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RESEARCH ARTICLE

Evaluation of Anti- Inflammatory effect of 4-Benzylpiperidine using Membrane Stabilization Method – an Invitro Study

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ABSTRACT:
The objective of this study was to evaluate the anti-inflammatory potential of 4-Benzylpiperidine. The anti-inflammatory activity was evaluated using invitro models such as heat induced membrane stabilization method, hypotonicity induced membrane stabilization method at different concentrations. Aspirin were used as standard drugs. The percentage of inhibition was compared with those of standard drugs. The results indicate that the 4-benzylpiperidine possess anti-inflammatory properties. The mode of action of the test sample and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells. Protective effect on heat and hypotonic saline, induced erythrocyte lysis is known to be a good index of anti-inflammatory activity of any agent. So 4-Benzylpiperidine can be used as a potential source of anti-inflammatory agents.

KEYWORDS: Anti- Inflammatory Activity, 4-Benzylpiperidine, Membrane Stabilization

INTRODUCTION:
Inflammation is a normal protective response to tissue injury and it involves a complex array of enzymes activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [1]. It is a bodily response to injury, infection or destruction characterized by heat, redness, swelling, pain and disturbed physiological functions. It is triggered by the release of chemical mediators from injured tissue and migrating cells. The inflammatory mediators are Histamine, 5-HT (serotonin), Bradykinin, Prostaglandins (eg PGE2), Interleukins, Substance P, Nitrous oxide[2].

In many inflammatory disorders there is excessive activation of phagocytes, production of 0_2-, OH radicals as well as non free radicals species (H_20_2) [3], which can harm severely tissues either by powerful direct oxidizing action or indirect with hydrogen peroxide and –OH radical formed from O_2- which initiates lipid per oxidation resulting in membrane destruction. Tissue damage then provokes inflammatory response by production of mediators and chemo tactic factors [4]. The reactive oxygen species are also known to activate matrix metello proteinase damage seen in various arthritic tissues [5]. Any form of injury to the human body can elicit a series of chemical changes in the injured area. Earlier it was believed that inflammation was contemplated as a single disease caused by disturbances of body fluids. According to the modern concept, inflammation is a healthy process resulting from some disturbance or disease[6].

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues.

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Chronic inflammation can also lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g. gallbladder carcinoma). It is for that reason that inflammation is normally closely regulated by the body[7]. Various physiological effects of PGs include acute and chronic inflammatory reactions, blood pressure (BP) change, platelet aggregation, induction of labor and intensification of pain and fever [8].

To treat the inflammatory diseases analgesic and anti-inflammatory agents are required. The drugs used in inflammatory disorders may be either with analgesic and insignificant anti-inflammatory effects or with analgesic and mild to moderate anti-inflammatory activity.4-Benzylpiperidine is a drug and research chemical used in scientific studies. It acts as a monoamine releasing agent with 20-40 fold selectivity for releasing dopamine versus serotonin. It has a fast onset of action and a short duration [9,10] . 4-Benzylpiperidine is used to synthesize pimetine, an hypolipidemic agent [11], for treatments for cocaine dependence, also used as anti inflammatory agent to treat joint pain, arthritis, fever and also used to treat Parkinson's disease by increasing the dopamine synthesis[9].

The present study was conducted to evaluate the in vitro anti inflammatory activity of 4-Benzylpiperidine using in vitro models such as Heat induced membrane stabilization method and hypotonicity induced membrane stabilization method.

EXPERIMENTAL DESIGN:

Drugs and Chemicals :

4-Benzylpiperidine were procured from Sigma Aldrich, Chennai, India. All other chemicals used were of analytical grade obtained commercially.

Assessment of Invitro Anti-Inflammatory Activity by Membrane Stabilization Method:

Heat induced hemolysis: [12, 13, 14, 15]

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100-1000µg/ml) and 1ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at2500 rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiments were performed in triplicates for test samples. The percentage of inhibition of Haemolysis was calculated as follows:Percentage inhibition = (Abs Control – Abs Sample) x 100 / Abs control.

