

INFLUENCE OF TETRACYCLINES ON INFLAMMATION AND THEIR INTERACTION WITH ASPIRIN IN MALE WISTAR RATS.

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Summary

Oxytetracycline (OXC), tetracycline (TRC), Doxycycline (DXC) and Minocycline (MNC) in their therapeutic equivalent doses significantly ($p < 0.01$) suppressed both acute as well as sub-acute inflammation. And the suppression was almost comparable to that of aspirin. DXC and MNC in their sub anti-inflammatory (SAI) dose when co-administered with SAI dose of aspirin produced synergistic anti-inflammatory activity without significant gastric toxicity in sub-acute model of inflammation. The results indicate that DXC or MNC when used concurrently with aspirin might reduce the anti-inflammatory dose and there by decrease the gastric toxicity.

Keywords: Aspirin, Inflammation, Interaction, Tetracycline

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Introduction

Intensive investigations have revealed that a number of agents, other than conventional anti-inflammatory drugs favourably influence the process of inflammation. Some such drugs reported to possess anti-inflammatory activity include ascorbic acid¹, calcium salts², calcium channel blockers³, and enzymes like chymotrypsin⁴, etc. In addition to these, some of the anti-microbials like sulfonamides⁵, antifungal agents⁶ and macrolide antibiotics⁷ have also been reported to possess anti-inflammatory activity. Similarly minocycline, a broad-spectrum antibiotic has been reported to favourably interact with NSAIDs as well as steroids and provide relief in rheumatoid arthritis.⁸ Logically such antimicrobials could suppress inflammation due to microbial infections, but it is surprising to observe their anti-inflammatory activity in non-infective inflammatory conditions.

However like minocycline, tetracycline failed to provide significant benefit for patients with rheumatoid arthritis.⁹ Since the mechanism involved in pathogenesis of acute and chronic inflammation are different and there are scanty reports regarding anti-inflammatory activity of minocycline and other tetracyclines in acute inflammation. The present study was therefore planned to investigate the influence of oxytetracycline (OXC), tetracycline (TRC), doxycycline (DXC) and minocycline (MNC) on experimentally induced acute and subacute inflammation in albino rats and also to explore their interaction with aspirin, a commonly used non-steroidal anti-inflammatory drug (NSAID).

Materials and Methods:

Wistar rats of either sex weighing between 150 ± 20 grams were acclimatized to the laboratory under 12:12 light dark cycle for a week with free access to standard diet (Amrut Brand) and water. The animals were divided into various groups (n=6, in each) to receive various treatments. Ethical clearance was obtained from Institutional Animal Ethical Committee constituted as per CPCSEA guidelines. For anti-inflammatory activity two models of inflammation *viz.* carrageenan induced paw edema (acute inflammation) and foreign body (grass pith and cotton pellet) induced granuloma formation (sub acute inflammation) were employed.

Acute inflammation was produced by injecting 0.05ml of 1% carrageenan in one of the hind paw as described by Winter *et al*¹⁰. Modified standard technique of D'Arcy

as described in earlier literature¹¹ was employed to induce sub acute inflammation by implanting 2 sterile cotton pellets (10 mg each) and 2 sterile grass piths (25x3mm) sub-cutaneously in axillae/groin randomly under light ether anaesthesia with strict aseptic precautions.

Considering the maximum human therapeutic equivalent dose, rat doses for each was calculated using Paget and Barnes¹² conversion table. The doses used were 200mg/kg of aspirin, 180mg/kg of oxytetracycline, 180mg/kg of tetracycline, 18mg/kg of doxycycline and 18mg/kg of minocycline. The sub-anti-inflammatory (SAI) were determined to be 54mg/kg of aspirin, 13mg/kg of doxycycline and 13mg/kg of minocycline. In sub acute study aspirin interaction with tetracycline or oxytetracycline were not considered as both (TRC and OXC) had short half life requiring repeated administration and also their bioavailability is only 60-70% after oral administration as compared to nearly 100% for doxycycline and minocycline.

