

**INFLUENCE OF CALCITRIOL & CALCIUM  
GLUCONATE ON WOUND HEALING – AN  
EXPERIMENTAL STUDY**

<sup>1</sup> Mathew T <sup>2</sup> Patil PA <sup>3</sup> Singh KR

<sup>1</sup> Dept of Pharmacology, KLE College of Pharmacy,  
Belgaum-590010. India.

<sup>2</sup> Dept of Pharmacology, J.N.Medical College, Belgaum  
-590010.India

<sup>3</sup>Dept of Pharmacology, T.N.Medical College & B.Y.L  
Nair Ch.Hospital, Mumbai-400008.India.

**Summary**

Calcitriol, an active metabolite of vitamin D<sub>3</sub> is known to influence calcium metabolism leading to hypercalcemia. It has been reported that calcitriol also possesses antiproliferative activity which may influence wound healing . Due to paucity of information about the action of calcitriol on wound healing , the present study was planned & calcium gluconate & calcitriol were investigated for their action in excision, resutured incision & dead space wounds in male Wistar rats. It was observed that calcitriol impeded healing significantly in all the three wound models as compared to olive oil controls whereas calcium gluconate enhanced healing in all the three wound models significantly as compared to normal saline controls.

Key words: Calcitriol, Calcium gluconate, Wound Healing

\*Corresponding author:

Patil P.A.,

Professor,

Dept of Pharmacology & Pharmacotherapeutics,

J N Medical College, Belgaum-590010, Karnataka,

India.

Phone: 0831- 24091828, Fax: 08312470759.

Email: [drpapatil@yahoo.co.in](mailto:drpapatil@yahoo.co.in)

### **Introduction**

Wound is one of the commonest clinical conditions met with in day to day practice. The principle of treatment has always been to achieve early healing without complications. Progress made in allied medical sciences viz physiology, pathology, biochemistry, immunology has contributed for better understanding of normal & abnormal wound healing.

The process of wound healing follows a certain sequence i.e. initial inflammatory phase, proliferative phase & remodeling phase<sup>1</sup>. Various factors like nutrients, vitamins like ascorbic acid<sup>2</sup>, vitamin D<sup>3</sup>, oxygen tension at the site<sup>4,5</sup>, promote wound healing; whereas a large number of drugs like 5-fluorouracil, colchicine & cyclophosphamide<sup>6,7</sup> retard wound healing. Anti-inflammatory drugs<sup>8</sup> also interfere with cell proliferation<sup>6,7</sup>. Agents inhibiting collagen synthesis also retard the healing process<sup>9</sup>.

Cell proliferation is the basic mechanism involved in healing of all types of wounds especially excision wounds. Calcitriol is an active biological metabolite of vitamin D<sub>3</sub> & known to influence calcium metabolism. It stimulates osteoclastic bone resorption as well as intestinal & renal calcium absorption which may lead to hypercalcemia<sup>10</sup>. In addition calcitriol also possesses antiproliferative activity<sup>11</sup> & its role in cell proliferation & differentiation has been

confirmed<sup>10</sup>. By virtue of this antiproliferative activity, calcitriol is expected to retard the healing process as observed by other antiproliferative agents<sup>6,7</sup>.

Though hypercalcemia & antiproliferative activities of calcitriol appear independent of each other it would be interesting to probe the influence of calcium on wound healing; since calcium channel blockers are found to promote the healing process. Literature survey indicates paucity of information on the influence of calcitriol on wound healing. The present study was thus planned to investigate the influence of calcitriol & calcium gluconate on various wound models viz excision, resutured incision dead space wounds in albino rats.

### **Materials & Methods.**

#### **Animals & drug treatment**

Healthy male Wistar rats weighing 175±25g were housed individually & acclimatized to the laboratory for a week under 12:12 light dark cycle. The animals were fed on standard pellet diet (Amrut brand) & water ad lib, where as they were starved overnight the day prior to experimentation. The study was approved by the institutional animal ethics committee constituted as per CPCSEA guidelines. Depilation at the wounding site was done a day before wounding.

