Pravara Med Rev; September 2024, 16 (03), 82 - 89
DOI: 10.36848/PMR/2024/11000.90111

Original article

Screening of Azithromycin for its anti-inflammatory potential in experimental Wistar rats

Mahesh M. Bharambe[1], Vandana M. Thorat[1*], Somnath M. Matule[2]

[1] P.G. Student, Department of Pharmacology, Krishna Institute of Medical Sciences, Karad, India, 415539
[1] Professor, Department of Pharmacology, Krishna Institute of Medical Sciences, Karad, India, 415539*
[2] Associate Professor, Department of Pharmacology, Ashwini Rural Medical College & Research Centre, Solapur, India, 413006
**Corresponding author : Dr. Vandana M. Thorat*

Abstract

Aim & Objectives: *To evaluate the effect of azithromycin on acute as well as sub-acute model of inflammation in experimental Wistar rats.*

Materials and Methods: *The study was conducted after animal ethics committee approval. Animals were divided into three groups each for acute as well as sub-acute model of inflammation. Control group received 1% gum acacia suspension (4ml/kg) while test groups received aspirin (200mg/kg) and azithromycin (20mg/kg). Acute model of inflammation consisting of carrageenan induced rat paw edema method and sub-acute model consisting of foreign body induced granuloma method were used to investigate anti-inflammatory activity of azithromycin.*

Results: *The present study clearly show significant anti-inflammatory activity of azithromycin ($p < 0.05$) in acute model of inflammation when compared to control and its activity was comparable to aspirin at 5hour interval ($p > 0.05$). In sub-acute model of inflammation, azithromycin showed significant anti-inflammatory activity ($p < 0.05$) as compared to control and it was comparable to aspirin ($p > 0.05$).*

Conclusion: *Azithromycin have significant anti-inflammatory potential in animal models of acute and sub-acute inflammation.*

Key words: *Azithromycin, aspirin, anti-inflammatory, Carrageenan, foreign body granuloma*

Introduction

Inflammation is the response of the immune system to deleterious stimuli, such as pathogens, injured cells, noxious compounds, or irradiation. The acute inflammatory response is mediated by three main vascular components like vasodilation, increase in vascular permeability, and migration of leukocytes to the injured tissues. Chronic inflammation may follow uncontrolled acute inflammation comprising of fibroblasts proliferation, mono nuclear cells infiltration and increased connective tissue contributing to diverse chronic inflammatory diseases. The inflammatory response acts by commencing the healing process and abolishing deleterious stimuli[1].

Therapy of inflammation is a matter of debate and is also insufficient since long. Multiple treatment modalities are available for the treatment of different inflammatory conditions consisting three major groups likely Corticosteroids, NSAIDs & DMARDs. These drugs yielded good results till now but they have got potential to produce serious adverse effects, sometimes even life-threatening events have resulted in widespread limitation of their use[2].

Some dipeptidyl peptidase 4 inhibitors[3], Angiotensin receptor blocker telmisartan[4,5], some adrenergic agonists[6] calcium channel blockers[7] and sulfonamides[8] have also been reported to possess anti-inflammatory activity in experimental studies. As these drugs are not completely devoid of adverse effects[2] there is a need to search for safer and better anti-inflammatory agents.

Azithromycin is a broad-spectrum macrolide antibiotic having bacteriostatic activity against many Gram-positive and Gram-negative bacteria including namely Hemophilus influenzae, Legionella pneumophilia, Chlamydia and Mycobacteria. Azithromycin has better pharmacokinetic profile of good tolerability, greater bioavailability and once a day administration[9] which is compatible with patient's compliance. Azithromycin not only possess

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

antimicrobial activity but also modify many components of the immune response[10].

According to a conclusion of 2005 Cochrane review, there is lack of sufficient evidence for the use of azithromycin and other macrolides in chronic asthma and hence additional studies are required in the same area in order to explore their anti-inflammatory potential[11].