In acute inflammation studies, 30min prior to carrageenan injection aspirin, oxytetracycline, tetracycline, minocycline, doxycycline in their therapeutic equivalent doses individually and SAI of doxycycline (13 mg/kg) or minocycline (13mg/kg) with that of aspirin (54mg/kg) together were administered orally in a single dose to different groups (n=6, in each) of animals in a volume of 10ml/kg. In subacute studies all the treatments were repeated every 24hrs for 10 days. Control animals in both models of inflammation received equal volume of 1% gum acacia suspension, per orally.

Carrageenan injected paw volume was measured at 0 (immediately after injection), 1, 3 & 5 hours after injection in all groups with help of plethysmometer (mercury displacement) and the actual edema volume was calculated by subtracting initial (0 hr) reading from subsequent corresponding readings. The percentage inhibition of paw edema was calculated using formula

$$\text{Percentage inhibition} = 100(1-V_t/V_c)$$

V_c - edema volume in control while V_t - edema volume in treated groups.

The foreign body implanted animals were sacrificed on day 11 by over anaesthesia to dissect out the foreign body granulomas, stomachs and adrenals. Ten day old cotton pellet granulomas were dried over night at 60°C in an incubator to note their dry weight and the same was expressed as mg/100gm body weight as suggested by Dipasquale & Meli¹³. Grass pith induced granulomas were preserved in 10% formalin for their histopathological studies. The stomach of control as well as treated animals

were opened along with greater curvature and after gently cleaning with normal saline the ulcer index was calculated as described by Gupta *et al.*¹⁴ The preserved granulomas were sectioned and stained with hematoxylin and eosin (H&E) for microscopic quantification of granulation tissue. Adrenal glands free from extraneous tissue were weighed immediately and the group mean of each treated group were calculated and compared to that of control group.

Statistical analysis: The results were statistically analysed by ANOVA followed by Dunnet's posthoc test and $p \leq 0.05$ was considered as significant.

Results

In acute model of inflammation, as expected aspirin showed significant ($p < 0.01$) anti-inflammatory activity from 1st hour onwards and so did TRC and MNC. The anti-inflammatory activity of OXC and DXC was observed from 30 minutes onwards indicating their quicker onset of action and the action was almost comparable to that of aspirin. (Table 1, Fig 1)

Combination treatment with sub anti-inflammatory doses of DXC or MNC with that of aspirin also showed significant ($p < 0.01$) anti-inflammatory activity, indicating mutual synergistic activity. (Table 1, Fig 2)

Fig 1. Effect of individual drugs on carrageenan induced paw edema

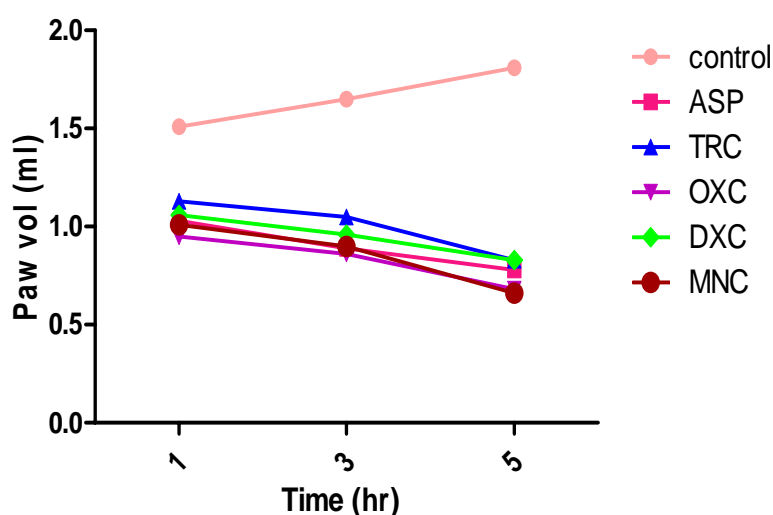
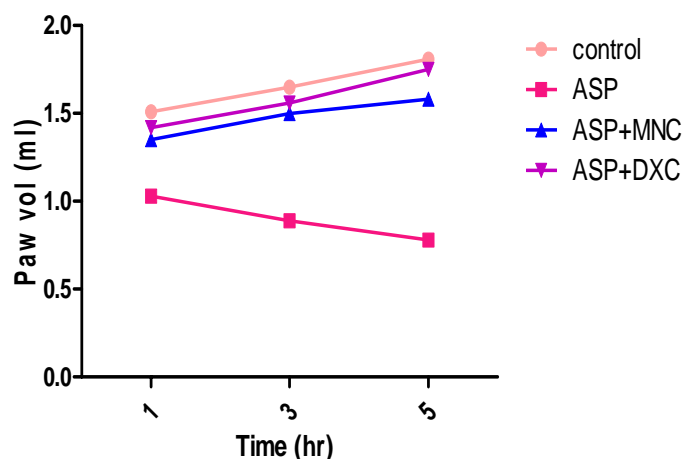