Wound Models: Resutured incision wounds were inflicted with two 6 cm long parallel para vertebral incisions under light ether anesthesia as described earlier<sup>12</sup>. Sutures were removed on the 7<sup>th</sup> day; breaking strength was measured on the 10<sup>th</sup> post wounding day, by the continuous water flow technique of Lee<sup>13</sup>.

Excision wounds were inflicted as described by the method of Morton & Malone<sup>14</sup>, by excising the full thickness (approximately 500 mm<sup>2</sup>) from the nape of the neck under

light ether anesthesia. Wound closure rate & epithelization time were assessed by tracing the wound on polythene paper from the wounding day, followed by 4, 8 & 12th day & subsequently on alternate days till complete epithelization (fall of scab without any raw area). Similarly scars were traced on complete epithelization to assess wound contraction by noting the scar shape & size.

Dead space wounds were inflicted by implanting sterile cotton pellets (10mg) & cylindrical grass piths (2.5 cm X 0.3 cm) subcutaneously in the groin & axilla alternatively by the technique of D'Arcy et al. as described by Turner<sup>15</sup>. On the 10<sup>th</sup> post wounding day, all the granulation tissues were removed under light ether anesthesia. Cotton pellet granulomas were dried overnight at 60<sup>o</sup>C to record the dry weight which was expressed as mg/ 100g body weight as suggested by Dipasquale & Meli<sup>16</sup>. One of the granulation tissue over the grass pith was opened & trimmed to a rectangular piece for estimation of breaking strength & subsequent estimation of hydroxyproline content colorimetrically<sup>17</sup>, whereas the other piece was preserved in 10% formalin for histological studies.

All the wounding procedures were carried out aseptically & none of the animals received any local or systemic antimicrobials.

After wounding, the animals were divided into control & treatment groups (n=6, in each) for each of the wound models to receive treatments. The drugs were administered in their therapeutically equivalent doses as calculated with the help of conversion table devised by Paget & Barnes<sup>18</sup>. Calcium gluconate (135mg/kg) intraperitoneally & calcitriol (25ng/kg) orally were administered suspended in normal saline & olive oil respectively. Control groups received normal saline & olive oil once a day in the volume of 5ml/kg.

The duration of treatment was 10 days for animals inflicted with incision & dead space wounds, whereas it was

continued till complete epithelization in animals bearing excision wounds.

#### Statistical analysis

The results were analysed by student 't' test expressed as mean  $\pm$  S.E.  $p < 0.05$  was considered as significant.

### Results

Resutured incision wounds: Breaking strength in the control animals (normal saline) was  $362 \pm 23.12g$ , whereas in calcium gluconate treated group it was  $544 \pm 13.8g$  indicating a significant ( $p \leq 0.001$ ) increase.(Table I)

Similarly the mean breaking strength of wounds in control animals (olive oil) was significantly ( $p \leq 0.001$ ) reduced as compared to calcitriol group.

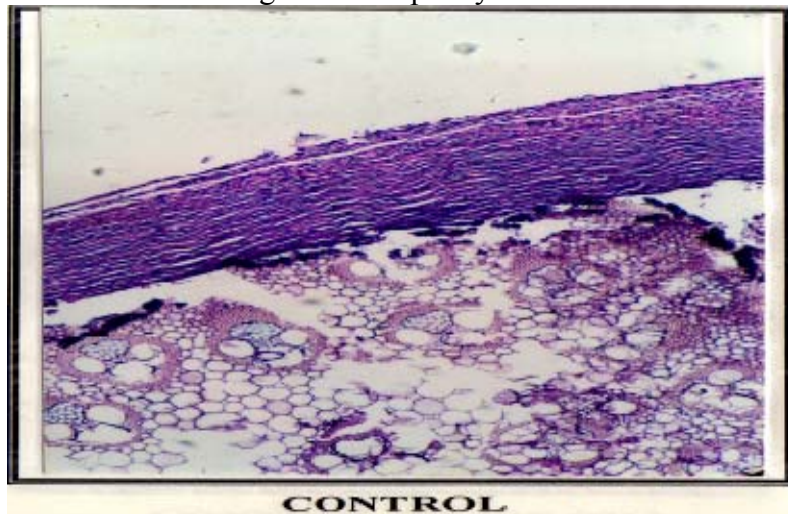
Dead Space wounds: Calcium gluconate significantly ( $p < 0.001$ ) increased the breaking strength of the granulation tissue compared to control similar to its effect on resutured incision wound(Table I), whereas the breaking strength of the granulation tissue in the calcitriol ( $128.3 \pm 11.92g$ ) group was significantly ( $p < 0.001$ ) decreased as compared to the olive oil control group ( $248 \pm 9.4g$ ). Cotton pellet granuloma weight was decreased significantly ( $p < 0.001$ ) in the calcitriol treated group as compared to olive oil control group.(Table I). On the other hand there was significant ( $p < 0.001$ ) increase of cotton pellet granuloma weight in the calcium gluconate treated group as compared to saline controls. Histopathological studies revealed markedly increased collagen content, subsequent fibroblastic proliferation in calcium gluconate treated group (Figure II) as compared to control.(Figure I) However the histological features of granulation tissue from calcitriol group (Figure IV) showed poor collagen content & fibroblastic proliferation as in the olive oil treated control groups.(Figure III).

