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Comparative Study of Anti-Inflammatory Activity of Piperine with Hydrocortisone in Albino Rats

Giri KR*, Totade SV, Giri RR, Tatkare SN

Dept of Pharmacology, J.N. Medical College, Sawangi (M), Wardha, Maharashatra, India

ABSTRACT

Since antiquity piperine has been widely used in Indian traditional medicine. Piperine is an alkaloid isolated from the fruit of Black pepper. To compare the anti-inflammatory property of Piperine with standard as hydrocortisone against Carrageenan induced paw edema model in albino rats. After approval from Institutional Animal Ethics Committee, albino rats of either sex (Wt. 150-250 gms) were divided into 3 groups with 6 animals in each. Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1% freshly prepared Carrageenan in normal saline in right hind paw of rats. Control groups were treated with normal saline, Test groups with Ethanolic root extract of Piperine (10 mg/kg p.o.), Standard groups with hydrocortisone (40mg s.c.) one hour before carrageenan injection. The paw volume was measured plethysmometrically at an interval of 1, 2, 3, 6 hrs after carrageenan injection. Mean paw volume increase in ml (Mean + SE) and % inhibition of paw swelling was evaluated and analyzed statistically. Mean + SE and % inhibition of paw swelling are as follows: Piperine 0.325+0.012, 57.23%; hydrocortisone 0.285+0.009, 65.3% after 3 hrs respectively. $p<0.05$ is significant for Piperine as compare to control group. Piperine exhibit potent acute anti-inflammatory activity comparable to hydrocortisone.

Keywords: piperine, carrageenan, Anti-inflammatory.

Corresponding author

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INTRODUCTION

Inflammation (Latin, inflammare, to set on fire) is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [1]. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Acute inflammation is the initial response achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues.

Medicinal herbs as a potential source of therapeutic aids have attained a significant role in health system all over the world not only in the diseased condition but also as potential drugs for maintaining good health. [2]

Piperine is an alkaloid found naturally in plants belonging to the Piperaceae family, such as Piper nigrum L, commonly known as black pepper, a household spice and Piper longum L, commonly known as long pepper. Piperine is the major pungent substance in these plants and is isolated from the fruit of the black pepper and long pepper plants. Piperine comprises 1 to 99% of these plants. [3] It has potential anti-inflammatory activity. There is also preliminary evidence that it may have some anticonvulsant and anticarcinogenic properties. Although this piperine has many useful claims, the mechanism of its medicinal effects are not understood. [4]

Currently available remedy for inflammation mainly includes Corticosteroids and Non-steroidal anti-inflammatory drugs for the relief of pain and inflammation. All these therapies are however associated with adverse effects. Adverse effect of hydrocortisone are hypokalaemia, hypertension, myopathy, immunosuppresion.

This study aims towards the comparison of the anti-inflammatory property of piperine with standard as hydrocortisone against carrageenan induced paw edema model in albino rats.

MATERIALS AND METHODS

Preparation of the extract

The powdered plant material of Black pepper were extracted with ethanol in a Soxhlet apparatus for 48 hrs. The extracts were filtered through Whatman filter paper (No.1) and concentrated by vacuum evaporation. The yield of extract as per solvent used was 3.25% w/w. The dried extracts were suspended in 2% gum acacia and used for experiments.

ANIMALS: Albino rats.

Inclusion criteria:

Sex : Male and Female rats

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Weight : 150-250 gms.(6 rats in each groups)

Rats were obtained from the animal house of J. N. Medical College, Sawangi (M), Wardha.

Exclusion criteria:

Pregnant female rats
Old rats
Unhealthy / Diseased rats

The study was undertaken after obtaining approval of Institutional Animal Ethics Committee.Rats were kept in Polypropylene cages in the animal house with 12/12 h light/dark cycle at 27±2°C and were provided with the standard environmental conditions including temperature, humidity, aeration and food. Animals were kept under fasting for overnight and weighed before the experiment. The guidelines recommended by the CPCSEA were taken into consideration throughout the study.

CHEMICALS AND DRUGS:

Drug Hydrocortisone was obtained from institutional medical store; carrageenan from Himedia chemicals Mumbai. Piperine were obtained from Shrishell Herberium Nagpur.

STUDY DESIGN:

The rats of either sex were randomly allocated into four groups of six rats in each.