Amongst the current perspectives to treat inflammation, azithromycin not only decreases infection but appears to be beneficial in decreasing the inflammation[12]. However, the mechanisms responsible for these actions are still unclear. There is a paucity of published literature regarding animal studies done to evaluate the anti-inflammatory activity of azithromycin, hence in the present study we planned to assess the anti-inflammatory activity of azithromycin in acute as well as sub-acute models of inflammation in Wistar rats.

Materials:

Experimental animals

Wistar rats of either sex weighing 200-250 grams were procured from the Central Animal House of the institution. The animals were housed under standard conditions and accommodated to 12h light/dark cycle for 10 days prior to experimentation day. They had free access to standard rat chow pellet and water ad libitum under strict hygienic conditions. The present study was approved by the IAEC (Institutional Animal Ethics Committee). CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) guidelines were strictly followed throughout the study

Drugs

Azithromycin was obtained in pure powder form Century Pharmaceuticals Ltd, Vadodara. Injection Ketamine (10ml vial of 50mg/ml) (Troikaa Pharmaceuticals Ltd), Aspirin (Reckitt Benckiser India Ltd), carrageenan and gum acacia powder were obtained from Central Pharmacy of KIMS, Karad

a. Azithromycin: 100 mg pure powder form was added in 10 ml of 1% gum acacia. Gum acacia was used as a suspending agent as azithromycin was poorly soluble in sterile water

b. Aspirin: The standard solution of aspirin was prepared by dissolving 325 mg of the dispersible tablet in 5 ml of sterile water.

c. Carrageenan was prepared as suspension (1% in 0.9% normal saline)[13].

Groups

The animals were divided into 3 groups. Each group consisted of 6 animals for acute as well as sub-acute model of inflammation. Total Wistar rats utilised for the study = 36.

Group	Treatment	Dose	Route
I	Gum Acacia	4ml/kg	Oral
II	Aspirin	200mg/kg [14]	Oral
III	Azithromycin	20mg/kg [15]	Oral

Methods:

1. Model of acute inflammation

It was done by Carrageenan induced rat paw edema[16]:

Mercury plethysmograph was used to evaluate anti-inflammatory activity by carrageenan induced rat paw edema model. A red ink mark was put on one paw of each animal at the level of lateral malleolus to enable uniform dipping at subsequent readings. One hour prior to the induction of edema, all the drugs were administered by oral gavage feeding needles to the respective groups. The paw edema was induced by injecting 0.05 ml of freshly prepared 1% suspension of carrageenan in normal saline intradermally into the plantar region of one of the hind paws of the rats. By mercury plethysmograph, the paw edema volume was measured in ml as the displacement of mercury at zero hour i.e., immediately after injecting carrageenan and the same procedure was repeated at 1, 2, 3, 4 and 5 hours. The actual edema volume was calculated by noting the difference between 0 hour and subsequent reading. The rise in mean paw edema is considered as a measure of inflammation and hence the potential to control this rise in edema as compared to the control group implies anti-inflammatory activity.

The percentage inhibition of edema for control and test groups was calculated by using the formula,

$$\frac{\text{Mean edema in control - mean edema in drug treated group}}{\text{Mean edema in control.}} \times 100$$

2. Sub-acute model of inflammation

It was carried out by foreign Body Induced Granuloma Method [17]:

All the drugs were administered to respective groups by oral gavage feeding needles on the first day of experiment. The rats were then anesthetized by ketamine (50mg/kg i.p)[18]. Under all aseptic precautions after clipping the hair over axillae, a small incision was given in bilateral axillae of the rats and after that two cotton pellets (sterilized by autoclaving at 120°C) weighing 10 mg each were implanted subcutaneously. Wounds were then sutured and animals were kept in clean cages individually after recovery from anaesthesia. On Day 1 of pellet implantation, the treatment was started and it was repeated once a day for the next ten days. The rats were sacrificed on eleventh day with an overdose of ketamine anaesthesia to remove the cotton pellets. After removing the extraneous tissue from the cotton pellets, the pellets were dried overnight at 60°C in hot air oven to note their dry weight. We calculated the net granuloma by recording the difference in the weight of the pellets which was recorded before and after the implantation. The mean dry weight of granuloma for study groups was calculated and expressed as mg/100 g of body weight of rat. The mean dry granuloma weight is taken as a measure of inflammation and thus the ability to decrease this mean dry granuloma weight when compared with control group implies anti-inflammatory activity.

