WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 6, 1563-1570. Research Article ISSN 2277– 7105

POSSIBLE ANTI-INFLAMMATORY MEDIATED WOUND HEALING ACTIVITY OF PYRAZINAMIDE AND CLOFAZEMINE IN WISTAR RATS

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Article Received on
19 April 2017,

Revised on 09 May 2017,
Accepted on 29 May 2017

DOI:10.20959/wjpr20176-8709

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ABSTRACT

Inflammation is defined as a sequence of events that occurs in response to noxious stimuli, infection or trauma. Symptoms of inflammations are local redness, swelling, pain, heat and loss of function. The present study was performed to evaluate the anti-inflammatory and wound healing activity of pyrazinamide and Clofazemine in Wistar rat. The aim of present work is to study the screening of synthetic compounds for its pharmacological aspects in inflammation and wound healing activity by using various preclinical models of wound healing and inflammation.The both drugs were studied for their anti-inflammatory activity by using carrageenan-induced paw edema in rats and the mean increase in paw volume in paw volume were measured by plethysmometer at different time intervals after carrageenan (1% w/v) injection. The pyrazinamide and Clofazemine were further evaluated for wound healing activity using incision wound model. The pyrazinamide and Clofazemine showed significant ($p<0.05$) reduction in the carrageenan-induced paw edema in rats and incision wound healing activity evidenced method in Wistar rats. The pyrazinamide and Clofazemine showed a greater anti-inflammatory and wound healing effect when compared with the standard drugs, indomethacin and povidone iodine respectively. The present observation indicated significant ($p<0.0001$) activity of the pyrazinamide and Clofazemine in the treatment of inflammation and wound healing.

KEYWORD: Inflammation is defined Clofazemine injection.

INTRODUCTION

Wound healing is a complex process of the replacement of the dead tissue by living one. Generally wound healing process consists of three basic phases, inflammatory, proliferative maturation phase. All these steps are in controlled manner by a variety of cytokines including growth factors such as epidermal growth factor (egf), fibroblast growth factor (fgf) and transforming growth factor beta (tgfβ).

The inflammatory process may be defined as a sequence of events that occurs in response to noxious stimuli, infection or trauma. The classic signs of inflammation are local redness, swelling, pain, heat and loss of function. The events of inflammation that underline these manifestations are induced and regulated by a large number of chemical mediators, including kinins, eicosanoids, complement proteins, histamine and monokines. NSAIDs are among the most commonly used drugs worldwide. They are prescribed for orthopaedic conditions such as osteoarthritis, soft-tissue injuries and fractures *etc*. NSAIDs are one of the best classes of drug to prevent and treat postoperative pain. The greatest disadvantage in presently available potent synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation. Therefore, thescreening and development of drugs for their anti-inflammatory activity is the need of hour and there are many efforts for finding anti inflammatory drugs from indigenous medicinal plants. The use of NSAIDs is associated with many side effects, but their unwanted effects on the gastrointestinal tract, the kidney and the cardiovascular system are considered as major issues with the use of these drugs.

MATERIAL AND METHOD

Drugs and chemicals

Pyrazinamide drug were gifted by Avanscure life sciences pvt. Ltd.**, Gurgaon,** Haryana - 122002, India. Clofazemine were gifted by elite international 320 sardar griha 198 l.t. Marg Mumbai, Maharashtra, India - 400002. Carrageenan was procured from new neeta chemicals pimpri, pune. Povidone iodine was purchased by shri krushna medico, near pune station pune -01.

Experimental animals

Male Wister rats weighing 175 - 200 g were used. The animals were fed with standard diet (AISSMS College of pharmacy, pune), had free access to water under well ventilated

condition of 12 h day light cycle. The animals were adapted to laboratory condition for 7 days prior to the experiments. Animals were kept in individual standard cages in standardized environmental condition with an ambient temperature of 22 ± 2ºc and a 12 h light-dark cycle. The studies were performed with the approval of institutional animal ethics committee (IAEC) of AISSMS College of pharmacy, Kennedy road, pune station, pune.