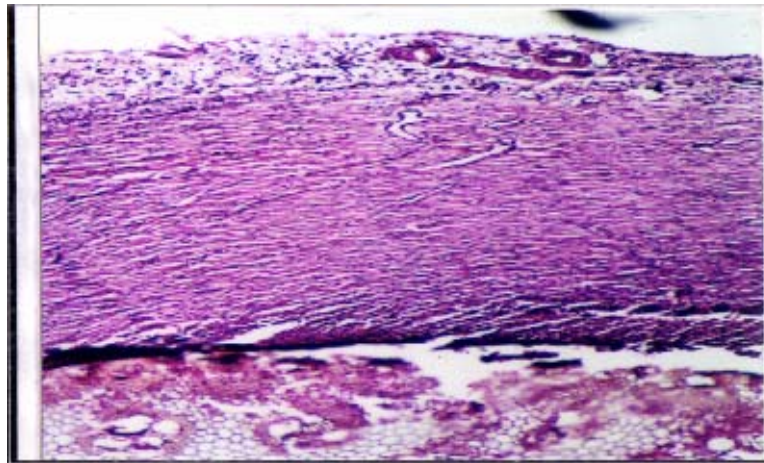
Table I : Effect of calcium gluconate & calcitriol on resutured incision & dead space wounds

Group n= 6 in each	Dose mg/kg	Resutured incision wound breaking strength (g)	Granulation tissue		
			Breaking Strength (g)	Dry weight (mg% of body wt)	Hydroxypro-line (mcg/900mg of wet granulation tissue)
Normal Saline Control	i.p. (5ml/kg)	362 ± 23.12	232 ± 14.6	41.7 ± 0.63	5.4 ± 0.08
Calcium gluconate	i.p.	544 ± 13.8 *	305 ± 4.6 *	57.4 ± 1.09 *	7.6 ± 0.13 *
Olive oil control	oral (5ml/kg)	408 ± 6.04	248 ± 9.4	43.8 ± 1.01	5.4 ± 0.08
Calcitriol	oral	192 ± 8.42 *	128.3 ± 11.92 *	32.3 ± 0.81 *	4.3 ± 0.07 *

p ≤ 0.001

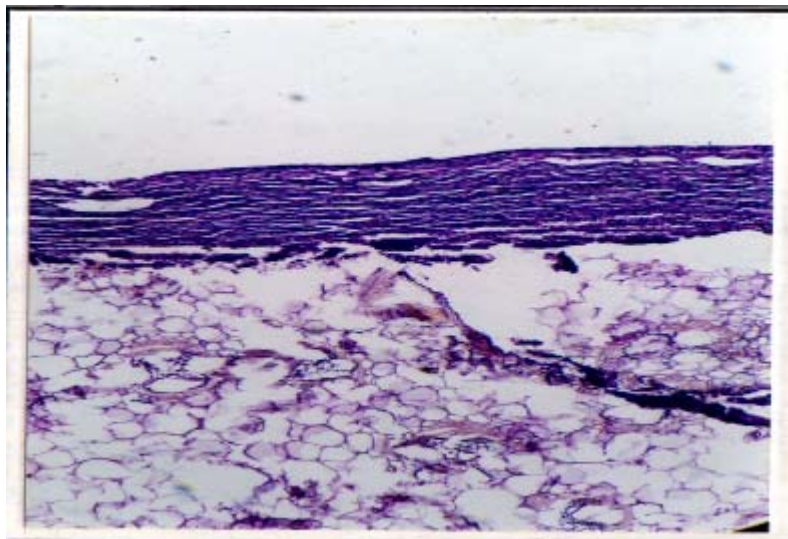
(I) Normal saline control group: Shows granulation tissue, moderate collagenation & plenty of fibroblasts.





