OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2018.144.148

Short Communication
Phytochemicals Improve Anti-inflammatory Activity of Diclofenac in Rat Paw Edema Model

[1]Supriya Pandurang Mortale, [1]Ramdas Ganpatrao Bhong, [2]Sushant Shivdas Sole and [1]Sankunny Mohan Karuppayil

[1]School of Life Sciences (DST-FIST and UGC-SAP sponsored), SRTM University, Nanded, 431606, Maharashtra, India
[2]Department of Pharmacology and Toxicology, Bombay Veterinary College, Mumbai 400012, Maharashtra, India

Abstract

Background and Objective: Great effort has been expended on the development of drugs for the treatment of inflammation. Therefore, combination of non-steroidal anti-inflammatory drug, Diclofenac sodium combination with Terpenoids can increase anti-inflammatory activity. As natural components have pharmacological potential to act as anti-inflammatory agents. Current study aimed to reduce Diclofenac sodium concentration in combination with phytochemicals. **Materials and Methods:** To examine the anti-inflammatory activity of Diclofenac sodium combination with Eugenol, Thymol and Carvacrol a Carrageenan induced paw edema was studied in rat model. **Results:** In this study it was found, Diclofenac-Thymol combination inhibited 80.88% of inflammation, whereas Diclofenac-carvacrol combination inhibited 89.68% of inflammation after 3 h of Carrageenan administration. Diclofenac-carvacrol combination brought up the inhibitory rate by two fold as compared to alone activity of drug. **Conclusion:** The present data confirmed that, components of essential oils like Eugenol, Thymol and Carvacrol are efficacious in anti-inflammatory action in combination with Diclofenac sodium in rat paw edema, permitting the use of lower doses and thus limiting the side effects.

Key words: Anti-inflammatory activity, diclofenac sodium, inflammation, paw edema, rat

Citation: Supriya Pandurang Mortale, Ramdas Ganpatrao Bhong, Sushant Shivdas Sole and Sankunny Mohan Karuppayil, 2018. Phytochemicals improve anti-inflammatory activity of diclofenac in rat paw edema model. Pharmacologia, 9: 144-148.

Corresponding Author: Sankunny Mohan Karuppayil, School of Life Sciences (DST-FIST and UGC-SAP Sponsored), SRTM University (NAAC Accredited with A Grade), Nanded, 431606, Maharashtra, India Tel: +91 9764386253

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A complex biological response given by vascular tissues against aggressive agents such as pathogens, irritants or damaged cells is called inflammation[1]. During inflammation process, inflammatory mediators like histamine, serotonin, cytokine, prostaglandin and leukotriene are released. These mediators are responsible for redness, edema and secretions[2,3]. Inflammatory mediators have ability to increase the vascular permeability, thus leucocytes migrate to the sites of inflammation[4]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the strong backbone for the management of pain which arises due to inflammatory diseases. A number of NSAIDs like Diclofenac sodium, Ketorolac, Aspirin, Piroxicam etc., are available in market, they suppress natural processes responsible for inflammation[5,6]. The NSAIDs are potential inhibitors of cyclooxygenase (COX) pathway which produces prostaglandin. Diclofenac sodium is an inhibitor of COX-1 and COX-2 enzyme, effective in treating acute and chronic pains. However repeated use of high doses of NSAIDs may induce bleeding, ulcers, renal disorders, nephro and hepatotoxic effect[2,7]. Therefore, new anti-inflammatory drugs lacking those toxic effects are being searched all over the world. For many years physicians have used herbal extracts for the treatment of pain and inflammation. Interaction between herbs and drugs may either increase or decrease the pharmacological or toxicological effects of each component in combination[8]. Plants possessing anti-inflammatory activity could be an alternative to anti-inflammatory therapeutics because of their best availability and their low toxicity in comparison to other drugs[9,10]. Eugenol, Thymol and Carvacrol are monoterpenes present in the essential oils of various plants and spices such as *Nigella sativa*, *Thymus. Vulgaris* L., Lamiaceae, *Thymus daenensis* L., Lamiaceae, *Origanum* species and *Eugenia caryophyllata*. These monoterpene are widely used and well known for its medicinal properties[2]. These plants are used in folk medicine for the treatment of pain, arthritis, asthma and headache. Their components also have biological effect like antioxidative, antimicrobial, antifungal, anticarcinogenic, antimutagenic, antiallergic, antispasmodic, expectorant, antibacterial and anti-inflammatory properties[9,10]. Eugenol is active against oral bacteria. It has been used in dental practice to relive pain due to variety of sources. Anti-inflammatory activity of eugenol was studied in rat model[11]. Thymol and carvacrol are reported to inhibit inflammatory edema .Thymol effectively inhibit COX-1 activity, where as Carvacrol and eugenol inhibit COX-2 activity[12]. NSAIDs have been used as external treatment but the uses of this kind of drugs are sometime restricted due to side effects. To avoid these adverse reactions many efforts have been made including development of new compounds derived from natural sources. In the present study, the effects of plant Terpinoids Thymol, Carvacrol and Eugenol were studied in combination with Diclofenac sodium in experimental models of Rat in Carrageenan induced paw edema. The main aim of this study is to reduce the concentration of doses of Diclofenac sodium.