The percentage inhibition of mean dry weight of granuloma for control and test groups was calculated using the formula,

$$\frac{\text{Mean dry weight of granuloma in control group - Mean dry weight of granuloma in drug treated}}{\text{Mean dry weight of granuloma in the control group}} \times 100$$

Statistical analysis:

The data analysis was carried out by one way ANOVA followed by post hoc Dunnett's test. Bonferroni's test was done to compare azithromycin and aspirin group. All the statistical methods were carried out through the software Graph pad Instat version 3.06 and $p < 0.05$ was considered statistically significant.

Results:

Acute inflammation (Carrageenan induced paw edema method):

The mean paw edema volumes measured using plethysmograph in 'ml' as mercury displacement for control, aspirin and azithromycin groups are shown in (Table 1 and Graph 1).

Aspirin (200mg/kg) as well as azithromycin (20mg/kg) showed statistically significant inhibition of paw edema volume as compared to control group ($p < 0.05$). Further anti-inflammatory activity of Azithromycin was compared with anti-inflammatory activity of aspirin. It was found that anti-inflammatory effect of Azithromycin was comparable to aspirin at 5hr ($p > 0.05$) (Table 1).

Percentage inhibition of paw edema of aspirin and azithromycin are mentioned in (Table 1and Graph 2).

There is no significant inhibition of paw edema with any of the study drugs at 1hr interval.

Sub-acute inflammation (Foreign body induced granuloma method):

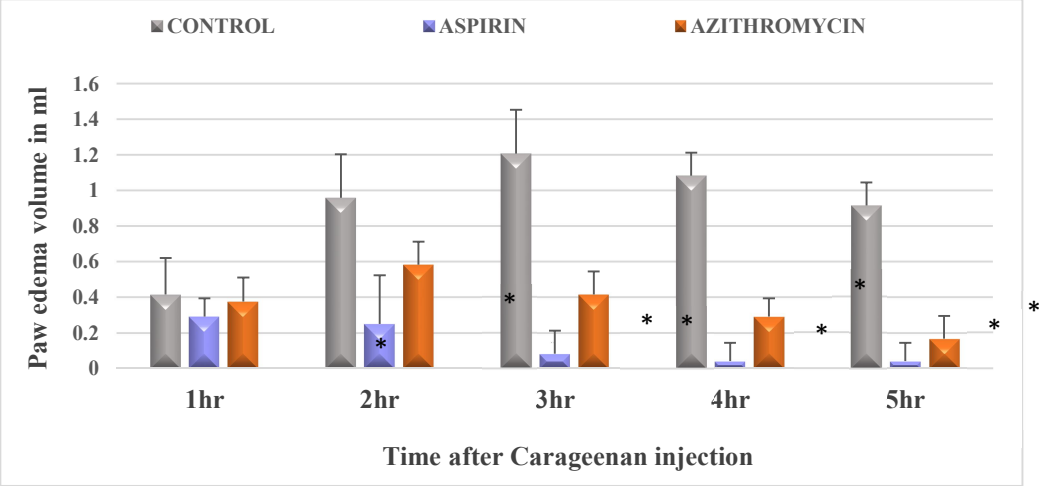
The mean dry weight of ten-day old granuloma, expressed as (mg/100 g) body weight of rat as well as percentage inhibition of mean dry granuloma weight in control, aspirin and azithromycin groups are shown in (Table 2, Graph 3 and Graph 4)

Azithromycin treated group exhibited statistically significant decrease in granuloma dry weight ($p < 0.05$) when compared to control. Further mean granuloma dry weight of Azithromycin was compared with mean granuloma dry weight of aspirin group. There was no statistically significant difference in mean granuloma dry weight of Azithromycin group when compared to mean granuloma dry weight of aspirin group ($p > 0.05$). It shows that the anti-inflammatory activity of Azithromycin was comparable to aspirin in sub-acute model of inflammation (Table 2).