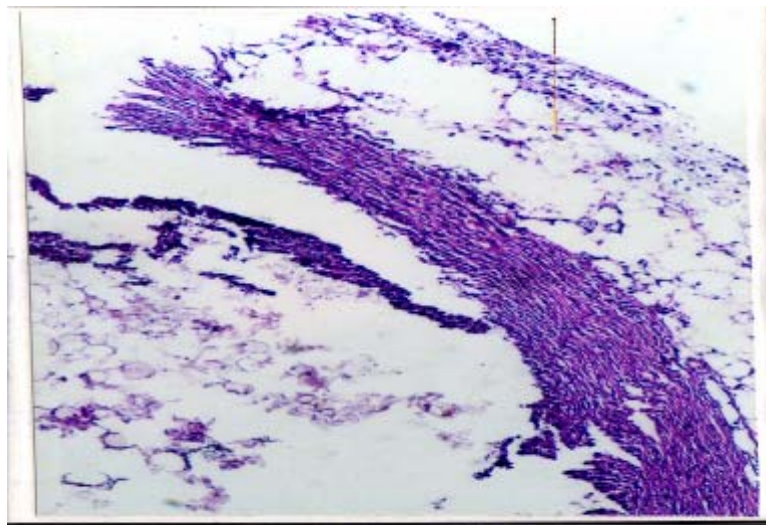
**CALCIUM GLUCONATE**

(II) Calcium gluconate group: Shows increased collagen content & fibroblastic proliferation as compared to normal saline controls.



**CONTROL (OLIVE OIL)**

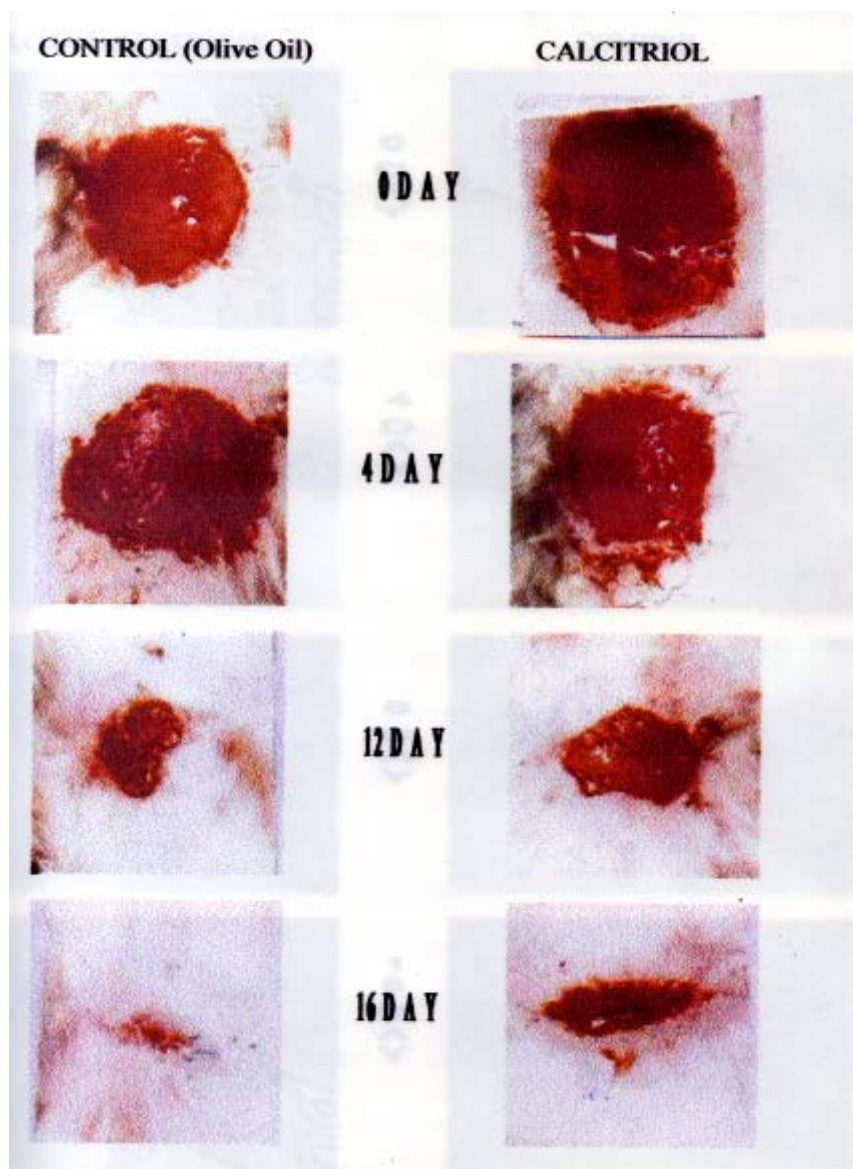
(III) Olive oil control group: Shows granulation tissue, moderate collagenation & plenty of fibroblasts.