MATERIALS AND METHODS

Molecules and drugs: Carvacrol (AR) and Carrageenan was purchased from Sigma Chem. Mumbai., India. While Eugenol (AR) and Thymol (AR) were obtained from HiMedia Chem. Ltd., Mumbai, India. Non-steroidal anti-inflammatory drug Diclofenac sodium was purchased from local market Mumbai, India. Dimethyl sulphoxide (DMSO-1%) was used as solvent for dissolving the Carvacrol, Thymol and Eugenol.

Animals: Male Wistar rats weighing between 150-200 g were purchased from Central Laboratory Animal House, Bombay Veterinary College, Mumbai. Rats were housed in colony cages (4 rats per cage), at an ambient temperature of 25°C with 12 h light: 12 h dark cycle. Rats had free access to standard food and water repetitive. The Principles of Laboratory Animal Care[13] were followed throughout the duration of experiment. All the experiments were performed according to CPCSEA guidelines for the care and experimentation on laboratory animals and the protocol was approved by the Institutional Animal Ethics Committee (Protocol No/. Registration. No of Animal House: 230/CPCSEA).

Carrageenan-induced paw edema in rats: The carrageenan-induced paw edema model[14,15] was used to evaluate the anti-inflammatory effect of Diclofenac sodium combination with Eugenol, Thymol and Carvacrol. The right and left hind paws of the rats marked on the skin at apposition that was located over the lateral malleolus and initial paw volume was recorded. The animals were divided for oral treatment in nine groups. Rat groups were orally administered by doses of molecule alone and in combination with Diclofenac sodium. In that, first group was saline (2 mL kg^{-1}) negative control. Second group was Carrageenan (100 µL of a 1% w/v solution prepared in sterile saline) positive control and three groups were combination of Diclofenac with eugenol, Diclofenac-Thymol and Diclofenac-Carvacrol. After 30 min of Carrageenan injection into the planter region of animals, the paw volumes of each rat was measured after 0, 1 and 3 h of time interval. The measurement of foot volume was accomplished

immediately by displacement technique using the plethysmometer[16]. The inhibition (%) of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the formula[17]:

$$\text{Inhibition paw edema (\%)} = \frac{\text{Mean paw volume in the control group} - \text{mean paw volume in the treated group}}{\text{Mean paw volume in the control group}} \times 100$$

Statistical analysis: Results of the paw edema of the rats were reported as Mean±Standard error of mean (SEM). The total variation was analyzed by performing one-way analysis of variance (ANOVA). Probability levels of less than 0.05 were considered b significant[18].