Table 1. Effect of various treatments on inflammation, ulcer index and adrenal weight.

Group	Drug (mg/kg)	Paw volume (ml) Mean \pm SEM			Granuloma dry wt (mg/100g body wt)	Ulcer index (Mean \pm SEM)	Adrenal wt (mg/100g body wt)
		1 st hr	3 rd hr	5 th hr			
1	Vehicle (control)	1.51 \pm 0.05	1.65 \pm 0.03	1.81 \pm 0.07	19.87 \pm 0.34	10.5 \pm 7.51	9.8 \pm 0.4
2	Aspirin(200)	1.03 \pm 0.03*	0.89 \pm 0.03*	0.78 \pm 0.04*	10.71 \pm 0.31**	35 \pm 0.0*	6.6 \pm 0.38**
3	Tetracycline(180)	1.13 \pm 0.04*	1.05 \pm 0.03*	0.83 \pm 0.06*	12.32 \pm 0.33**	9.7 \pm 6.14*	7.5 \pm 0.35*
4	Oxytetracycline(180)	0.95 \pm 0.04*	0.86 \pm 0.05*	0.68 \pm 0.05*	10.88 \pm 0.34**	5.3 \pm 5.33*	6.7 \pm 0.33**
5	Doxycycline (18)	1.06 \pm 0.02*	0.96 \pm 0.03*	0.83 \pm 0.03*	14.14 \pm 0.35**	9.51 \pm 6.03*	7.6 \pm 0.35*
6	Minocycline (18)	1.01 \pm 0.03*	0.9 \pm 0.03*	0.66 \pm 0.33*	11.63 \pm 0.39**	6.0 \pm 6.00	7.1 \pm 0.34**
7	ASP + MNC (54+13)	1.35 \pm 0.02*	1.5 \pm 0.04*	1.58 \pm 0.03*	12.49 \pm 0.26**	10.0 \pm 6.34*	7.3 \pm 0.34**
8	ASP + DXC (54+13)	1.42 \pm 0.03*	1.56 \pm 0.03*	1.75 \pm 0.05*	18.08 \pm 0.4*	10.0 \pm 6.34*	8.1 \pm 0.42*

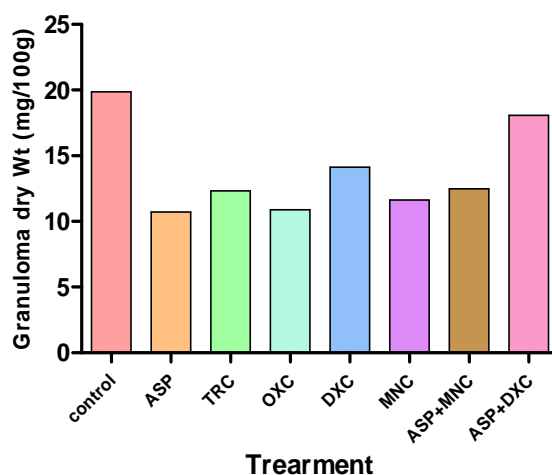
* $p < 0.01$, ** $p < 0.001$ when compared to controls.

