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The Comparative Effect of Medicinal Herb Feverfew with that of a Synthetic Parthenolide to Assess the Expression of Inducible Cyclo-oxygenase and Anti-inflammatory Activity

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: This paper describes the expression of inducible cyclooxygenase and anti-inflammatory of medicinal herb feverfew with a synthetic parthenolide.
Study Design: *In vivo* model to study the expression of inducible cyclooxygenase and anti-inflammatory activity of leaf extract of *T. parthenium* and synthetic compound parthenolide.
Place and Duration of Study: Central Research Laboratory, K. S. Hegde Medical Academy, Derlakatte, Mangalore, Karnataka, India between August 2015 and November 2015.
Methodology: The inhibitory effect of COX-1 and COX -2 were assessed in the serum of mice with the treated groups of aqueous and ethanolic extract of *T. parthenium* (100 mg/kg) and synthetic compound parthenoilde (4 mg/kg) using Cayman's COX activity assay kit.

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The anti-inflammatory activity was evaluated by Ethyl phenylpropiolate induced ear edema and carrageenan induced hind paw edema.

Results: The ability of the aqueous and ethanolic extract of *T. parthenium* (100 mg/kg) and synthetic compound parthenoilde (4 mg/kg) to inhibit cyclooxygenase enzymes (COX-1 and COX-2) was significant when compared to control. *T. parthenium* leaf extracts and synthetic compound reduced the Ethyl phenylpropiolate induced ear edema and carrageenan induced hind paw edema respectively.

Conclusion: The result indicates that the inhibitory effect of aqueous and ethanolic extract of *T. parthenium* and synthetic compound parthenoilde anti-inflammatory activity by Carrageenan induced paw edema and Ethyl phenylpropiolate induced ear edema reduced the inflammation demonstrated through the reduction of vascular permeability may be due to the inhibition of cyclo-oxygenase leading to inhibition of prostaglandin synthesis. Hence the study suggests that selected plants can be considered as a resource for searching novel anti-inflammatory agents possessing COX-2 inhibition.

Keywords: Pain; anti-inflammatory activity; cyclooxygenase; caragennan; ethyl phenylpropiolate.

1. INTRODUCTION

Cyclooxygenase also abbreviated as COX, is a prostaglandin endoperoxide synthase (E.C. 1.14.99.1) enzyme involved in the metabolism of arachidonic acid and synthesis of prostanoid including potent pro inflammatory prostaglandins (PGE2, PGF2a) [1-2]. In mammalian cells, COX exist in at least two isoforms COX-1 and COX-2 [3-5]. COX-1 is expressed constitutively in almost all cell types, including platelets and those present in stomach, kidney, vascular endothelium, forebrain and uterine epithelium and is regulated as a house keeping enzyme for various physiological functions, whereas COX-2 is inducible and expressed during tissue damage or inflammation in response to pro inflammatory cytokines such as IL-1b, interferon gamma and TNF-alfa [6-8].

Inflammatory diseases are a major cause of morbidity and mortality in the world. These diseases are mainly treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and steroidal drugs, which have proven effective with negative side effects. For instance, NSAIDs may induce gastric and intestinal ulcers, anaemia, platelet inhibition in uterine motility. In some reported cases, an increased risk of myocardial infarction [9]. Drugs that are currently used in management of pain are opioids or nonopioids while for that of inflammatory conditions non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are used. But some of these drugs carry potential toxic effects. Although NSAIDS provide good therapeutic relief against inflammation, some of these drugs currently in use have various side effects, particularly in the gastrointestinal tract ulceration and kidney [10]. It is also reported that the prolonged use of nonselective NSAIDs have adverse effects such as nausea, dyspepsia, gastritis, abdominal pain, peptic ulceration, gastrointestinal bleeding and/or perforation of gastroduodenal ulcers [11].