RESULTS

Carrageenan administration produced an increase in the volume of paws. Diclofenac sodium and three Terpenoids exerted a dose dependent anti-inflammatory effect ($p<0.001$). Diclofenac sodium combination with Thymol and with Carvacrol showed significant anti-inflammatory activity in the model of paw edema induced by carrageenan in rat compared to positive control of Carrageenan self and alone activity of Diclofenac sodium, eugenol, Thymol and Carvacrol. Carrageenans induce 99.35% inflammation after 3 h of administration. Diclofenac sodium at 3 mg kg^{-1} alone inhibited 100% of inflammation. But at 1.5 mg kg^{-1} of dose, Diclofenac alone inhibited 75.09% of inflammation after 3 h. This same concentration (1.5 mg kg^{-1}) of Diclofenac sodium was used in combination with terpinoids to study anti-inflammatory activity (Table 1).

Diclofenac-Eugenol (1.5, Diclo+75 mg kg^{-1} eugenol) combination inhibited 55.96% of inflammation after 3 h. This combination brought down two fold inhibition as compare to Diclofenac and eugenol alone activity. Diclofenac-Thymol (1.5, Diclo+200 mg kg^{-1} Thymol) combination inhibited 80.88% of inflammation after 3 h. Diclofenac-Thymol was an effective anti-inflammatory combination because one fold increases the inhibitory rate as compared to Diclofenac sodium was observed. Diclofenac-carvacrol was a most effective anti-inflammatory combination in this study. Diclofenac-carvacrol (1.5, Diclo+10 mg kg^{-1} Carvacrol) combination inhibited 89.68% of inflammation after 3 h. Diclofenac-carvacrol combination brought up the inhibitory rate by two fold as compared to alone activity of drug used (Table 1). Diclofenac combinations with Thymol and with Carvacrol were significantly effective against inflammation at reduced doses of molecules in combination as compared to alone activity of molecules and drug against inflammation paw edema.

DISCUSSION

Present study showed that Diclofenac combination with Thymol and with carvacrol has significant anti-inflammatory effect at low concentrations on the paw edema caused by Carrageenan. Diclofenac combination with Eugenol demonstrated a weak anti-inflammatory effect (Table 1). Different mechanisms are well known to be involved in the inflammatory reactions. The development of inflammatory edema induced by intraplanter way injection of carrageenan is characterized by an initial stage (1-2 h) and release of histamine, serotonin and bradykinin, followed by a later stage (3-4 h) that is maintained by the release of kinin, lysozymes and prostaglandin E2, provoking significant increase in edema[19].

Interaction between Diclofenac and Curcumin against anti-nociceptive effect was synergistic due to a pharmacodynamics interaction[20]. Naproxen combination with citral can produce minor gastric damage and may have therapeutic advantages for the clinical treatment of inflammation by the mechanism of inhibition of synthesis of prostaglandin[15]. Carvacrol and Thymol combination with Diclofenac sodium showed significant anti-inflammatory activity in the model of paw edema induced by carrageenan in rat. It is probable that the anti-inflammatory effect observed was produced by the inhibition of prostaglandin

Table 1: Anti-inflammatory activity of drug combination on carrageenan-induced paw edema in rat

	Inhibition of carrageenan-induced paw edema in rat (%)					
Diclofenac alone and in combination	Inhibition (%) (after 0 h)	Mean±SEM	Inhibition (%) (after 2 h)	Mean±SEM	Inhibition (%) (after 3 h)	Mean±SEM
Diclofenac alone	0.00	0.485±0.012	71.32	0.595±0.026	75.09	0.605±0.023
Diclofenac+Eugenol	0.00	0.522±0.022	49.00	0.548±0.013	55.96	0.562±0.016
Diclofenac+Thymol	0.00	0.562±0.024	72.23	0.685±0.023	80.88	0.668±0.007
Diclofenac+Carvacrol	0.00	0.517±0.024	79.61	0.600±0.019	89.28	0.572±0.016

Each value represents the mean number and±Slandered Error of Mean (SEM) from 1- 6 for each group (Positive control-Carrageenan, Negative control-saline)

synthesis. Present result demonstrated that Carvacrol was ability to inhibit vascular permeability, which might be related to the reduction of Cox-2 and tumor necrosis factor-α. Cox-2 is an inducible enzyme found in activated inflammatory cells, plays role in production of cytokine and prostanoid mediator release. The inhibition cox-2 protein expression has been used to demonstrate anti-inflammatory effects of molecule *in vivo*[21,12].