Fig 2. Effect of combination treatment on carrageenan induced paw edema



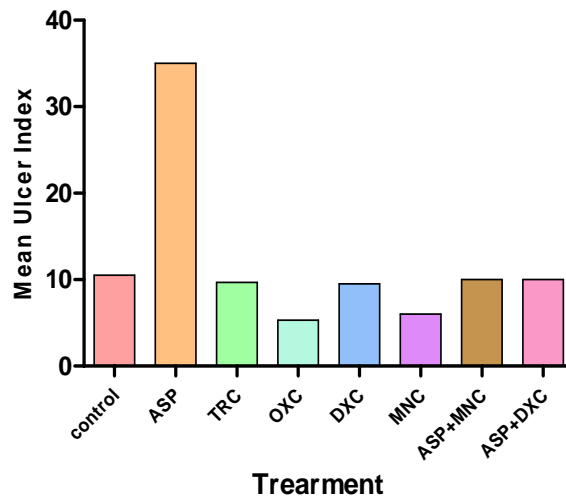
In foreign body induced inflammation (sub acute model) all the tetracyclines individually, as well as sub-anti-inflammatory dose combination of MNC or DXC with that of aspirin showed significant ($p < 0.01$, $p < 0.001$) anti-inflammatory activity as denoted by significant reduction in granuloma formation. (Table 1, Fig 3)

Fig 3. Effect of various treatments on granuloma dry weight.



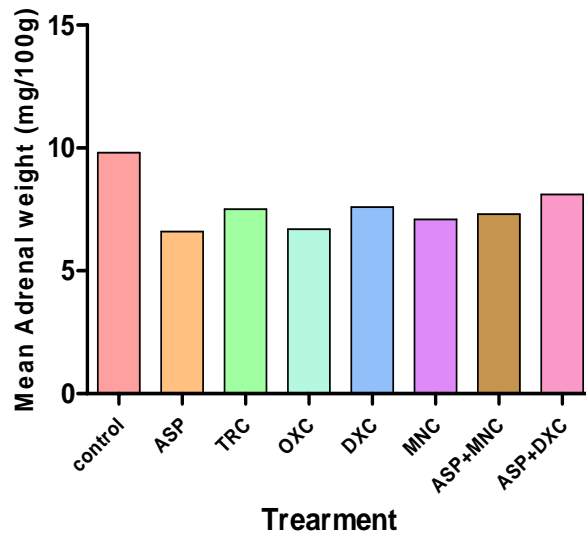
Compared to control animals aspirin treated rats showed significantly ($p < 0.01$) higher gastric ulcer index, while the gastric ulcer index in rats treated with various tetracyclines individually or their combination with aspirin did not significantly differ from that of control animals. (Table 1, Fig 4)

Fig 4. Effect of various treatments on Ulcer Index.



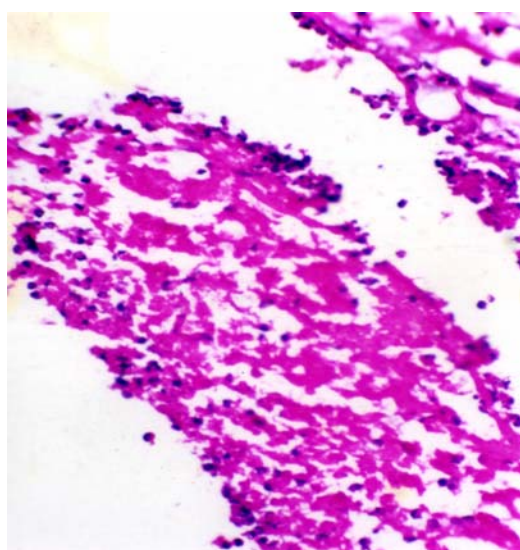
The adrenal weights of treated groups with various tetracyclines individually and their combination with aspirin showed significant ($p < 0.01$, $p < 0.001$) decrease as compared to that of control. (Table 1, Fig 5)

Fig 5. Effect of various treatments on Adrenal weight.

