The Pharma Innovation Journal 2022; SP-11(4): 1315-1319

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(4): 1315-1319

www.thepharmajournal.com
Received: 22-01-2022
Accepted: 11-03-2022

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Haemato-biochemical changes of external skeletal fixation on long bone fractures in sheep and goats

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Abstract
The present clinical study on the management of long bone fractures of tibia, metacarpal and metatarsal in sheep and goat using Stainless steel, Mild steel, and Aluminium implants of Type Ia, Linear External Skeletal Fixators and Hybrid circular External Fixation (HEF)-Type Ia, was conducted. A total of 36 clinical cases of sheep and goat of either sex aged between 2 months to 4 years 6 months with fractures of tibia, metacarpal and metatarsal were assigned into three groups with two subdivided groups total six groups based on the metal used, type and location of fracture and the surgical procedure adopted for fracture fixation. Clinical, radiological and heamato-biochemical effects were studied. Statistical analysis of the data revealed that the haemoglobin level and packed cell volume on 15th post-operative day decreased initially and then the values gradually increased within normal physiological limits by 60th post-operative day. The total erythrocyte count in three groups was elevated non significantly which were within normal physiological limits. This might be due to the physical stress at the time of fracture, loss of blood during surgery, as well as haemodilution and anesthesia during internal fixation procedure. The total leukocyte count was higher on the day before surgery when compared to the post-operative period. Physiological leucocytopenia seen was suggestive of gradual decrease in inflammatory reaction. The differential leucocyte count like neutrophil count decreased on 15th and 60th post-operative day when compared to the day before surgery in all the groups. Contrary to this, the lymphocyte count increased on 15th and 60th post-operative days. This indicated gradual decrease of inflammatory reaction. However, they were within normal physiological limits on different post-operative days in all the groups. The monocytic and eosinophilic count showed nonsignificant variations at different time interval in the animals of all the three groups. However, it fluctuated within normal physiological limits. The serum calcium values showed a gradual decrease by 15th post-operative day followed by increase in the value and reaching normal at 60th post-operativeday. The serum phosphorous values showed a gradual increase without any significant variation and the values were within the normal range. The serum alkaline phosphatase values showed a gradual increase by 15th post-operative day followed by increase in the value and reaching normal at 60th post-operative day. Serum alkaline phosphatase values increased non significantly from pre-operative day to 15th post-operative day indicating increased chondroblastic proliferation to cause bone formation during bone repair with good fracture stability till the completion of the bone healing in all the sheep and goats. Results of the present clinical study revealed that the Type Ia ESF and and Hybrid External Fixators Type Ia of stainless steel, mild steel and aluminium can be used with excellent outcome for treatment of long bone fractures of tibia, metacarpal and metatarsal in sheep and goats.

Keywords: ESF, steel, mild steel, aluminium, haematology & biochemical, sheep & goat

Introduction
Long bone fractures are common in sheep and goats and among all long bone fractures, tibia, metatarsal and metacarpal fractures are frequently encountered. (Aithal *et al.*, 1999 and Seaman and Simpson, 2004). There are many methods for fixation of long bone fractures in sheep and goats like intramedullry (IM), pinning, bone plating, interlocking nailing (Piermattei *et al*., 2006 and Fossum, 2007).

The goals of fracture treatment are to encourage healing, restore function to affected bone and surrounding soft tissue and obtain a cosmetically accepted appearance. Stabilization by internal fixation of long bone fractures have some limitations that IM pinning is not suitable for long bones like radius, ulna and tibia as this technique may damage the joints associated with them (Pope, 1998 and Probst, 1998). That is why external skeletal fixation (ESF) is gaining importance in the treatment of long bone fractures in small animals. Fixator stiffness must be sufficient but not excessive to maintain fracture alignment, so healing is not inhibited. (Paley *et al*., 1990 and Calhoun *et al*., 1992).

Use of ESF in small animals has also been impeded by economic constraints and difficulties with post-operative care in developing countries.

ESF provides better opportunity to maximize the biologic potential for healing within the fracture zone (Toombs, 2014). There are three major types of external skeletal fixators (ESF). a. Linear External Skeletal Fixators (LESF), b. Circular External Skeletal Fixators (CESF) and c. Hybrid External Fixators (HEF). Fixator rigidity depends on the design and the material used to construct the fixator, and hence it varies among different fixator constructs. Thus, our purpose was to evaluate Haematological and biochemical changes following ESF with stainless steel, mild steel and aluminium for long bone fractures in sheep and goats to determine if these ESF material could provide stable fracture fixation in sheep and goats.