Carvacrol have an activity of vascular events of inflammation possibly by suppressing the release of histamine and serotonin from mast cell[22,18]. Similarly, there finding suggested that Nitric oxide take part in the regulation of increased vascular permeability that was apparent in Carrageenan induced paw edema that was inhibited by Diclofenac[23]. Thymol acts by inactivating calcium channel machinery means it reduces intracellular calcium in human neutrophils. This leads to the reduction of elastase, a serine proteinase released by human neutrophils. Elastase is considered as marker of inflammatory diseases[24,12].

CONCLUSION

This experiment showed that carrageenan successfully induced edema in paws. Tested molecules Diclofenac-thymol and Diclofenac-carvacrol combination reduced paw volume, the response being greater than Diclofenac and terpenoids alone effect. Due to high doses of Diclofenac undesirable and sometimes short and long term organ toxicities are come forward, to overcome this problem this combinations are effective. These combinations appear to be a promising candidate for further preclinical and clinical trial in inflammation.

SIGNIFICANCE STATEMENT

Management of pain and inflammatory conditions are unmet need of our society however the current allopathic treatment include the agents whose side effects to living system are more dangerous viz Cox inhibitors are responsible for gastric ulcers and cardiac arrhythmia due to PGI2 inhibition. Therefore, the current investigation is novel as the phytochemicals used in the study are never before tested in combination with Diclofenac. In addition the present study findings showed reduction in dose of diclofenac in combination with tested phytochemicals which can be applied for translational research.

ACKNOWLEDGMENTS

Authors are thankful to Dr. PanditVidyasagar, Hon. Vice Chancellor of SRTM University for the facilities and encouragement. SMK thank UGC, Govt. of India, for infrastructural grants for the School of life Sciences under UGC SAP-DRS II program.

REFERENCES

1. Da Silveira e Sa, R.D.C., L.N. Andrade and D.P. de Sousa, 2013. A review on anti-inflammatory activity of monoterpenes. Molecules, 18: 1227-1254.
2. Azab, A., A. Nassar and A.N. Azab, 2016. Anti-inflammatory activity of natural products. Molecules, Vol. 21. 10.3390/molecules21101321.
3. Ben Khedir, S., M. Mzid, S. Bardaa, D. Moalla, Z. Sahnoun and T. Rebai, 2016. *In vivo* evaluation of the anti-inflammatory effect of *Pistacia lentiscus* fruit oil and its effects on oxidative stress. Evidence-Based Complement. Alter. Med., Vol. 2016. 10.1155/2016/6108203.
4. El Youbi, A.E.H., L. El Mansouri, S. Boukhira, A. Daoudi and D. Bousta, 2016. *In vivo* anti-inflammatory and analgesic effects of aqueous extract of *Cistus ladanifer* L. From morocco. Am. J. Ther., 23: e1554-e1559.
5. Bink, A., S. Kucharikova, B. Neirinck, J. Vleugels, P. van Dijck, B.P. Cammue and K. Thevissen, 2012. The nonsteroidal antiinflammatory drug diclofenac potentiates the *in vivo* activity of caspofungin against *Candida albicans* biofilms. J. Infect. Dis., 206: 1790-1797.
6. Sondhi, S.M., M. Dinodia, J. Singh and R. Rani, 2007. Heterocyclic compounds as anti-inflammatory agents. Curr. Bioact. Compd., 3: 91-108.
7. O'Neil, C.K., J.T. Hanlon and Z.A. Marcum, 2012. Adverse effects of analgesics commonly used by older adults with osteoarthritis: Focus on non-opioid and opioid analgesics. Am. J. Geriatr. Pharmacol., 10: 331-342.
8. Wagner, H. and G. Ulrich-Merzenich, 2009. Synergy research: Approaching a new generation of phytopharmaceuticals. Phytomedicine, 16: 97-110.
9. Raut, J.S. and S.M. Karuppayil, 2014. A status review on the medicinal properties of essential oils. Ind. Crops Prod., 62: 250-264.
10. Falcao, H.D.S., I.O. Lima, V.L. dos Santos, H.D.F. Dantas, M.D.F.F.M., Diniz, J.M. Barbosa-Filho and L.M. Batista, 2005. Review of the plants with anti-inflammatory activity studied in Brazil. Rev. Bras. Farmacogn., 15: 381-391.
11. Daniel, A.N., S.M. Sartoretto, G. Schmidt, S.M. Caparroz-Assef, C.A. Bersani-Amado and R.N. Cuman, 2009. Anti-inflammatory and antinociceptive activities A of eugenol essential oil in experimental animal models. Rev. Bras. Farmacogn., 19: 212-217.