Tanacetum parthenium (L.) is an aromatic perennial plant which is basically found in the northern hemisphere. The plant is also been cultured in gardens [12]. It is commonly known as feverfew [13]. This plant has long been used to treat headache, stomach-ache, menstrual irregularities etc. by the western herbal experts [14]. Feverfew can be used in anti-bacterial treatments. *T. parthenium* can also be used to cure fever, toothache and insect bite. Compounds found in *T. parthenium* and alcoholic extract of *T. parthenium* have metal chelating and free radical scavenging property [15]. However the mechanism of migraine prevention by feverfew has not been completely established. Many researchers have stated that bioactive component corresponding for the pharmacological functionality is parthenolide [16]. Parthenolide helps to prevent excessive clumping of platelets and inhibits the release of certain chemicals, including serotonin and some inflammatory mediators [17].

2. MATERIALS AND METHODS

2.1 Experimental Animals

Adult male Swiss Albino mice (20-25 g) were procured from the Institutional Animal House, K.S Hegde Medical Academy, Nitte University, Mangalore. Animal care and handling was carried out according to the guidelines set by WHO (World Health Organization, Geneva, Switzerland). They were housed under standard animal house conditions and fed with standard

laboratory pellets and water *ad libitum.* The experimental protocol was approved by the Institutional animal ethical committee.

2.2 Test drugs and Drug Administration

For anti-inflammatory experiments all test substances viz., ATPL (Aqueous extract of *Tanacetum parthenium* leaves), ETPL (Ethanolic extract of *Tanacetum parthenium* leaves) were diluted in distilled water and SCP (Synthetic compound parthenolide) were diluted with 3% ethanol. They were orally administered in an equivalent volume of 0.1 ml/10 g body weight of the mice, at the concentration of 100 mg/kg body weight except in the ear edema model where a local application of the test drug to outer and inner surfaces of the ear was performed. Control groups received vehicle only in the same volume and as same route of administration.

2.3 Plant Materials

The entire experiment was carried out at the Central Research Laboratory (CRL), Nitte University, Mangalore, India. The Ethanolic and Aqueous leaf extracts of *T. parthenium* was obtained from Organic Inc China. The leaf extracts are stored in air tight container for future reference. The synthethic compound parthenolide (98% min; HPLC graded) was obtained from Shanghai Better BioChem Co., Limited China.

2.4 Chemicals for Biological Activities

Carrageenan solution (1%w/v), Ethyl phenylpropiolate (vehicle for anti-inflammatory activity), Indomethacin (standard), Methanol (HPLCGrade), Cayman's COX activity assay kit. All the other chemicals used were of analytical grade.

2.5 Instrumentation

Vernier callipers (Kayco), Plethysmograph apparatus Digital Plethysmometer (Paw Volume) IITC Life Science Inc to measure the paw volume, ELISA (LISA plus).

2.6 Cayman's COX Activity

Cyclooxygenase activity in the serum of mice was assessed using Cayman's COX activity assay kit which measures the peroxidase activity of COX. The peroxidase activity was determined colorimetrically in a ELISA (LISA plus) plate reader by monitoring the appearance of oxidized N, N, N1, N1 - Tetramethyl -*p*-phenyldiamine (TMPD) at 590 nm. The kit includes isozyme specific inhibitors for distinguishing COX-2 activity from COX-1 activity.

2.7 Ethyl Phenylpropiolate (EPP) Induced Ear Edema

Topical anti-inflammatory activity of the extract was assessed by the method described by Young et al. [18] and Winter et al. [19]. Selected 24 Male mice weighing 20–25 g were used. The inflammogen EPP was dissolved in acetone and ear edema was induced by topical application of EPP (1 mg/20 µl/ear) to the inner and outer surfaces of both ears using an automatic microliter pipette.

Test substances dissolved in distilled water were administered topically (20 µl/ear) just before the inflammogen. The thickness of each ear was measured with vernier calipers in the beginning followed by 15^{th}, 30^{th}, 60^{th} and 120^{th} min after EPP induction. The inhibitory effect on the edema formation in the treatment groups was compared with that of the vehicle-control group and the percentage inhibition was calculated (Table 1).