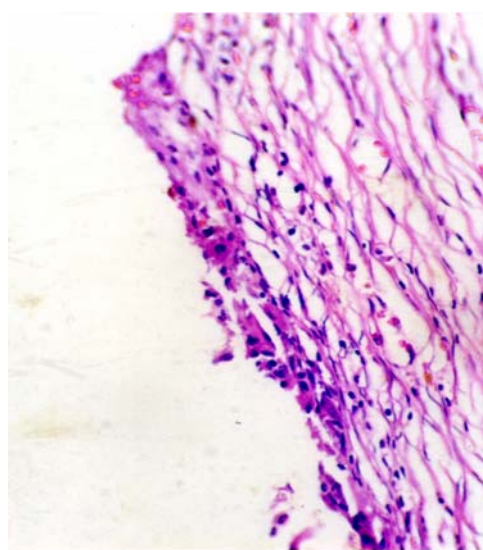


Histological studies of granulation tissue revealed decrease in fibroblasts, collagen content, etc. in various tetracycline treated groups and similar changes were observed in the group treated with SAI dose of DXC or MNC with SAI dose of aspirin, confirming the anti-inflammatory activity. (Fig 6)

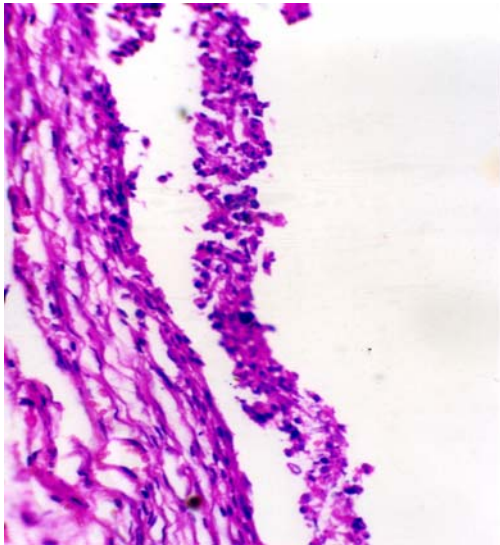
Fig 6. Microphotographs of granulation tissue stained with H & E (400X)