12. Fachini-Queiroz, F.C., R. Kummer, C.F. Estevao-Silva, M.D.D.B. Carvalho and J.M. Cunha *et al*., 2012. Effects of thymol and carvacrol, constituents of *Thymus vulgaris* L. essential oil, on the inflammatory response. Evidence-Based Complement. Alter. Med., Vol. 2012. 10.1155/2012/657026.
13. NIH., 1985. The principles of laboratory animal care. National Institutes of Health Publication, No. 86-23.
14. Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Exp. Biol. Med., 111: 544-547.
15. Ortiz, M.I., M.P. Gonzalez-Garcia, H.A. Ponce-Monter, G. Castaneda-Hernandez and P. Aguilar-Robles, 2010. Synergistic effect of the interaction between naproxen and citral on inflammation in rats. Phytomedicine, 18: 74-79.
16. Halici, Z., G.O. Dengiz, F. Odabasoglu, H. Suleyman, E. Cadirci and M. Halici, 2007. Amiodarone has anti-inflammatory and anti-oxidative properties: An experimental study in rats with carrageenan-induced paw edema. Eur. J. Pharmacol., 566: 215-221.
17. Ozturk, A. and H. Ozbek, 2005. The anti-inflammatory activity of *Eugenia caryophllata* essential oil: An animal model of anti-inflammatory activity. Eur. J. Med., 2: 159-163.
18. Silva, F.V., A.G. Guimaraes, E.R.S. Silva, B.P. Sousa-Neto and F.D.F. Machado *et al*., 2012. Anti-inflammatory and anti-ulcer activities of carvacrol, a monoterpene present in the essential oil of oregano. J. Med. Food, 15: 984-991.
19. Rodrigues, L.B., A.O.B.P.B. Martins, F.R.A.S. Cesario, F.F.E. Castro and T.R. Albuquerque *et al*., 2016. Anti-inflammatory and antiedematogenic activity of the *Ocimum basilicum* essential oil and its main compound estragole: *In vivo* mouse models. Chem. Biol. Int., 257: 14-25.
20. De Paz-Campos, M.A., M.I. Ortiz, A.E.C. Pina, L. Zazueta-Beltran and G. Castaneda-Hernandez, 2014. Synergistic effect of the interaction between curcumin and diclofenac on the formalin test in rats. Phytomedicine, 21: 1543-1548.
21. Hotta, M., R. Nakata, M. Katsukawa, K. Hori, S. Takahashi and H. Inoue, 2010. Carvacrol, a component of thyme oil, activates PPARα and γ and suppresses COX-2 expression. J. Lipid Res., 51: 132-139.
22. Guimaraes, A.G., M.A. Xavier, M.T. de Santana, E.A. Camargo and C.A. Santos *et al*., 2012. Carvacrol attenuates mechanical hypernociception and inflammatory response. Naunyn Schmiedeberg's Arch. Pharmacol., 385: 253-263.
23. Nacife, V.P., M.D.N.C. Soeiro, R.N. Gomes, H. D'Avila, H.C.C.F. Neto and M.D.N.L. Meirelles, 2004. Morphological and biochemical characterization of macrophages activated by carrageenan and lipopolysaccharide *in vivo*. Cell Struct. Funct., 29: 27-34.
24. Braga, P.C., M.D. Sasso, M. Culici, T. Bianchi, L. Bordoni and L. Marabini, 2006. Anti-inflammatory activity of thymol: Inhibitory effect on the release of human neutrophil elastase. Pharmacology, 77: 130-136.