2.8 Carrageenan-induced Hind Paw Edema

Paw edema was induced by an intradermal injection with a volume of 0.05 ml carrageenan (1% in normal saline solution) [19]. Into the plantar surface of the right hind paw of the selected male mice weighing 20-25 g were used. Test substances were given to all the selected mice 1 hour prior to carrageenan injection. The control group received vehicle only. The edema volume was determined using a Digital plethysmometer at 1, 3 and 5 hour intervals after carrageenan injection. The inhibitory effect on the edema formation was compared with that of the vehicle-control group and the percentage inhibition was calculated (Table 2).

2.9 Statistical Analysis

The statistical analysis of the data for significant variations within the groups was performed by using SPSS statistical software. The values were expressed as mean ± S.D for six samples in each group. It was done by using the one way analysis of variance (ANOVA), Repeated measures (ANOVA) was carried out. Further multiple group comparisons was carried out by using Dunnett's test. $P< 0.05$ was considered as significant.

Table 1. Effects of ATPL, ETPL and SCP on Ethylphenylpropiolate Induced Ear Edema in Mice at different time of observation

Treatment groups	Time after Topical Application of EPP Ear edema Thickness (mm)							
	15min	%Inhibition	30min	%Inhibition	60min	%Inhibition	120min	%Inhibition
***Control	0.525±0.05	____	0.4±0.08	____	0.3±0.08	____	0.325±0.09	____
ATP(100 mg/kg p.o.)	0.325±0.12	38.09	0.25±0.05	37.5	0.225±0.09	25	0.125±0.05	61.53
ETP(100 mg/kg p.o.)	0.375±0.15	28.57	0.275±0.05	31.25	0.175±0.05	41.6	0.125±0.05	61.53
SP(4 mg/kg p.o.)	0.375±0.05	28.57	0.275±0.05	31.25	0.2±0.0	33.33	0.1±0.0	69.23

*Values are expressed as mean ± SD, n = 6. Significantly different from control, **P≤0.01*

Table 2. Effects of ATPL, ETPL and SCP on 1% carrageenan paw edema in Mice at different time of observation

Treatment groups	Time after 1%carrageenan injection Paw edema volume thickness (ml)					
	1hour	%Inhibition	2hour	%Inhibition	5hour	%Inhibition
Control	0.475±0.05	____	0.4±0.08	____	0.35±0.05	____
*Indomethacin	0.375±0.05	21.05	0.275±0.05	31.25	0.175±0.05	50
ATP(100 mg/kg p.o.)	0.375±0.15	21.05	0.3±0.08	25	0.225±0.05	35.7
*ETP(100 mg/kg p.o.)	0.375±0.15	21.05	0.275±0.09	31.25	0.225±0.05	35.71
*SP(4 mg/kg p.o.)	0.35±0.05	26.31	0.25±0.05	37.5	0.2±0.0	42.85

*Values are expressed as mean ± SD, n = 6. Significantly different from control, **P≤0.01*

3. RESULTS

3.1 Cayman's COX Activity

The results of the COX inhibition using serum were compared for COX-1 & 2 inhibition was calculated by taking the mean of COX inhibition activity in triplicates. It was found that ATPL COX activity (COX-1 50%) (COX-2 52.7%), ETPL COX activity(COX-1 45.2 %) (COX-2 49.7 %) and SCP COX activity (COX-1 51.3%) (COX-2 49.8 %) were observed to be significant inhibitors of COX-1 and COX-2. A look at the COX inhibition profile by the selected leaf extracts and synthetic compound with more selectivity towards COX-2 inhibition as compared to COX-1 inhibition which showed moderate inhibition. The results were compared with indomethacin showing (COX-1 39.4%) and (COX-2 46.5%) The percentage of COX activity was depicted by using using multiple bar diagram (Graph 1), the results were significantly different from normal control at P< 0.001.