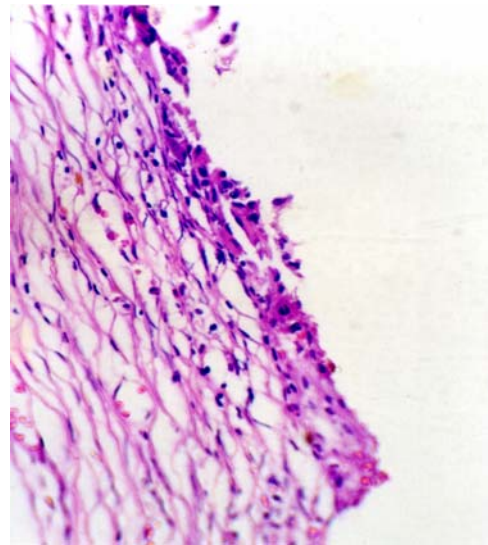
a. Control



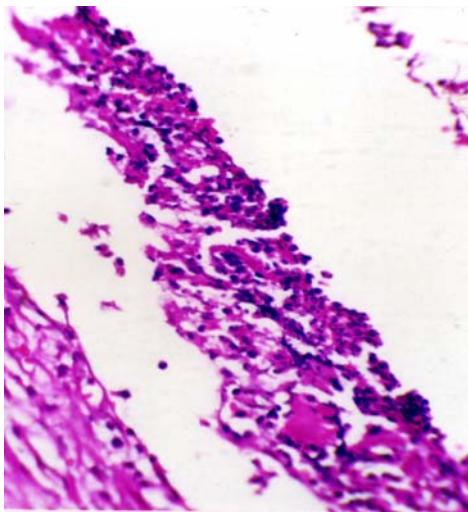
b. Aspirin



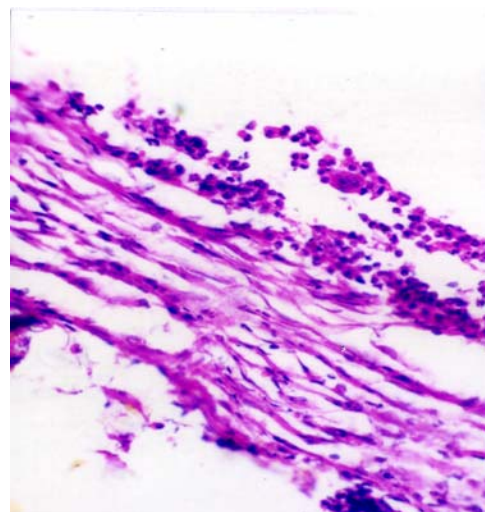
C. Tetracycline



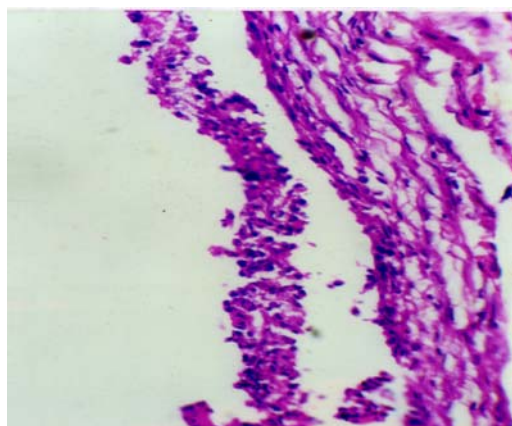
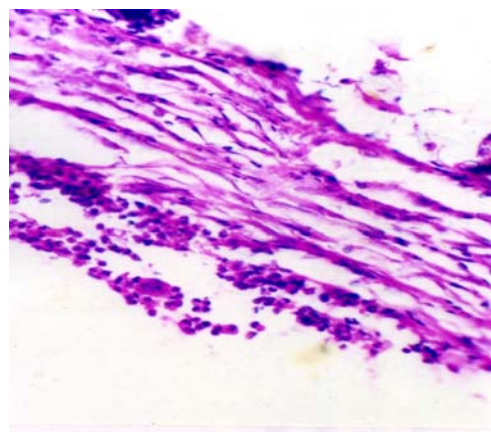
d. Minocycline



e. Doxycycline



f. Oxytetracycline



g. Aspirin + Doxycycline

h. Aspirin + Minocycline

Discussion

Observed anti-inflammatory activity of tetracyclines in the present study is almost comparable to that of aspirin. Anti-inflammatory activity of MNC as observed in the present study agrees with earlier clinical reports.^{8,15,16} However, Skinner et al.⁹ failed to demonstrate anti-inflammatory activity of TRC in the dose of 250mg daily for one year in rheumatoid arthritis patients. The failure of treatment could be due to lower dose of TRC. The information regarding the influence of DXC, OXC and the interaction of these tetracyclines with aspirin (on inflammation) is not well documented.

Though the exact mechanism by which tetracyclines produce anti-inflammatory action is not known, Harold (1995)¹⁷ proposed anti-microbial (antimycoplasma) activity of MNC to explain its anti-rheumatoid activity. Other proposed mechanisms involved include, superoxide scavenging¹⁸ ; inhibition of metalloproteinases like collagenase, gellatinase and others¹⁹, phospholipase A₂ –inhibition, suppression of T-lymphocytes as well as inhibition of cytokine production²⁰ ; and decreased leukotaxis²¹.

Sub anti-inflammatory dose of aspirin with subtherapeutic doses of various tetracyclines used in the present study showed mutual synergistic anti-inflammatory activity, without significant ulcerogenicity of gastric mucosa. Such interactions are scantily reported in the literature. Decreased adrenal weights in aspirin; in various tetracyclines treatment individually and in the combination of DXC or MNC with aspirin treated groups indicate their anti-inflammatory activity at least in part could be mediated through the endogenous steroids. The nature of interactions appears to be of pharmacodynamic rather than pharmacokinetic, since no pharmacokinetic interaction between aspirin and tetracyclines has been identified.

Mutual synergistic anti-inflammatory activity of aspirin and tetracyclines as observed in the present study if could be extrapolated to clinical situation indicates that patients on tetracycline therapy require lower dose of aspirin to suppress the inflammation.