Materials and Methods

The present study was undertaken in 36 sheep and goats presented with fractures of tibia, Metatarsal and Metacarpal for treatment at Department of Veterinary Surgery and Radiology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana. All the fractures of long bones were included in the present study as external skeletal fixation is suitable in treatment of fractures of these bones. And were assigned into three groups with two subdivided groups each of six sheep and goats.

The sheep and goats of the Group-Ia were treated with Type Ia Linear External Skeletal Fixator with Stainless Steel. The sheep and goats of Group Ib - Hybrid External Fixator Type Ia (HEF Type Ia) with Stainless Steel. The sheep and goats of Group IIa – Type Ia Linear External Skeletal Fixator with Mild steel. The sheep and goats of Group IIb – Hybrid External Fixator Type Ia (HEF Type Ia) with Mild Steel. The sheep and goats of Group IIIa – Type Ia Linear External Skeletal Fixator with Aluminium. The sheep and goats of Group IIIb – Hybrid External Fixator Type Ia (HEF Type Ia) with Aluminium.

In the present study on 34 goats and 2 sheep, 9 goats had tibial fractures, 12 goats had metatarsal fractures, 13 goats had metacarpal fractures and two sheep had metatarsal fracture. All the fractures were open and closed fractures. Out of thirty six sheep and goats of the three groups, the radiographs revealed 14 goats with transverse factures, 10 goats and 2 sheep with oblique fractures, 3 goats with short oblique fractures, 6 goats with multi-fragmentary or comminuted fractures, 1 goat with compound fracture. 30 fractures were closed fractures and 6 fractures were open fractures.

Limited open approach in 18 sheep and goats and closed approach in 18 sheep and goats were adopted. For reduction of fracture fragments, hanging limb technique with the animal in dorsal recumbency with the affected limb suspended from the ceiling by a sterile snap hook system facilitated reduction and alignment of the fracture fragments. C-arm adopted for indirect reduction of fractures through hanging limb technique for the tibial, metacarpal and metatarsal closed fractures to enable indirect fracture reduction and proper pin placement.

Blood sample was collected in EDTA coated vials in all the cases of Group Ia & Ib Group IIa & IIb and Group IIIa & IIIb before surgery and on the 15th, 30th, 45th and 60th postoperative days of surgery. Haemoglobin (gm/dl), packed cell volume (%), total erythrocyte count (x106/µl), total leucocyte count (x103/µl) and differential leucocyte count (%) were estimated on the said day as per the methods described by Jain (1986).

Blood sample was collected and serum was separated in all the cases of Group Ia & Ib Group IIa & IIb and Group IIIa & IIIb before surgery and on the 15th, 30th, 45th and 60th postoperative days of surgery. Serum alkaline phosphatase, calcium and phosphorous were estimated on the said day.

Results and discussions

Haematological observations

In order to evaluate the animal pre-operatively and to monitor the health status of animals the following haematological examinations were conducted on 0th day (on day before surgery), 15th day, and 45th and 60th post-operative day. The mean ± SE values of haematological parameters of haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count of three groups were presented below. (Table1).

The mean ±SE values of haemoglobin, Packed cell volume on different post- operative days in different groups fluctuated non-significantly (P> 0.05) around their base value. These values were higher on 60th post-operative day when compared to pre-operative values. There was a non-significant decrease in haemoglobin and Packed cell volume in three groups on 15th post-operative day and then the values gradually increased within normal physiological limits by 60th post-operative day.

The mean ±SE values of total erythrocyte count on different post-operative days in different groups fluctuated non-significantly (P> 0.05) around their base value. There was a non-significant gradual increase in total erythrocyte count in three groups which were within normal physiological limits. This might be due to the physical stress at the time of fracture, loss of blood during surgery, as well as haemodilution and anesthesia during external fixation procedure. This findings were in agreement with the findings of Uwagie-Ero *et al* (2016) The mean ±SE values of total leukocyte count on different post-operative days in three groups fluctuated non-significantly (P> 0.05) around their base value.

The total leukocyte count was higher on day before surgery when compared to post- operative days in all the three groups. Leucocytosis on initial days of surgery occurred in conditions where there was corticosteroid release in state of stress, pain, anaesthesia, trauma and surgical manipulation. Physiological reduction in total leukocyte count was reported to be suggestive of gradual decrease in inflammatory reaction. This was in correlation with Khillare (2006) [7].

The mean ±SE values of neutrophil count on different post-operative days in three groups fluctuated non- significantly (P> 0.05) around their base value. The neutrophil count was higher on day before surgery when compared to post-operative days in all the three groups. This may be due to reduced inflammatory response leading to progressive fracture healing without any exudation. Similar findings were reported by Shinde (1994) [19] and Hoque (1996) [5].