Control Group 1: Treated with Normal Saline.
Test groups Group 2: Treated with piperine one hour before Carrageenan injection
Standard groups Group 3: Treated with hydrocortisone one hour before Carrageenan injection

Anti-inflammatory activity (Carrageenan-induced paw edema)

Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1% freshly prepared Carrageenan in normal saline in right hind paw of rats. Control groups were treated with normal saline, Test groups with Ethanolic root extract of Piperine (10 mg/kg p.o.), Standard groups with hydrocortisone (40mg s.c.) one hour before carrageenan injection.The paw volume was measured plethysmometrically at an interval of 1, 2, 3, 6 hrs after Carrageenan injection. Mean paw volume increase in ml (Mean + SE) and % inhibition of paw swelling was evaluated. [5]

The right hind paw edema inhibition at different doses of Test drug and Standard drug were calculated by comparing with vehicle treated control rats. Following formula was used:

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$$\% \text{ inhibition of paw edema} = \frac{(Vt\text{-}Vo)_{control} - (Vt\text{-}Vo)_{treated}}{(Vt\text{-}Vo)_{control}} \times 100$$

Where,

Vt is the rat paw volume at time't', Vo is the initial rat paw volume (before Carrageenan injection), $(Vt\text{-}Vo)_{control}$ is edema produced in control group and $(Vt\text{-}Vo)_{treated}$ is edema produced in treatment groups.

Statistical Analysis

All values were shown as Mean + SE. Statistical analysis was performed using one-way analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

RESULTS

The results obtained as Mean + SE and % inhibition of paw swelling. The results of effect of Piperine, hydrocortisone on carrageenan induced oedema in rat hind paw are summarized in Table 1,2,3,4.

Mean + SE and % inhibition of paw swelling are as follows: Piperine 0.325+0.012, 57.23%; hydrocortisone 0.285+0.009, 65.3% after 3 hrs respectively which were maximum.

Table -1 – Anti-inflammatory activity 1hr (Carrageenan Induced Paw Edema Method)

Group	Treatment Dose(mg/kg, p.o)	Increase in paw volume (Mean + SE) in ml	% inhibition of paw Edema
1	Control	0.57+0.004	
2.	Piperine (10 mg/kg p.o.)	0.291+0.007	48.8%*
3.	Hydrocortisone (40mg s.c.)	0.228+0.01	59.9%**

One Way ANOVA, N=6 in each group; $p < 0.05$*; $p<0.001$** compared to control

Table -2 – Anti-inflammatory activity 2hr (Carrageenan Induced Paw Edema Method)

Group	Treatment Dose(mg/kg, p.o)	Increase in paw volume (Mean + SE) in ml	% inhibition of paw Edema
1	Control	0.61+0.01	
2	Piperine (10 mg/kg p.o.)	0.266+0.0192	56.28%*
3	Hydrocortisone (40mg s.c.)	0.23+0.01	62.2%**

One Way ANOVA, N=6 in each group; $p < 0.05$*; $p<0.001$** compared to control

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Table -3 - Anti-inflammatory activity 3hr (Carrageenan Induced Paw Edema Method)

Group	Treatment Dose(mg/kg, p.o)	Increase in paw volume (Mean + SE) in ml	% inhibition of paw Edema
1	Control	0.76+0.006	
2	Piperine (10 mg/kg p.o.)	0.325+0.012	57.23%*
3	Hydrocortisone (40mg s.c.)	0.266+0.009	65.5%**

One Way ANOVA, N=6 in each group; $p < 0.05$*; $p<0.001$** compared to control

Table -4 – Anti-inflammatory activity 6hr (Carrageenan Induced Paw Edema Method)

Group	Treatment Dose(mg/kg, p.o)	Increase in paw volume (Mean + SE) in ml	% inhibition of paw Edema
1	Control	0.8+0.0135	-
5	Piperine (10 mg/kg p.o.)	0.349+0.009	56.3%*
6	Hydrocortisone (40mg s.c.)	0.295+0.013	63.1%**

One Way ANOVA, N=6 in each group; $p < 0.05$*; $p<0.001$** compared to control

DISCUSSION

The carrageenan-induced edema in the rat hind paw most widely used for the screening of new anti-inflammatory agents [6] .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 mediated through the release prostaglandin and slow reacting substances which peak at 3 hrs [6].The piperine in doses of 10 mg/kg, p.o. produced significant inhibition of paw edema as compared to the control. These results indicate that piperine possesses inhibition of prostaglandin release mediated anti-inflammatory properties.

CONCLUSION

Ethanolic extract of Piperine an alkaloid obtained from a household spice exhibit potent acute anti-inflammatory activity comparable to hydrocortisone. Piperine can be used as a household remedy for inflammation.

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