Carrageenan-induced paw edema model

Initially 0hr paw volume measured for the carrageenan induced paw edema. After half an hour carrageenan Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat. After the carrageenan induced inflammation after half an hour topical application was applied to the animal. Rats were divided into five groups; each group consisting of six animals. First group consist of carrageenan control animal only given normal saline. Second group consist of pyrazinamide drug with honey. Animal only given pyrazinamide with honey. Third group consist of Clofazemine drug with honey. Animal only given Clofazemine with honey. Fourth group consist of standard povidone iodine drug with honey. Animal only given standard povidone iodine. Fifth group consist of only honey. The paw thickness was measured before injecting the carrageenan and after repetitive time interval Using Plethismometer apparatus.

Incision wound model

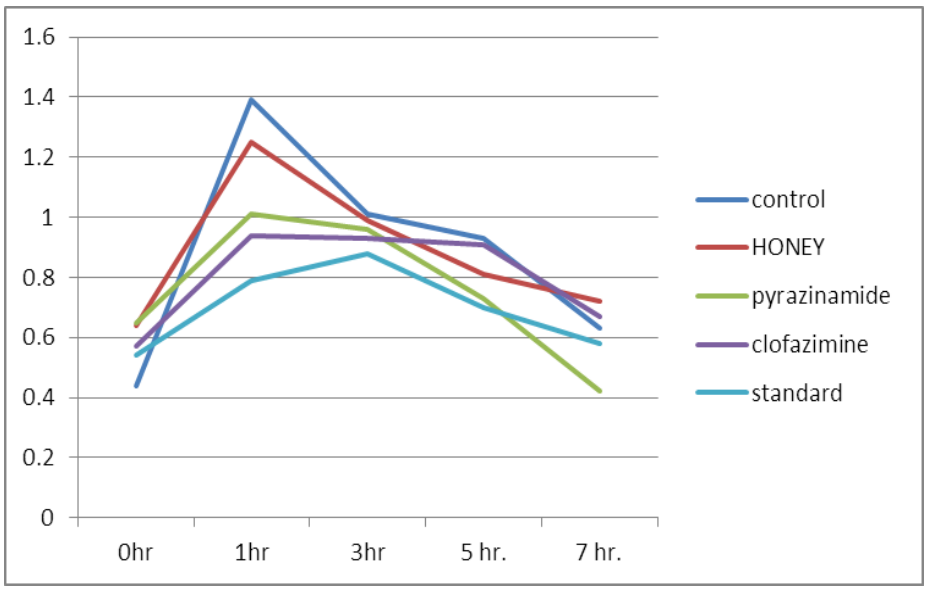
B. Incision wound model

The animals were divided in to a 5 groups each group consist of six animals. The animals were anaesthetized by using xylenthine (20 mg/kg *i.p*). Para vertebral straight incision of 6 cm length was made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete homeostasis, the wound was closed by means of interrupted sutures placed at equidistant points about 1 cm apart. 1st group was taken as a control this group is only vehicle. Group 2nd were taken as a standard this group is treated with standard drug povidone iodine, group 3rd were taken as a test this group is treated with pyrazinamide drug, group 4th were taken as a test this group is treated with Clofazemine drug, and group 5th were taken as a test this group is treated with honey. Animals were treated once a day with drugs from 0 day to 9 post-wounding day. The wound breaking strength was estimated on 10 day by tensiometer apparatus. The breaking strength was expressed as minimum weight necessary to bring about gaping of area.