The mean ±SE values of lymphocyte count on different post-operative days in three groups fluctuated non-significantly (P> 0.05) around their base value. The lymphocytic count was higher on 60th post-operative day than the day before surgery in all the three groups which showed a gradual increase in lymphocytic count. This can be attributed to relative variation in neutrophil count which increases initially after surgical intervention and further activates the production of immune regulatory cytokines by macrophages and

monocyte. Cytokines are responsible for activation of adrenal axes and increase production of glucocorticoids, which might be responsible for lyses of lymphoid tissue and increased circulating lymphocytes. This was in correlation to Pawar (1999).The mean monocytic and eosinophilic count showed nonsignificant variations at different time interval in the animals of all the three groups. Vinay Kumar (1997) [23] Mahmood Ahmadi-hamedani *et al*. (2015) reported that the monocyte and eosinophil count showed non significant variations at different time intervals in all the groups during fracture healing in sheep and goats.

Serum Biochemical Observations

The mean ± SE values of serum biochemical parameters of serum calcium, serum phosphorus and serum alkaline phosphatase are presented below (Table 2) The mean ±SE values of serum calcium on different post-operative days in three groups fluctuated non-significantly ($P>$ 0.05) around their base value within and among the groups. The mean serum calcium values showed a gradual decrease by 15thpost-operative day followed by increase in the value and reaching normal at 60 days of postoperative interval period. The serum calcium level in all the animals fluctuated within normal physiological range. All these values were within reference range (10.70-12.16 mg/dl) as documented by PK Sriraman (2009) [17]. Meller *et al*. (1984) [10] reported the changes observed in levels of calcium regulating hormones (calcitonin and 24, 25–dihydroxy vitamin D3) in response to bone injury might be associated with the process of callus formation. Further, the trend was almost consistent with findings of Soliman and Hassan (1964) [16] who reported that the mean serum calcium concentration initially decreased and reached a minimum level on 10th day, then increased again and returned to initial values or higher in union groups. The present observations were in accordance with those of Pandey and Udupa (1981) [14] Chaudhari *et al*., (2000) and Saraswathy *et al*., (2004) [20]. Bush (1991) [2] and Uma Rani and Ganesh (2003) [22].

The mean ±SE values of serum phosphorus on different post-operative days in three groups fluctuated non-significantly ($P>$ 0.05) around their base value within and among the groups.In all the three groups the serum phosphorous mean values showed a gradual increase in serum phosphorus without any significant variation and the values were within the normal range. The present observations were in accordance with those of Chandy (2000) [4] Chaudhari *et al*., (2000) Uma Rani and Ganesh (2003) [22] and Saraswathy *et al*., (2004) [20].

The mean ±SE values of serum alkaline phosphatase on different post-operative days in three groups fluctuated non-significantly ($P>$ 0.05) around their base value within and among the groups. The mean alkaline phosphatase values showed a gradual increase by 15th post-operative day followed by increase in the value and reaching normal at 60 days of postoperative interval period. Serum alkaline phosphatase values increased non significantly ($P>$ 0.05) from pre-operative day to 15th post-operative day indicating increased chondroblastic proliferation to cause bone formation during bone repair (Maiti *et al*., 1999) [9]. Increase in the serum alkaline phosphatase was from the periosteumof destructed bone which was a rich source of serum alkaline phosphatase. Normal fracture healing is generated by increased osteoblastic activity. Osteoblasts are responsible for both new tissue (bone matrix) formation and its mineralization and secrete large quantities of ALP which was involved bone healing process. The findings were in concurrence with those of Chaudhari *et al*., (2000), Kulkarni (2000) [8] Uma Rani and Ganesh (2003) [22], Saraswathy *et al*., (2004) [20] and Julie (2005) [6] and the serum alkaline phosphatase levels reached normal levels by the 60th post-operative day indicating quiescence at the fracture site.

