respectively, showed significant anti-inflammatory activity by decreasing paw volume from 1st hour itself. The {piperine (8mg/kg) +trans-cinnamaldehyde (3mg/kg)} group V had shown significant(**P<0.01) activity against inflammation at 3rd and 4th hour when compared with control group I. Also, the anti-inflammatory action of the combination {piperine (8mg/kg) + trans-cinnamaldehyde (3mg/kg)} was comparable with the anti-inflammatory action of standard drug indomethacin (3mg/kg).

CONCLUSION: The research done supports the use of phytoconstituents - piperine and trans-cinnamaldehyde in combination for their anti-inflammatory action. However, studies on biological mechanisms and bioavailability are needed to fully evaluate their role in human health.

ACKNOWLEDGEMENT: It is my privilege to express my grateful acknowledgement to Dr. Munira Momin, Principal In-Charge, for providing me all the necessary facilities required during the entire course of my research work. And, I am thankful to my guide Dr. Kedar Prabhavalkar, for his help, support and invaluable suggestions during my entire research study.

REFERENCES:

1. Senthil Kumar, Ganeshan R. Anand: Evaluation of anti-inflammatory activity of *Nerium oleander*. PHARMACIA-July 2010; 1(1):33-36.
2. Hayliyar J, Macpherson A, and Bjarnason I: Gastro protection and non-steroidal anti-inflammatory drugs. Drug safety 1992; 4:1323-6.
3. Gupta M, Mazumdar UK, Gomathi P, Thamilselvan V: Antiinflammatory evaluation of leaves of *Plumeria acuminate*. BMC Complementary and Alternative medicine 2006; Volume 6:1472-6882.
4. Pasupuleti Visweswara Rao and Siew Hua Gan: Cinnamon - A Multifaceted Medicinal Plant. Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine 2014; 12.
5. Priyanga Ranasinghe, Shehani Pigera, GA Sirimal Premakumara, Priyadarshani Galappaththy, Godwin R Constantine and Prasad Katulanda: Medicinal properties of 'true' cinnamon (Cinnamomum zeylanicum)-a systematic review. BMC Complementary and Alternative Medicine 2013, 13:275.
6. Jung-Chun Liao, Jeng-Shyan Deng, Chuan-Sung Chiu, Wen-Chi Hou, Shyh-Shyun Huang, Pei-Hsin Shie, and Guang-Jhong Huang: Anti-Inflammatory Activities of Cinnamomum cassia Constituents In Vitro and In Vivo.

Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine 2012.
7. Yu-Tang Tung, Pei-Ling Yen, Chun-Ya Lin, and Shang-Tzen Chang: Anti-inflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*) leaves. *Pharmaceutical Biology* 2010; 48(10):1130–1136.
8. Vaibhavi Jakhetia, Rakesh Patel, Pankaj Khatri, Neeraj Pahuja, Sunil Garg, Anupriya Pandey, Sonu Sharma: Cinnamon - a pharmacological review. Journal of Advanced Scientific Research 2010; 1(2):19-23.
9. Deepthi Swapna P R, Junise V, Shibin P, Senthila S, Rajesh R S: Isolation, identification and antimycobacterial evaluation of piperine from *Piper longum.* Der Pharmacia Letter 2012; 4(3):863-868.
10. K. Vasavirama, Mahesh Upender: Piperine - A valuable alkaloid from piper species. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(4):34-38.
11. Sri Agus Sudjarwo: The Potency of Piperine As Anti-inflammatory And Analgesic In Rats And Mice. Folia Medica Indonesiana, July – September 2005; Vol. 41, No. 3.
12. Rui-Xin Zhang and Ke Ren: Animal Models of Inflammatory Pain. Neuromethods LLC 2011; Springer Science + Business Media, vol. 49.
13. Gerhard vogel H, Wolfganga H V, Bernward A S, Jurgen S, Gunter M, Wolfganga F V: Drug discovery and evaluation pharmacological assays, 2nd edition, Berlin, Germany: Spinger, 2002:725-771.
14. Mitul Patel, Murugananthan, Shivalinge Gowda K P: In Vivo Animal Models in Preclinical Evaluation of Anti-Inflammatory Activity-A Review. International Journal of Pharmaceutical Research & Allied Sciences 2012; 1(2):01-05.
15. S. Thanga Krishna Kumari, M. Packia Lincy, S. Muthukumarasamy and V. R. Mohan: Anti- inflammatory activity of *Sarcostemma secamone* (L) Bennet whole plant against carrageenan induced paw edema. Bioscience Discovery 2012; 3(3):288-291.
16. Vasu Kandati, P Govardhan, Ch Siva Reddy, A Ravinder Nath , R R Reddy: In-vitro and in-vivo anti-inflammatory activity of Andrographis serpyllifolia (Rottl. Ex Vahl.) Wt.. International Current Pharmaceutical Journal 2012; 1(8):199-204.
17. Winters WD, Hance AJ, Cadd GG, Quam DD, Benthuysen JL: Study of analgesic and anti-inflammatory activity from plant extract of *Lactuca Scariola* and *Artemisia Absinthium.* Journal of Pharmacology and Experimental Therapeutics 1987; 244:51-57

How to cite this article:
Jadhav SA and Prabhavalkar K: Anti-Inflammatory Activity of Trans-Cinnamaldehyde and Piperine Combination against Carrageenan Induced Paw Edema. Int J Pharm Sci Res 2015; 6(12): 5188-92.doi: 10.13040/IJPSR.0975-8232.6(12).5188-92.