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Reference

1. Spillert CR, Spillert KR, Hollinshead MB, Lazaro EJ. Inhibitory effect of high dose ascorbic acid on inflammatory edema. *Agents action* 1989; 27(3-4): 401-2.
2. Binder LS. Acute arthropod envenomation: incidence, clinical features and management. *Med Toxicol Adverse Drug Exp* 1989; 4: 163-73.
3. Shrivastava VK, Saxena KK, Gupta B. Calcium channel blockers in acute inflammation. *Indian J Exp Biol* 1988; 26: 70-1.
4. David A Cooney, Richard J Rosen. Enzymes as a therapeutic agents. Chapter 40, *Advanced Pharmacology*, vol 12, Sivio Garatini and A Gold, E Hawking T Kopin, academic press: Newyork, Sanfrancisco 1975: 266-9.

5. Hiremath SV, Gouripur VV and Patil PA. The anti inflammatory activity of some sulphonamides in albino rats. *Ind J Med Res* 1996; 103: 120-5.
6. Puig Parallada P. Analgesics, anti-inflammatory and oxyradical scavenger activity of imidazole derivatives. *Int J Tissue Reac* 1985; 7(6): 507-12.
7. Ianaro A, Ialenti A, Maffia P, Sautebin L, Rombolà L, Carnuccio R, et al. Anti inflammatory activity of macrolide antibiotics. *J Pharmacol Exp Ther* 2000; 292: 156-63.
8. Tilly BC, Alarcon GS, Heyse SP, et al. Minocycline in Rheumatoid Arthritis. *Ann Intern Med* 1995; 122(2): 81-9.
9. Skinner M, Cathcart ES, Mill JA, Pinols RS. Tetracycline in the treatment of rheumatoid arthritis, A double blind controlled study. *Arthritis Rheum* 1971; 14: 727-32.
10. Winter CA, Risely EA, Nuss GW. Carrageenan induced oedema in hind paw of rat as an assay for anti inflammatory drugs. *Prock Soc Exp Biol Med* 1962;111: 544.
11. Ch. Kasi V, Patil PA. Enhancement of anti-inflammatory activity of aspirin by verapamil. *Indian J Med Res(B)*98 1993 Feb: 34-6.
12. Paget GE, Barnes JM. Toxicity tests. In : Laurence DR and Bacharach AL, Evaluation of drug activities pharmacometrics. London and New York; Academic Press, 1964.
13. Dipasquale G, Mali A. Effect of body weight changes on the formation of cotton pellet induced granuloma. *J Pharm Pharmacol*, 1965; 17: 379-82.
14. Gupta MB, Nath R, Gupta GB, Bhargava KP. Role of opioid receptors in stress induced gastric ulceration in rat. *Ind J Med Res* 1986; 83: 532-35.
15. Kloppenburg M, Breedveld FC, Terwiel JP, Mallee C, Dijkmans BA. Minocycline in active rheumatoid arthritis, A double blind placebo controlled trial. *Arthritis and Rheumatism* 1994; 37: 629-36.
16. Sebastian GC, Slots J, Reynolds HS, Zambon JJ and McKenna JD. The effect of short administration of minocycline HCl on gingival inflammation and subgingival microflora. *J of Periodontol* 1982; 53(9): 557-61.
17. Harold EP. Minocycline treatment of rheumatoid arthritis. *Ann Int Med* 1995; 122(2): 147-8.
18. Van Barr HM, van de Kerkhof PC, Mier PD, Happle R. Tetracyclines are potent scavengers of the superoxide radical. *Br J Dermatol* 1987; 117(1): 131-2.
19. Greenwald RA, Moak SA, Ramamurthy NS, Golub LM. Tetracycline suppress matrix metalloproteinase activity in adjuvant arthritis and in combination with flurbiprofen, ameliorate bone damage. *J Rheumatol* 1992; 19(6): 927-38.
20. Pruzanski W, Greenwald RA, Street JP, Laliberfe F, Stefanski E, Veda P. Inhibition of enzymatic activity of phospholipase A₂ by minocycline and doxycycline. *Biochem Pharmacol* 1992; 44(6): 1165-70.
21. Veda P, Pruzanski W, Kim J and Fornasier V. The pro-inflammatory effect of intra articular injection of soluble human and venom phospholipase A₂. *Am J Pathol* 1989; 134: 807-11.