Time after carrageenan injection	Control (4ml/kg) Paw edema in ml (±SD)	Aspirin (200mg/kg)		Azithromycin (20mg/kg)		ANOVA	
		Paw edema in ml (±SD)	Percentage inhibition (%)	Paw edema in ml (±SD)	Percentage inhibition (%)	F value	*p* Value
1 hr	0.416±0.204	0.291±0.102	30.95	0.375±0.136	11.90	1.029	0.3811
2 hr	0.958±0.245	0.250± 0.273 *	73.95	0.583±0.129*, #	39.58	14.863	0.0003
3 hr	1.208±0.245	0.083± 0.129 *	93.33	0.416±0.129*, #	65.83	64.111	<0.0001
4 hr	1.083±0.129	0.041±0.102 *	96.29	0.291±0.102*, #	73.14	141.94	<0.0001
5hr	0.916±0.129	0.041±0.102 *	95.65	0.166±0.129*	82.60	92.143	<0.0001

Table 1: Effect of Control, Aspirin and Azithromycin treatments on carrageenan induced paw edema
Post hoc analysis: - by Dunnett's Test: *$p < 0.05$; by Bonferroni's test: # $p < 0.05$

Graph 1: Effect of various treatments on carrageenan induced paw edema volume



Graph 2: Percentage inhibition of carrageenan induced paw edema at different time interval

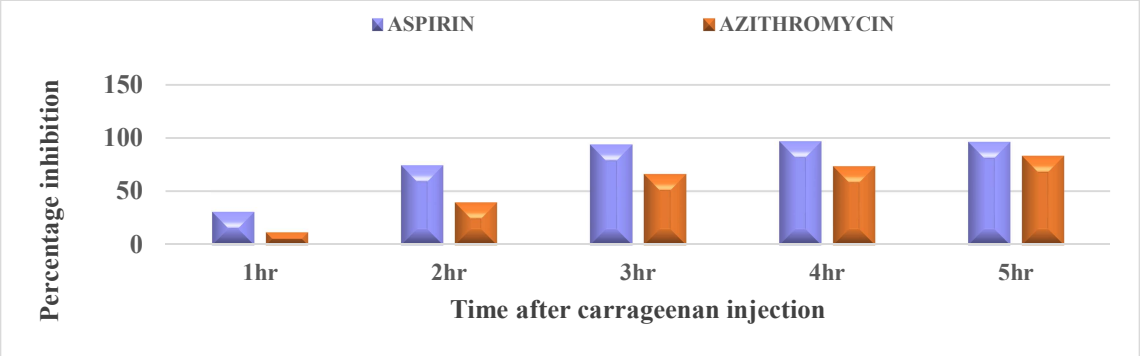


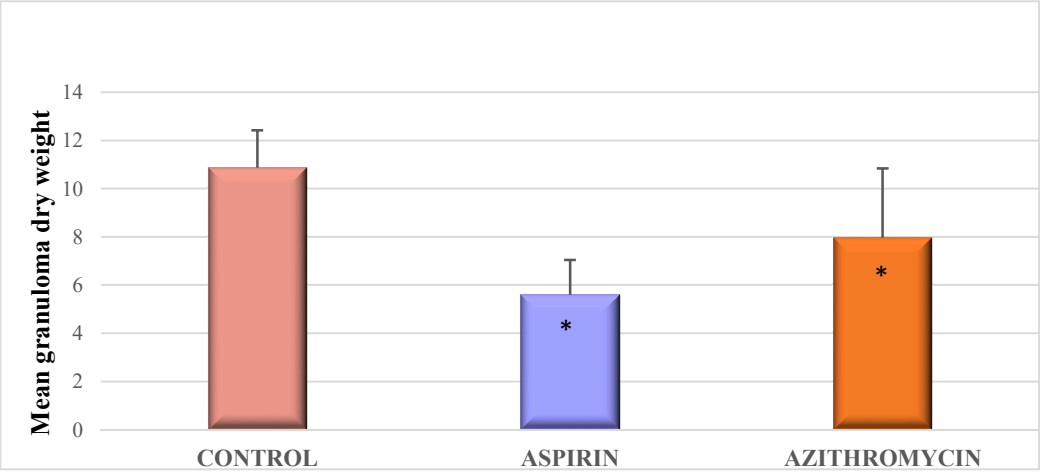
Table 2: Effect of Control, Aspirin and Azithromycin treatments on granuloma dry weight