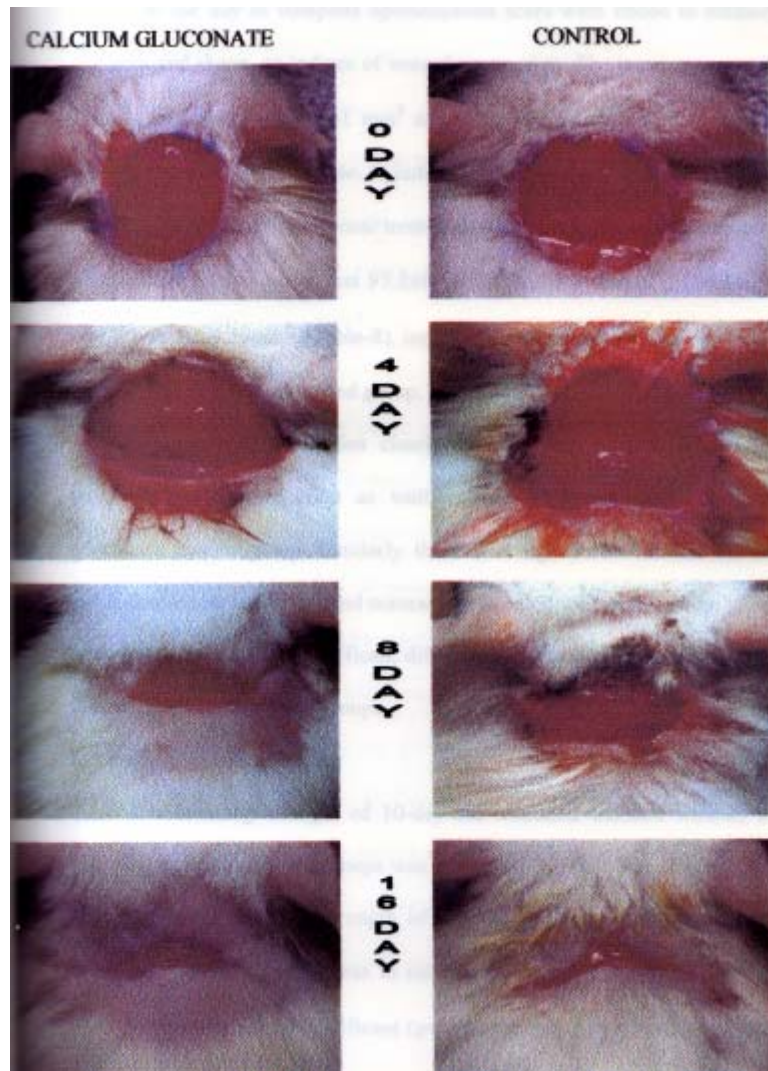
**CALCITRIOL**

(IV) Calcitriol group : Poor collagen content & subsequent fibroblastic proliferation as compared to olive oil controls.





