Original Article

A COMPARATIVE STUDY OF ANTI-INFLAMMATORY EFFECT OF THIOCOLCHICOSIDE, DICLOFENAC AND THEIR COMBINATION IN WISTAR RATS USING CARRAGEENAN INDUCED PAW EDEMA METHOD

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DOI: 10.47750/pnr.2023.14.S01.152

Abstract

AIM: To study anti-inflammatory effects of thiocolchicoside in wistar rats in comparison with the standard drugs.

OBJECTIVES:

1. To study anti-inflammatory effect of thiocolchicoside, diclofenac and combination of thiocolchicoside & diclofenac in wistar rats.

2. To compare anti-inflammatory effect of thiocolchicoside, diclofenac and combination of thiocolchicoside & diclofenac in wistar rats.

MATERIAL AND METHODS: Anti-inflammatory activity was evaluated by using carrageenan induced paw edema method on plethysmograph. Wistar rats divided into 5 groups (n =6) Group 1 (control group) which was given 0.9% Normal Saline. Group 2 (test group) was treated with Thiocolchicoside 2mg/kg (ip). Group 3 (test group) was treated with Thiocolchicoside 4mg/kg (ip). Group 4 is standard treatment group where Diclofenac 10mg/kg (ip) was given. Group 5 was treated with Thiocolchicoside 2mg/kg + Diclofenac 10mg/kg. Inhibition of paw edema was recorded at 1 hr, 2 hr, 3 hr, 4 hr time intervals after treatment.

RESULTS: Significant anti-inflammatory activity was observed in group 3 (Thiocolchicoside 4mg/kg) and Group 4 (Diclofenac 10mg/kg), and except at 1st hr interval in Group 5 (Thiocolchicoside 2mg/kg + Diclofenac 10mg/kg) Statistically significant anti-inflammatory activity was observed ($p<0.05$) in test drug treatment group 3 (Thiocolchicoside 4mg/kg) and group 4 (Diclofenac 10mg/kg) and except at 1st hr interval in Group 5 (combination of Thiocolchicoside 2mg/kg + Diclofenac 10mg/kg) when compared to group 1 (Normal Saline 0.1ml/100gm). Thiocolchicoside 2mg/kg did not add to anti-inflammatory activity of Diclofenac 10mg/kg.

CONCLUSION: Thiocolchicoside showed time dependent and dose dependent rise in anti-inflammatory activity.

Keywords: Anti-inflammatory property, Diclofenac, Plethysmograph, Thiocolchicoside, Carrageenan induced paw edema method, Wistar rats.

INTRODUCTION:

The word inflammation is derived from the Latin word "inflammacio," which meaning "to ignite a fire."[1] Inflammation is a response of vascularized tissues to infection and damaged tissues that brings cells and molecules of host defense from the circulation to the sites where they are needed, in order to eliminate the offending agents.[2] Rubor, tumour, calor, and dolor are the four cardinal symptoms of inflammation that were first described by Celsus, a Roman author from the first century AD. Rubor denotes redness, tumour denotes swelling, calor denotes heat, and dolor denotes pain. The fifth clinical sign, "loss of function," was added by Virchow.[3] Currently, Thiocolchicoside is used in CNS disorders for the symptomatic treatment of neurological disorders such multiple sclerosis, cerebral palsy, and stroke that cause muscle spasm. It is used in treatment of local tissue injuries and pain. Ointment form of Thiocolchicoside is used in myofascial pain syndrome for those patients who are unable to take injections. The pilot studies have shown its use as an adjuvant therapy in oral submucosal fibrosis with muscle relaxants. It is used in preventing bone loss as it suppresses osteoclastogenesis and in breast cancer, multiple myeloma because it inhibits inflammatory pathways. [4] Also it is used in treating dental pains.[5] It is proposed to be used as anti-cancer as it works by modulating NF- κB regulating proteins [6]

Restrictions have been put on by European Medical Agency for not using thiocolchicoside more than 7 days orally, or 5 days by injection as it can cause aneuploidy by its metabolite M2 following its breakdown in the body. If used during pregnancy, the developing baby is harmed to a greater extent. It is also known to cause infertility in men.[7]

Although numerous clinical studies prove anti-inflammatory activity of thiocolchicoside [8], [9]in most of these studies thiocolchicoside is used in combination with standard anti-inflammatory drugs like diclofenac or any other non-steroidal anti-inflammatory drug. There are scanty animal studies available showing anti-inflammatory activity of thiocolchicoside though the drug is sold as anti-inflammatory for many decades. Consequently, it was deemed valuable to evaluate anti-inflammatory activity of thiocolchicoside by Carrageenan induced paw edema method on Plethysmograph in animal model using Wistar rats.