S. No	Drug Treatment	Mean granuloma dry weight mg/100g body weight (Mean ± SD)	Percentage inhibition (%)
1.	CONTROL	10.86 ± 1.555	____
2.	ASPIRIN (200mg/kg)	5.60 ± 1.436*	48.43
3.	AZITHROMYCIN (20mg/kg)	7.96 ± 2.884 *	26.70

ANOVA: F = 9.744, p =0.0019

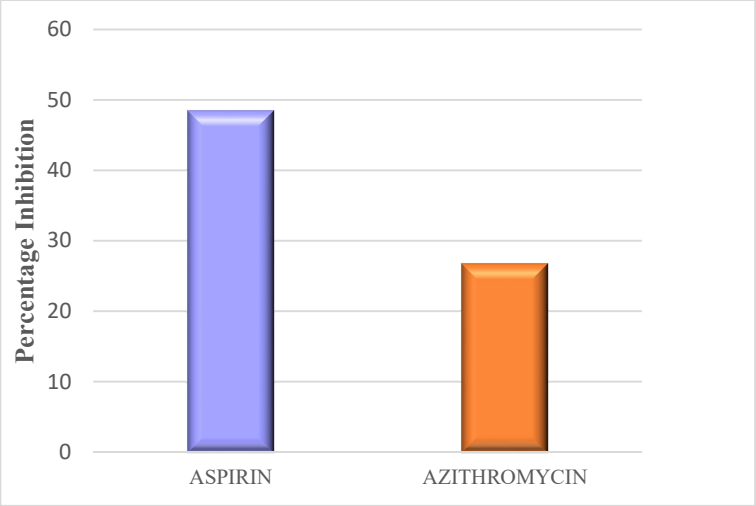
Post hoc analysis: - by Dunnett's Test: *p < 0.05; by Bonferroni's test: p > 0.05

Graph 3: Effect of aspirin and azithromycin on mean granuloma dry weight compared to control



PMR P ISSN: 0975-0533, E ISSN: 0976-0164

Graph 4: Percentage inhibition of granuloma dry weight



Discussion

Acute inflammation (Carrageenan induced paw edema method):

Carrageenan-induced hind paw edema comprises of biphasic response in which the first phase response is related to the release of serotonin, histamine and kinins, whereas the second phase response is related to prostaglandins and slow reacting substances release[19]. We estimated the mean paw volume in acute inflammatory model. The reduction in mean paw volume after inflammation induction was considered as anti-inflammatory activity. In this model, it was found that azithromycin (20mg/kg) has significant anti-inflammatory activity in acute model of inflammation when compared to control group. The anti-inflammatory activity of azithromycin was comparable at 5 hours when compared to aspirin (200mg/kg). This indicates that azithromycin might have delayed anti-inflammatory effect beyond 5 hours. The most plausible mechanisms of azithromycin in acute model of inflammation could be due to the inhibition of synthesis of histamine, serotonin prostaglandins, and other inflammatory mediators[12,13,20]

There is no significant inhibition of paw edema with any of the study drugs at 1hr interval. This might be either due to inadequate progression of inflammation at 1 hr or due to delayed onset of action of study drugs or by both the mechanisms. According to Winter et al.[16], after injecting the phlogistic agent into the hind paw of rat, paw edema reaches a peak in 3-5hrs and then the same degree of edema is retained for few hours. This might explain that sufficient inflammation was not produced at 1hr after injecting carrageenan and hence the activity of azithromycin was comparable to aspirin at 1hr. As the inflammation progressed from 2nd hour, the individual drugs effects were also seen in the form of suppression of the edema till the 5th hour.