(V) Excision wound model: Bigger raw wound area in the calcitriol group as compared to olive oil control group.



(VI) Excision wound model: Smaller raw wound area in the calcium gluconate group as compared to normal saline control group.

Excision wounds: The rate of wound closure in the calcium gluconate group (Figure VI) was significantly ( $p \leq 0.001$ ) more on the 8<sup>th</sup>, 12<sup>th</sup> day as compared to that of saline control group. However significant ( $p \leq 0.0001$ ) decrease in the rate of wound closure in all the

calcitriol treated group (Figure V) was observed on the 8<sup>th</sup> 12<sup>th</sup> & 16<sup>th</sup> days as compared to saline control (Table II). The time taken for epithelization was  $16.6 \pm 0.34$  days in the normal saline control group, while it was significantly ( $p < 0.001$ ) decreased in the calcium gluconate to  $14 \pm 0.37$  days. Whereas the time for epithelization was significantly ( $p \leq 0.01$ ) increased in the calcitriol treated group to  $18.8 \pm 0.66$  days as compared to  $15.6 \pm 0.82$  days in the olive oil treated controls. (Table II). The scar areas were significantly ( $p < 0.001$ ) decreased in the calcium gluconate group compared to normal saline controls & significantly increased in the calcitriol group as compared to olive oil controls. (Table II).

Table II : Effect of calcium gluconate & calcitriol on excision wounds

Group n=6 in each	Dose (mg/kg) orally	Wound closure (in mm <sup>2</sup> ) on day ( Mean +- SE)					
		4	8	12	16	Days for complete closure	Scar area
Normal saline control	5ml/kg i.p.	74 ± 16.9	214 ± 37.7	368 ± 41.5	474 ± 0.05	16.6 ± 0.34	44.5 ± 3.49
Calcium gluconate	i.p.	112 ±20.3	269 ± 7.02***	381 ± 18.6***	481 ± 0.10	14 ± 0.37***	28.6 ± 3.41 ***
Olive oil control	oral	80 ± 18.9	320 ± 11.1	435 ± 10.5	475 ± 13.9	15.6 ± 0.82	50 ± 3.9
Calcitriol	oral	32± 6.14	266± 24.3****	405±20.8***	453 ± 18.9*	18.8 ± 0.66**	97.2 ± 5.8 ***

\*  $p \leq 0.02$ , \*\*  $p \leq 0.01$  \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$

### **Discussion**

In the present study calcitriol, an active form of vitamin D<sub>3</sub> was investigated for its influence on wound healing. The results of the present study clearly indicate that calcitriol suppressed wound closure after day 4 & also prolonged time for complete closure in the excision wound model as compared to the olive oil treated control group. The scar area was also larger in the calcitriol group as compared to controls.

In the resutured incision wound model calcitriol significantly decreased the wound breaking strength & also suppressed granuloma breaking strength, granuloma formation & hydroxyproline content in the dead space wound model. The decreased granuloma & collagen formation was in agreement with the histological studies conducted. Thus it can be concluded that calcitriol has anti-healing activity in all the three wound models. This could be attributed to the antiproliferative activity of calcitriol in normal & a variety of tumour cells<sup>10</sup>.

Suppression of granuloma formation by interfering with the proliferation of fibroblasts probably explains the delayed rate of wound healing. The above finding is in coherence with the poor collagen content of the granulation tissue, since collagen is formed by the fibroblasts.

Wound contraction is a function of myofibroblasts (modified fibroblasts) present in the wound margin, which behave as smooth muscles. Further to ascertain the association of hypercalcemic activity of calcitriol with its antihealing activity, calcium gluconate was investigated for its influence on the healing process. Contrary to expectations it enhanced healing in all the three wound models. Literature survey revealed paucity of information though calcium channel blockers like verapamil & diltiazem promoted healing of wounds. It was thus puzzling to note the prohealing activity of both calcium channel blockers as well as calcium gluconate.

Increased wound contraction may be attributed to increased myofibroblasts since calcium is known to enhance the contraction of smooth muscles; which the myofibroblasts mimic. Calcitriol by its hypercalcemic activity could be expected to promote healing as did calcium gluconate. But the antiproliferative action of calcitriol appears to dominate over its hypercalcemia. . It could also be hypothesized that decreased wound contraction in the calcitriol group is due to scanty myofibroblasts (though excess calcium is available).