RESULTS

Table 1: effect of drugs on carrageenan induced paw edema in rats

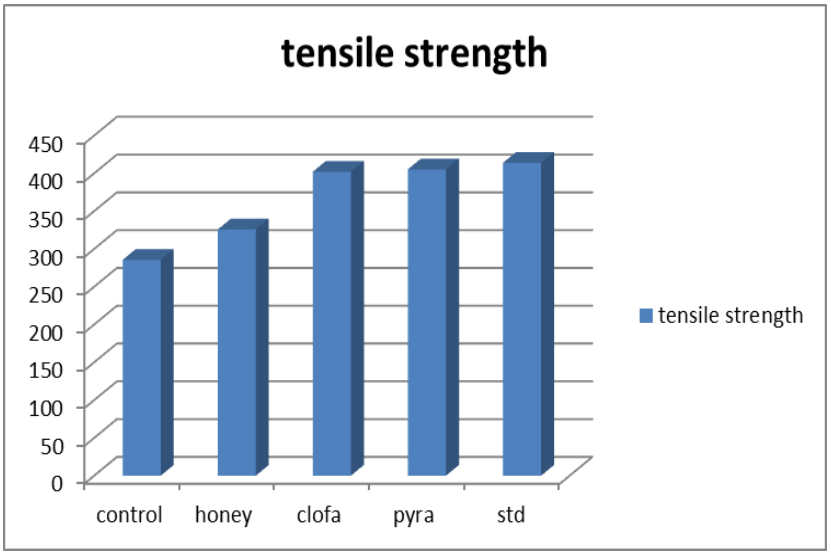
Group	0 hr.	1 hr.	3 hr.	5 hr.	7 hr.	% Inhibition
Group I (Control)	0.44 ±0.30	1.39 ±0.63	1.01 ±0.25	0.93 ±0.62	0.63 ±0.30	43%
Group II (HONEY)	0.64 ±0.15	1.06 ±0.18	0.93±0.13	0.81 ±0.14	0.44 ±0.09	48.50%
Group III (Pyrazinamide)	0.65 ±0.12	1.013 ±0.08	0.96 ±0.16	0.73±0.12	0.42±0.02	38%
Group IV(Clofazemine)	0.57 ±0.15	0.94±0.20	0.93±0.31	0.91±0.20	0.67±0.09	60%
Group V(Indomethacin std)	0.54±0.07	0.79±0.14	0.66±0.07	0.66±0.07	0.58±0.03	70%



Graph-1 Anti-inflammatory effect of pyrazinamide, Clofazemine and honey shows significant effect by ANNOVA (Values are expressed as mean ± SD, *P < 0.05* When compared with control)

Table 2: effect of drugs on incision model in rat

drugs	tensile strength ± S.D
control	285.6±6.69
honey	325.8±9.73
Clofazemine	402.2±5.45
pyrazinamide	405.4±6.950
Povidone iodine	414±8.124



Graph-2 incision model of pyrazinamide, Clofazemine and honey shows very significant effect by graph pad prism software

RESULTS AND DISCUSSION

The present study demonstrates the anti-inflammatory action of Clofazemine, pyrazinamide, in rats. The activity of **pyrazinamide and Clofazemine** drugs ($p < 0.05$, ANNOVA) was comparable to indomethacin when tested against carrageenan induced paw edema on rat. Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by means of development of proliferative cells which can either spread or form granuloma. Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts during granular tissue formation. In the study indomethacin as a standard and pyrazinamide and Clofazemine, as a test drugs shows a significant decline in paw edema on paw edema the anti-inflammatory action of *pyrazinamide and Clofazemine* was revealed to be via prostaglandin inhibition, the most frequently encountered mechanism of action amongst anti-inflammatory drugs.

The present study demonstrates the incision wound model of Clofazemine, pyrazinamide, in Wistar rats. The activity of **pyrazinamide and Clofazemine** drugs ($p < 0.0001$, ANNOVA) was comparable to povidone iodine in Wistar rat. Whereas, in the wound healing activity topical application of *pyrazinamide and Clofazemine with honey* significantly accelerated wound healing with drugs having the highest percentage wound contraction ability followed by povidone (a commercially sold topical antibiotic agent used in wound dressing) and honey

with the both drugs. This may be due to the stimulation of interlukin-8 and inflammatory α-chemokine, which in turn enhances the function of various inflammatory cells, fibroblast and keratinocytes wound healing due to their astringent and antimicrobial property and drugs having due to their antioxidant and antimicrobial activity, which appear to be responsible for wound contraction and elevated rate of epithelialization. Pyrazinamide and Clofazemine compounds are also responsible for wound healing due to free radical-scavenging and antioxidant activity, which are known to reduce lipid peroxidation, by reducing cell necrosis and improving vascularity.

CONCLUSION

This experiment showed that the carrageenan successfully induced edema in the paw. Clofazemine and pyrazinamide reduced the paw volume indicated anti-inflammatory property. The findings of the present experimental study appear to be clinically relevant since such drugs are likely to be used chronically in leprosy and TB patients who are prone for injury. Though Clofazemine and Pyrazinamide is commonly used as an anti-leprosy drug and anti-TB drugs.

ACKNOWLEDGMENT

The authors are grateful to the Principal Dr. Mrs. A. R. Madgulkar, AISSMS of Pharmacy, Pune and Dr. Rahul Somani and Dr. Sachin Tembhurne for providing the necessary support and guidance.

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