Sub-acute inflammation (Foreign body induced granuloma):

Foreign body induced granuloma model comprises of response phases of transudative phase, exudative phase and proliferative phase. The increase in dry weight of the granuloma is the measure of proliferative phase[21]. We evaluated mean dry granuloma weight. Reduction in mean dry granuloma weight was considered as anti-inflammatory action. In this model, it was found that azithromycin (20mg/kg) caused significant decrease in granuloma dry weight when compared to control and also its anti-inflammatory effect was comparable with aspirin (200mg/kg). Significant anti-inflammatory activity of azithromycin in sub-acute model of inflammation could be due to promotion of apoptosis of neutrophils or inhibition of fibroblast proliferation[12,22].

Observations of the present study are in agreement with Gosavi et al.[12] and Scaglione et al.[20] stating that azithromycin have anti-inflammatory activity using various animal models of inflammation. According to Gosavi et al.[12], they didn't get any significant anti-inflammatory activity in cotton pellet induced granuloma model whereas we found

azithromycin to be effective in decreasing the mean granuloma dry weight. In view of results obtained from our study, we propose that azithromycin have anti-inflammatory activity. Moreover, it has better pharmacokinetic profile making it a potential new anti-inflammatory drug for inflammatory conditions associated with acute infections. As this is an animal study and animal data cannot be directly hypothesized on humans, well planned human studies are required further to support these findings in order to disclose the role of azithromycin in inflammation and in the treatment of asthma, COPD, bronchiolitis, bronchiectasis and osteomyelitis.

A recent study performed in WT Mice proposed that azithromycin therapy reduces cardiac inflammation post myocardial infarction by downregulating the pro-inflammatory cytokines and upregulating the anti-inflammatory cytokines, reduces neutrophil counts after cardiac ischemia through enhancing apoptosis and improves cardiac recovery by mitigating adverse cardiac remodeling[23].

Conclusion

From the findings of the present experimental study, we conclude that Azithromycin has significant anti-inflammatory activity as compared to control and modest anti-inflammatory activity in animal model of acute inflammation as compared to aspirin. Azithromycin has significant and modest anti-inflammatory activity in sub-acute model of inflammation as compared to control and aspirin respectively. The present study predicts that the use of azithromycin either as monotherapy or along with the conventional medications may have an added benefit of anti-inflammatory activity in various inflammatory disorders. However, we suggest that azithromycin should not be given in chronic inflammatory conditions as long term use in chronic conditions will lead to development of resistance but it will be beneficial to treat acute infections developed in chronic inflammatory conditions. Hence, we suggest that azithromycin along with its anti-infective, immunomodulatory and additional anti-inflammatory property might be more suitable for patients in diseased conditions where there is infection associated with inflammation like COPD, asthma, chronic sinusitis, bronchiolitis, bronchiectasis, cystic fibrosis and osteomyelitis.

Acknowledgement:

We would like to acknowledge Century Pharmaceuticals Ltd, Vadodara., Gujarat, for providing drugs in pure powder form. We acknowledge the support given by the Central animal house of the institution and the technical staff of Department of Pharmacology during study.