MATERIAL AND METHODS:

Drugs used in the study used were Thiocolchicoside which was procured from Indo Phytochem Pharmaceuticals Pvt. Limited, India. Carrageenan, Diclofenac and Normal Saline were procured from Central Pharmacy, Krishna Institute of Medical Sciences, Karad. All the drugs were administered intra-peritoneally except Carrageenan which was administered intradermally into sub-plantar region.

EQUIPMENTS:

Plethysmograph, Tuberculin syringe, Digital Weighing Machine, Animal Cages, Marker were the equipments used in the study.

Animals:

Thirty Wistar Rats of either sex weighing 150 -250 g used for this study. Animals were maintained under standard husbandry conditions. Before beginning the study, approval of Institutional Animal Ethical Committee (IAEC) of KIMS Karad was obtained (IAEC/KIMS/2021/1). This study was conducted in Research lab of Department of Pharmacology, Krishna Institute of Medical Sciences, Karad, following all Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines

Experimental Model:

Carrageenan induced paw edema using Plethysmograph [10]

The animals were weighed and marked properly. Carrageenan 0.1 ml of 1% solution [11] was administered intradermally into sub-plantar region 30 mins before injecting the drugs to each group. Then, the drugs were administered accordingly. Observations were made at 0 hr ,1hr , 2hr, 3hr , 4hr respectively. Edema was measured with the help of plethysmograph. Percentage inhibition (protection) against edema formation is taken as an index of acute anti-inflammatory activity. It is calculated as follows: Percentage Inhibition = (Vc – Vt / Vc) x 100

where,

Vc = Volume of paw edema in control animals

Vt = Volume of paw edema in treated animals.

Group	**No. of animals**	**Treatment**	**Abbreviation used**	**Route**
1	6	Normal Saline 0.9%	N.S 0.9%	i.p
2	6	Thiocolchicoside 2mg/kg	TH 2	i.p
3	6	Thiocolchicoside 4mg/kg	TH 4	i.p
4	6	Diclofenac 10mg/kg	DF 10	i.p
5	6	Thiocolchicoside 2mg/kg + Diclofenac 10mg/kg	TH 2 + DF 10	i.p

i.p: Intra-peritoneal

Statistical analysis:

Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used in statistical analysis to acquire the results, and post-hoc analysis by Dunnett's was used to compare the results to those of the control and standard drug.

Value of $p < 0.05$ is considered statistically significant as is indicated by asterisk sign. Instat GraphPad software version 3.06 was used for statistical analysis

RESULTS:

Table 1: Effect of Thiocolchicoside, Diclofenac, and combination of Thiocolchicoside with Diclofenac on Carrageenan induced paw edema compared to control in Wistar rats.

	Control (ml)	TH 2 (ml)	TH 4 (ml)	DF 10 (ml)	TH 2+ DF 10 (ml)	f value	p value
0 hr	1.95 ± 0.18	2.04 ± 0.18	2.04 ± 0.18	2.16 ± 0.12	2.16 ± 0.12	1.75	0.169

1 hr	0.45 ± 0.24	0.45 ± 0.24	0.29* ± 0.29	0.04 * ± 0.10	0.16 ± 0.20	4.550 0.0067
2 hr	1.25 ± 0.50	0.95 ± 0.33	0.5* ± 0.31	0.29* ± 0.24	0.25* ± 0.22	10.06 <0.0001
3 hr	1.33 ± 0.40	0.91 ± 0.58	0.83* ± 0.34	0.29* ± 0.24	0.16* ± 0.12	9.792 <0.0001
4 hr	1.08 ± 0.58	0.58 ± 0.43	0.37* ± 0.34	0.00 * ± 0.00	0.00 * ± 0.00	4.698 <0.0001

Post hoc analysis by Dunnett's test: * p<0.05

The study showed following results.

ANOVA revealed statistically significant difference in study group; TH 2, TH 4, DF 10, TH 2+ DF 10. Post hoc analysis by Dunnett's revealed no statistically significant difference in Carrageenan induced paw edema by TH 2 whereas rest of the study group showed statistically significant difference at all time intervals except for group TH 2 + DF 10 at first hour. Post hoc analysis by Dunnett's test revealed statistically significant difference in Carrageenan induced paw edema by TH 2. Whereas TH 4, TH 2 + DF 10 showed no statistically significant difference when compared with DF 10 at all-time intervals. Bonferroni test revealed statistically significant difference in Carrageenan induced paw edema by TH 4 when compared with TH 2 + DF 10 at 2 hr and 3 hr time interval, whereas no statistically significant difference was found at 1st and 4th hr.