3.2 Ethyl Phenylpropiolate (EPP) Induced Ear Edema

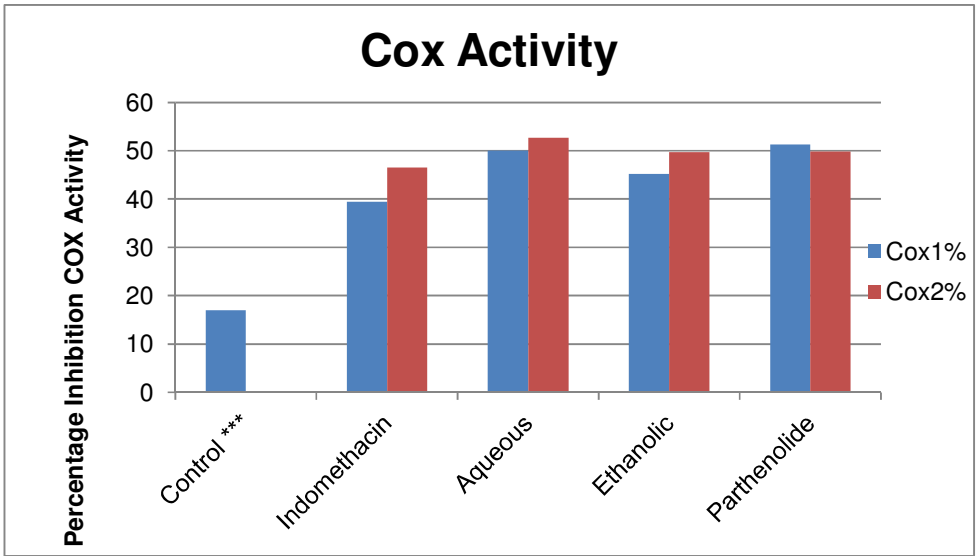
The mouse ear edema reached a maximum at 120 minutes after EPP application. The percentage inhibition at 120 minutes showed by the *Tanacetum parthenium* Linn leaves extracts was ATPL 61.53 %, ETPL 61.53% and Synthetic compound SCP 69.23% Thus ear edema showed significant difference among the groups (P < 0.001). (Table 1) the inhibitory results of plant extracts when compared to that of synthetic compound was at the higer dose considerably reducing the vascular permeability response to EPP application.

3.3 Carrageenan Induced Paw Edema (*Acute Inflammatory Model*)

The results of anti-inflammatory studies carried out reveal the percentage inhibition were given in (Table 2) The paw edema, reduced in both aqueous extract ATPL (35.7%) ,ethanol extract ETPL (35.71%) and synthetic parthenolide (42.85%) treated animals when compared with standard Indomethacin (50%) and control animals. The results are found to be statistically significant (p<0.01).

4. DISCUSSION

Medicinal plants and drug discovery has remained a very successful combination for the inventorization of new therapeutic agents. The main intention of the present study was to perform the COX activity guided standardization of selected medicinal plants with focus on antioxidant and cytotoxicity profile. Variety of phytochemicals like flavonoids, sesquiterpene lactone and volatile oils have been described to possess significant anti-inflammatory activity [17].



Graph 1. Effect of ATPL, ETPL and SCP on percentage inhibition activity of COX1 and COX2
*Values are expressed as mean ± SD, n = 6. Significantly different from control, ***P<0.001*

Several studies proved that naturally occurring coumarins [20] and flavonoids [21] act as dual inhibitors of cyclooxygenase and 5- lipoxygenase activities. The Indian spice turmeric, (*Curcuma longa* L.) possessing curcumin (and synthetic analogs) have established reputation as an anti-inflammatory agent by inhibiting COX-1 and COX-2 [22]. Flavonoids inhibit biosynthesis of prostaglandins (the end products of the COX and lipoxygenase pathways), which acts as a secondary messengers and are involved in various immunologic responses [23]. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory disorders [24].