Hypotonicity- induced hemolysis: [16]

The reaction mixture contains different concentration of test sample (100-1000µg/ml), reference sample and control were separately mixed with 1ml of phosphate buffer, 2ml of hypo saline and 0.5ml of HRBC suspension. Aspirin (100-1000µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37ºC for 30 min and centrifuged at 3000rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560mm. The percentage haemolysis was estimated by assuming the haemolysis produced in the control as 100%.

% Inhibition of haemolysis = 100 x [A 1 –A 2 /A 1]

Where,

A 1 = Absorbance of hypotonic buffered solution alone

A 2 = Absorbance of test /standard sample in hypotonic solution

RESULT:

In the study of membrane stabilization activity of 4-Benzylpiperidine at concentration range of 100, 200, 400, 800, 1000 µg/ml protected significantly in a concentration dependent manner the erythrocyte membrane against heat induced hemolysis and hypotonicity induced hemolysis. Aspirin in the concentration of 100, 200, 400, 800, 1000 µg/ml used as standard also offered protection of HRBC membrane against damaging effect induced by heat and hypotonic solution. In this study, the 4-Benzylpiperidine was effective in inhibiting the heat induced and hypotonicity induced hemolysis at different concentrations as shown in Table 3 and 4. In heat induced hemolysis, the results showed that 4-Benzylpiperidine at 1000µg/ml concentration exhibit 56.25% of inhibition and Aspirin showed 68.75% inhibition at 1000µg/ml concentration. In the hypotonicity induced hemolysis 4-Benzylpiperidine showed 44.18% of inhibition at 1000µg/ml and aspirin showed 55.81% inhibition at 1000µg/ml concentration.

Table 1. Invitro anti inflammatory activity of 4-benzylpiperidine and aspirin of heat induced membrane stabilization method

Treatments	Concentration (µg/ml)	Absorbance (660nm))	% Inhibition
Control	-	0.32+0.08	-
Benzylpiperidine	100	0.23±0.05	28.12
	200	0.22±0.06	31.25
	400	0.20±0.04	37.50
	800	0.17±0.01	46.87
	1000	0.14±0.05	56.25
Aspirin (Standard)	100	0.16±0.02	50.00
	200	0.15±0.03	53.12
	400	0.13±0.06	59.37
	800	0.12±0.02	62.50
	1000	0.10±0.03	68.75

Each value represents the mean ± SD

Table 2: Invitro anti inflammatory activity of 4-benzylpiperidine and aspirin of Hypotonicity induced membrane stabilization method

Treatments	Concentration (µg/ml)	Absorbance (660nm))	% Inhibition
Control	-	0.43±0.02	-
4-Benzylpiperidine	100	0.34±0.01	20.93
	200	0.32±0.08	25.58
	400	0.30±0.02	30.23
	800	0.28±0.05	34.88
	1000	0.24±0.03	44.18
Aspirin (Standard)	100	0.27±0.04	37.20
	200	0.25±0.07	41.96
	400	0.23±0.01	46.51
	800	0.21±0.03	51.16
	1000	0.19±0.01	55.81

Each value represents the mean ± SD

DISCUSSION:

During inflammation, lysosomal hydrolytic enzymes are released which causes damages of the surrounding organelles and tissues with attendance variety of disorders. The erythrocyte membrane is analogous to the lysosomal membrane [17] and its stabilization implies that the extract may stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release[18]. The lysosomal enzyme released during inflammation produce a various disorders. The extracellular activity of these enzymes are said to be related to acute to chronic inflammation. The non steroidal drugs act either by inhibiting the lysosomal enzymes or by stabilizing the lysosomal membrane[19].

In the present study, results indicate that the 4-benzylpiperidine possess anti-inflammatory activity. The mode of action of the test sample and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membranes with subsequent alteration of the surface charges of the cells [20]. Protective effect on heat and hypotonic saline, induced erythrocytelysis is known to be a good index of anti-inflammatory activity of any agent. Since the membrane of RBC is structurally similar to the lysosomal membrane the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane. Hence, this study gives an idea that the 4-Benzylpiperidine can be used as a lead compound for designing a potent anti-inflammatory drug which can be used to cure inflammation.

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