It has been reported that the antiproliferative action of calcitriol is independent of its hypercalcemic effect<sup>10</sup> & the present study indicates that calcium gluconate enhances the healing process probably through its hypercalcemic action.

### **Conclusion**

Calcium salts are often combined with vitamin D & advised in clinical practice to promote healing of bone fractures. If, the observations of the present experimental study could be extrapolated to the clinical scenario, the use of calcitriol in bone fracture patients could be expected to delay the healing process.

The above findings though need to be confirmed by further clinical studies.

### **Acknowledgements**

The authors are grateful to the Principal, J.N.Medical College, Belgaum for providing facilities & Dr.P.R.Malur, Professor of Pathology for his guidance in histopathological studies. Thanks to Mr. A. V. Karvekar & Mr. M. D. Kankanwadi for their skillful assistance.

**References**

1. Mckay IA, Leigh IM. Epidermal cytokines & their role in cutaneous wound healing. *Br. J Dermatol*, 1991;124:513-518.
2. Dunphey JE, Udupa KN, Edwards LC. Wound healing : A new perspective with particular references to ascorbic acid deficiency. *Ann Surg*, 1956; 144(3): 304 - 317.
3. Tauber C, Naff M, et al. Blood levels of active metabolites of Vitamin D in fracture repair in humans. *Arch. Ortho. Trauma. Surg*, 1990; 265: 109.
4. Goldberg B, Green H. Relation between collagen synthesis & collagen prolinehydroxylase activity in mammalian cells. *Nature*, 1969;221: 267-68.
5. Hunt TK, Pai MP. The effect of varying oxygen tension on healing of open wounds.. *Surg. Gynaecol. Obstet*, 1972;135:756-58.
6. Patil PA, Kulkarni DR. Effect of antiproliferative agents on healing of excision wounds in rats. *Ind. J Exp Biol*, 1985;23: 149- 150.
7. Patil PA, Kulkarni DR. Effect of antiproliferative agents on healing of dead space wounds in rats. *Ind J Med Res*,1984;79:445-447.
8. Lee KH. Studies on mechanism of action of salicylates. II Retardation of wound healing by aspirin. *J Pharm Sci*, 1968; 57: 1042.
9. Hunt TK. Disorders of repair & their management . In: Hunt TK, Dunphy ES eds. *Fundamental of wound Management*. Appleton Century- Crofte: New York, 1979. 103-07.
10. Bischof MG, Redlich K. Growth inhibitory effects on human colon adenocarcinoma derived Coco 2 cells & calo-2 cells & calcemic potential of 1  $\alpha$ 25 dihydroxy vitamin D<sub>3</sub> & analogs: structure-functional relationship. *J Pharmacol Exp Ther*. 1995; 275: 1254-60.
11. Haussler MR. Vitamin D receptors : Nature & function. *Ann Rev Nutr*, 1986; 6: 527-62.
12. Ehrlich HP, Hunt TK. The effects of cortisone & anabolic steroids on the tensile strength of healing wounds. *Ann Surg*, 1969;170:203-6.

13. Lee KH. Studies on the mechanism of action of salicylates II. Retardation of wound healing by aspirin. *J Pharm Sci*, 1968;57: 1942-3.
14. Morton JJ, Malone MH. Evaluation of vulnerary activity by an open procedure in rats. *Arch Int Pharmacodyn Ther*, 1972;196:117-26.
15. Turner RA. 'Anti-inflammatory agent' in screening methods of Pharmacology, 2<sup>nd</sup> ed. Academic Press; New York, 1965.
16. Dipasquale G, Meli A. Effect of body weight changes on the formation of cotton pellet induced granuloma. *J Pharm Pharmacol*, 1965;17:379-82.
17. Woessner JF Jr. The determination of hydroxproline in tissue & protein samples containing small proportions of this imino acid. *Arch Biochem*. 1961; 93: 440-47.
18. Paget GE, Barnes JM. Toxicity tests in Lawrence DR & Bacharach AL, Evaluation of drug activities pharmacometrics. London & New York; Academic Press, 1964.