Developing novel, effective and safe anti-inflammatory agents has remained a major thrust area in the main stream of 'finding alternatives to NSAID's. Anti-inflammatory agents possessing selective COX-2 inhibition and showing no or negligible effect on COX-1 activity are more appreciated as safe drugs as these agents have minimum gastrointestinal side effects [10].

The result of this study indicates the inhibitory effect of the leaf extracts and synthetic compound on carrageenan induced inflammation may be due to the inhibition of cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

The ability of ATPL, ETPL and SCP to inhibit cyclooxygenase enzymes (COX-1 and COX-2) was significant when compared to control respectively which proves to be a promising agent to counter numerous medical conditions which induce oxidative stress. The results obtained may be useful in strengthening the standardization of the selected botanicals. Moreover the selected plants can be considered as a resource for searching novel anti-inflammatory agents possessing COX-2 inhibition.

Ethylphenylpropiolate (EPP) induced ear edema model was selected because it produces local response and also avoids drug metabolism [25] EPP-induced mice ear edema is a useful model for screening and investigating the anti-inflammatory activity of test compounds. The inflammatory mediators released in this model include histamine, serotonin, bradykinin and prostaglandin (PGs). These mediators are capable of promoting vasodilation and increasing vascular permeability as well as synergistically producing edema [26].

Furthermore, Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is a biphasic response.

The first phase occurs within an hour of injection and is the result of the concurrent release of histamine, serotonin and kinins; the second phase is associated with elevated production of prostaglandins, oxygen-free radicals and inducible cyclooxygenase (COX-2) and the local infiltration and activation of neutrophils. Prostagladins play a major role in the development of the second phase, which usually occurs after 3 hours [27,28].

In this study, the *Tanacetum parthenium* ethanolic, aqueous extract of leaves and synthetic parthenolide significantly inhibited the edema formation induced by carrageenan at 1hr 2 hr to 5 hr of the experiment. This suggests that leaf extract and synthetic compound possesses anti-inflammatory activity probably by inhibiting the release or synthesis of various inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins and leucotrienes. In addition, the extract was also found to reduce the edema produced by histamine. This result confirms the activity of the extract at the first phase of carrageenan induced paw edema in mice and suggests that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine activity.

Preliminary phytochemical investigation of this plant showed the presence flavonoids, sesquiterpene lactone and volatile oils which might be in part responsible for anti-inflammatory effects [14]. The results obtained in the present study reflects that the ethanolic extracts of plant exhibit anti-inflammatory effects comparable with standard drugs, which is effective against pain of humans. Further scope for the study is to identify and isolate the possible active phytoconstituents responsible for the anti-inflammatory effects activity and study its pharmacological actions.

5. CONCLUSION

The present study may strengthen the process of standardization of botanicals containing the

selected plants as one of the ingredients. In many instances, the actual compounds isolated from the plants may not serve as drug, but leads to the development of potential novel therapeutic agents. The comparative study of ATPL, ETPL, SCP can be explored as a potential source of high-value bioactive metabolites and could be used in the pharmaceutical industry. The results of the present study showed significant pharmacological properties such as anti-inflammatory and cox inhibition activities. A plant product *Tanacetum parthenium* leaf extract have shown significant anti-inflammatory effects supporting traditional use proving to be important agents in the mainstream of drug discovery marathon. Hence with the rapid identification of new molecules from plant resources for having promising effect than that of the synthetic compounds, commercial benefits can be gained from natural plant products.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Animal Studies Committee of the nitte University approved the animal ethical clearance on 06.11.2013 Ref. KSHEMA/IAEC/14/2013. The experimental protocols were conducted following the technical and ethical principles recommended by the norms of the National Institute of Health Guide for Care and Use of Laboratory Animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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