Graph 1: Effect of Thiocolchicoside, Diclofenac, and combination of Thiocolchicoside with Diclofenac on Carrageenan induced paw edema compared to control in wistar rats (vol):

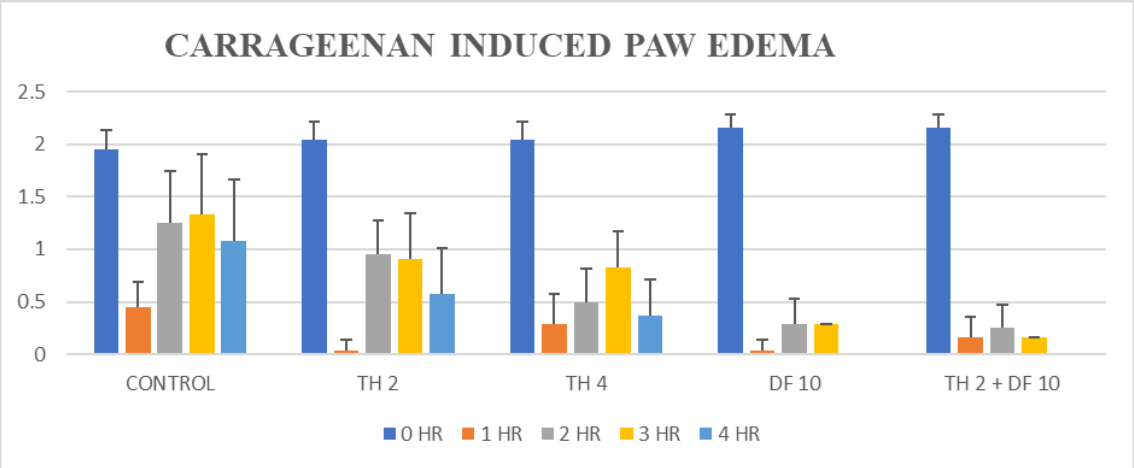
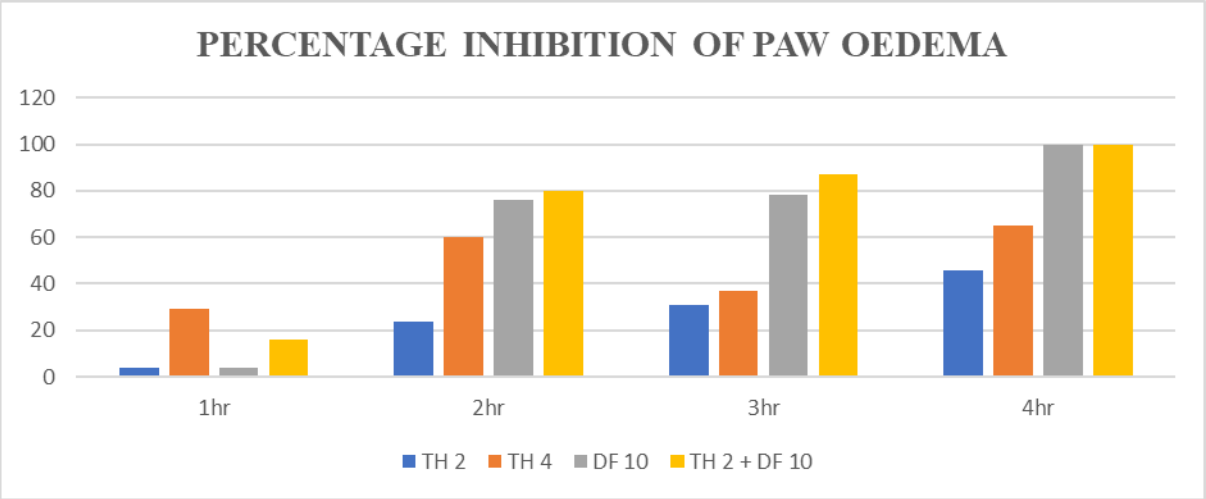


Table 2: Percentage inhibition of paw oedema in Study Groups at different time intervals

Group	1hr	2hr	3hr	4hr
TH 2	**4%**	**24%**	**31%**	**46%**
TH 4	**29%**	**60%**	**57%**	**65%**
DF 10	**4%**	**76%**	**78%**	**100%**
TH 2 + DF 10	**16%**	**80%**	**87%**	**100%**

Graph 2: Percentage inhibition of paw oedema in Study Groups at different time intervals



DISCUSSION:

Vascularized tissues in the body respond to infection and damaged tissues with inflammation, which transports cells and host defense chemicals from the circulation to the places where they are needed in order to get rid of the invading agents.[2] The four primary symptoms of inflammation are rubor, tumor, calor, and dolor.[3] Inflammatory mediators are found in a variety of forms, such as mast cells and basophils, which release histamine and enhance permeability. There are many inflammatory cells that, when activated by prostaglandins, leukotrienes, platelet activating factor, and cytokines, can dilate blood vessels, increase permeability, and harm tissue. Increased permeability is also caused by mediators such as kinins like bradykinin, and anaphylatoxins.[3]

Thiocolchicoside is a semisynthetic derivative of colchicoside and a natural derivative of colchicine both are extracted from seeds of Gloriosa superba Liliaceae.[12]

The anti-inflammatory activity of different doses of Thiocolchicoside were examined in the current study using Plethysmograph as an experimental model. Carrageenan induced paw edema method was used to evaluate anti-inflammatory activity. All the experiments were conducted as per CPCSEA guidelines. Each group contained 6 rats.

Thiocolchicoside acts as anti-inflammatory by down regulating NF-κB and suppresses the expression of NF-κB–regulated gene products. The reasons for NF-κB activation are: cytokines such as Tumour Necrosis Factor

(TNF), carcinogens, tobacco smoke, environmental contaminants, ionizing radiation and stress. Thiocolchicoside also suppressed TNF promoted COX 2 activity in a dose dependent manner. This suggests that thiocolchicoside inhibits NF-κB–regulated gene expression by suppressing NF-κB that binds to the COX-2 promoter [6]

In our study, all three treatment groups Thiocolchicoside 4mg/kg, Diclofenac 10mg/kg, and combination of Thiocolchicoside 2mg/kg +Diclofenac 10mg/kg showed good anti-inflammatory activity. When anti-inflammatory activity at 2hr, 3hr and 4hr interval of Thiocolchicoside 4mg/kg and combination of Thiocolchicoside 2mg/kg + Diclofenac 10mg/kg was done using Bonferroni test, it was observed that at 2hr and 3hr interval, the combination Thiocolchicoside 2mg/kg + Diclofenac 10mg/kg was having significantly more anti-inflammatory activity than Thiocolchicoside 4mg/kg.

This suggests that addition of Thiocolchicoside might potentiate anti-inflammatory activity of Diclofenac if used in higher doses. And therefore, if we had used combination of Thiocolchicoside 4mg/kg and Diclofenac 10mg/kg we would have got significant increase in anti-inflammatory activity at all time intervals.

This can be explained on the basis of different mechanism of anti-inflammatory activity of Diclofenac and Thiocolchicoside. Thiocolchicoside might be acting as anti-inflammatory because of its inhibitory effect on NF-κB pathway of inflammation.

Thiocolchicoside showed time and dose dependent inhibition of paw edema.

In animal study of anti-inflammatory activity of Thiocolchicoside which was conducted by L. Akramas, et al .in year 2019 showed Thiocolchicoside combined with Methylsulfonylmethane at a dose twice the lower dose considerably slowed the progression of the disease, supporting our results. [13]

Considering all the results in our experiments we can say that Thiocolchicoside has anti-inflammatory activity only in higher doses in wistar rats. But the fact is for many decades it has been used as well as recommended for anti-inflammatory activity in human being particularly for arthritis, low back pain, and myofascial pain.

In terms of Fixed dose Combination, when the price of one strip (10 tablets) of Thiocolchicoside 4mg, diclofenac 50mg, and combination of both was compared across five different brands, the combination was found to be 4 times more expensive than diclofenac 50mg alone.

Considering the lack of adequate proof in animal studies and restrictions put on its use, due to different adverse effects of Thiocolchicoside, it is recommended that more animal studies using different models of evaluation and large-scale clinical studies pertaining to anti-inflammatory activities should be performed. This will give us scientific evidence for use of Thiocolchicoside in clinical practice.

CONCLUSION:

Based on the results of the current experimental study, we conclude that:

Thiocolchicoside 4mg/kg showed anti-inflammatory activity whereas lower dose of Thiocolchicoside has not shown anti-inflammatory activity. Thiocolchicoside has shown time dependant and dose dependant rise in anti-inflammatory activity. Maximum percentage inhibition of paw edema is shown by Diclofenac 10mg/kg and the combination (Thiocolchicoside 2mg/kg + Diclofenac 10mg/kg) at 4^{th} hr (100 %). Addition of Thiocolchicoside 2mg/kg did not potentiate anti-inflammatory effect of Diclofenac 10mg/kg at all intervals. It is recommended that animal and human studies for anti-inflammatory activity should be carried out to authenticate the widespread use of this previously marketed medicine in light of the negative effects, restrictions on drug use, and higher cost.

Conflict of Interest: None

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