References:

1) Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease. 9th ed. Philadelphia: Saunders An imprint of Elsevier; 2015.
2) Brunton L, Chabner BA, Knollmann BC. Goodman and Gilman's the pharmacological basis of therapeutics. Twelfth.
3) Kagal UA, Angadi NB, Matule SM. Effect of dipeptidyl peptidase 4 inhibitors on acute and subacute models of inflammation in male Wistar rats: an experimental study. International Journal of Applied and Basic Medical Research. 2017 Jan;7(1):26.
4) Matule SM, Hogade AP, Kangle RP, Torgal SS, Kothari N. Effect of telmisartan on acute model of inflammation in male Wistar rats: an experimental study. Int J Res Med Sci. 2016;4:135e138.
5) Matule, S., Hogade, A., Kangle, R., B., N., & Kolla, R. (2016a). Effect of telmisartan on sub-acute model of inflammation in male Wistar rats - an experimental study. International Journal of Research in Medical Sciences, 1988–1992. https://doi.org/10.18
6) Green KL. The anti-inflammatory effect of catecholamines in peritoneal cavity and hind paw of mouse. Br J Pharmacol 1972;45(2):322-32.
7) Shrivastava VK, Saxena KK, Gupta B. Calcium channel blockers in acute inflammation. Indian J Exp Biol 1988;26(1):70-1.
8) Hiremath SV, Gouripur VV, Patil PA. The anti-inflammatory activity of some sulphonamides in albino rats. Ind J Med Res 1996;103:120-5.
9) Ungureanu V.: Macrolides, lincosamides, streptogramines (MLS):mechanisms of action and resistance. Bacteriol Virusol Parazitol Epidemiol., 55, 131, 2010.

10) Altenburg J. et al.: Immunomodulatory effects of macrolide antibiotic-part 1: biological mechanisms. Respiration, 81, 67, 2011
11) Richeldi L, Ferrara G, Fabbri LM, Lasserson TJ, Gibson PG. Macrólidos para el asma crónica (revisión Cochrane traducida). En: La Biblioteca Cochrane Plus. 2007(4).
12) Gosavi PA, Jaju JB, Ubale VM, Pawar GR, Dharmadhikari SC. Study of evaluation of anti-inflammatory activity of macrolide antibiotics in rats: an experimental study.
13) Morris CJ. Carrageenin induced paw edema in the rat and mouse. In: Winyard PG, Willoughby DA, editors. Inflammation Protocols. UK: Humana Press; 2003: 115-21.
14) Laurence DR, Bacharach AL. Evaluation of Drug Activities:Pharmacometrics Vol. 2. New York and London: Academic Press Inc; 1964.
15) Gajendra NJ, Madhavi E, Konda VG, Venkata RY, Rajeshwaramma G. Comparative study of anti-inflammatory activity of newer macrolides with etoricoxib. Journal of Evolution of Medical and Dental Sciences. 2014 Mar 10;3(10):2413-20.
16) Winter CA, Risley EA, Nuss GW. Carrageenan induced edema in hindpaw of the rat as an assay for anti-inflammatory drugs. Proc Soc exp Biol Med. 1962;111:544-7.
17) Meier R, Schuler W, Desaulles P. Zur Frage des mechanismus der hemmung des Bindegewebswachstums durch cortisone. Experientia 1950;6:469-71.
18) Karwacki Z, Kowiański P, Moryś J. General anaesthesia in rats undergoing experiments on central nervous system. Folia morphologica. 2001;60(4):235-42.
19) Brahmbhatt MR, Patel JM, Patel VB, Saluja AK.Analgesic and anti-inflammatory activity of leaves of rivea hypocrateriformis. J Pharm Phytother. 2010;1(1):001-3.
20) Scaglione F, Rossoni G. Comparative anti-inflammatory effects of roxithromycin, azithromycin and clarithromycin.J Antimicrob Chemother. 1998;41 Suppl B:47-50.
21) Swingle KF, Shideman FE. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents.J Pharmacol Exp Ther. 1972;183(1):226-34.
22) Kanoh S, Rubin BK. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clinical microbiology reviews. 2010 Jul;23(3):590-615.
23) Al-Darraji A, Haydar D, Chelvarajan L, Tripathi H, Levitan B, Gao E, Venditto VJ, Gensel JC, Feola DJ, Abdel-Latif A. Azithromycin therapy reduces cardiac inflammation and mitigates adverse cardiac remodeling after myocardial infarction:potential therapeutic targets in ischemic heart disease. PloS one. 2018 Jul 12;13(7):e0200474.