Table 1: Haemotological observations in three groups

	Days	Group Ia	Group Ib	Group IIa	Group IIb	Group IIIa	Group IIIb
haemoglobin (g/dl)	0	9.84±0.64	10.24±0.23	9.78±0.43	10.30±0.42	10.20±0.24	10.28±0.46
	15	9.72±0.24	10.08±0.24	9.69±0.41	10.01±0.42	10.00±0.24	10.04±0.23
	45	10.26±0.12	10.12±0.17	10.12±0.38	10.11±0.39	10.10±0.18	10.12±0.36
	60	10.32±0.39	10.34±0.24	10.24±0.37	10.55±0.39	10.43±0.23	10.45±0.48
Packed Cell Volume (%)	0	29.47±0.83	33.48±1.62	29.26±0.74	34.16±1.74	32.35±1.57	34.35±1.57
	15	29.84±0.64	34.26±1.46	29.64±0.76	35.80±1.38	34.45±1.74	35.45±1.74
	45	32.28±0.78	36.34±1.54	32.55±0.79	38.37±1.27	35.82±1.28	37.82±1.28
	60	32.86±1.26	37.52±1.28	33.86±1.19	39.47±1.42	37.21±1.42	39.21±1.42
Total Erythrocyte Count (x10^6/µl)	0	11.75±0.27	11.86±0.17	10.84±0.12	11.78±0.17	11.76±0.25	11.86±0.28
	15	11.21±0.18	11.58±0.28	10.12±0.18	11.16±0.18	11.43±0.26	11.17±0.18
	45	12.32±0.42	12.31±0.13	11.28±0.09	12.29±0.14	12.34±0.12	12.24±0.09
	60	12.47±0.29	12.42±0.10	11.62±0.32	12.47±0.13	12.41±0.09	12.63±0.12
Total Leukocyte Count (x10^3/µl)	0	8.38±0.24	8.42±0.27	8.89±0.46	7.68±0.46	9.16±0.35	8.86±0.45
	15	8.61±0.26	8.15±0.25	8.78±0.31	7.43±0.53	8.91±0.34	8.31±0.32
	45	7.72±0.34	7.74±0.37	8.33±0.42	7.38±0.48	8.38±0.29	7.86±0.27
	60	7.42±0.23	7.36±0.23	7.86±0.39	6.76±0.39	8.16±0.33	7.26±0.23
Neutrophil Count (%)	0	48.23±1.43	49.03±1.44	49.82±1.74	49.23±1.70	49.96±2.23	49.98±2.13
	15	47.82±1.64	47.43±1.68	48.86±1.82	47.88±1.80	48.51±2.23	48.81±2.03
	45	45.53±1.48	45.43±1.46	47.64±1.89	47.28±1.84	47.35±2.18	47.36±2.14
	60	42.84±1.49	42.85±1.42	45.42±2.27	45.94±2.17	45.83±2.97	45.63±1.97
Lymphocyte count (%)	0	52.32±1.31	53.34±1.20	53.35±1.24	52.30±1.32	53.35±1.25	52.33±0.74
	15	53.63±1.35	54.62±1.34	55.28±1.32	53.61±1.34	54.28±1.37	53.70±0.65
	45	55.20±1.38	56.24±1.36	57.91±1.48	54.20±1.36	57.91±1.43	55.91±0.62
	60	57.30±1.33	57.32±1.32	58.25±0.76	56.30±1.34	58.25±0.78	56.36±0.63
Monocyte count (%)	0	2.56±0.17	2.32±0.02	1.98±0.18	1.98±0.16	2.20±0.12	2.24±0.14
	15	2.48±0.12	2.58±0.18	1.88±0.16	1.86±0.14	2.03±0.11	2.08±0.21

	45	2.08±0.24	2.02±0.14	2.11±0.14	2.16±0.12	1.81±0.17	1.68±0.18
	60	2.16±0.13	2.18±0.16	2.32±0.12	2.34±0.09	2.21±0.07	2.32±0.17
Eosinophil count (%)	0	2.08±0.24	2.04±0.26	2.16±0.22	2.06±0.22	2.35±0.22	2.32±0.22
	15	2.50±0.22	2.45±0.12	2.68±0.25	2.20±0.25	2.40±0.23	2.48±0.26
	45	2.41±0.33	2.33±0.43	2.71±0.27	2.31±0.27	2.31±0.24	2.36±0.23
	60	2.64 ± 0.15	2.65±0.15	2.43±0.19	2.23±0.19	2.21±0.15	2.30±0.15

Table 2: Serum biochemical changes in three groups

	Days	Group Ia	Group Ib	Group IIa	Group IIb	Group IIIa	Group IIIb
Serum Calcium (mg/dl	0	10.70±0.27	10.76±0.30	10.74±0.29	10.30±0.27	10.81±0.30	10.82±0.31
	15	10.30±0.16	10.68±0.24	10.26±0.27	10.24±0.16	10.68±0.27	10.64±0.25
	45	11.63±0.23	11.76±0.23	11.70±0.28	11.73±0.23	11.76±0.26	11.46±0.28
	60	10.76±0.33	10.23±0.27	10.80±0.24	10.26±0.33	10.23±0.24	10.76±0.21
Serum Phosphorus (mg/dl)	0	4.46±0.24	4.46±0.34	4.65±0.26	4.64±0.21	4.32±0.26	4.34±0.24
	15	4.72±0.19	4.68±0.14	4.76±0.24	4.78±0.22	4.45±0.29	4.43±0.21
	30	4.86±0.26	4.89±0.26	4.91±0.22	4.94±0.23	4.73±0.24	4.72±0.26
	60	4.92±0.13	4.94±0.23	5.11±0.18	5.21±0.18	4.86±0.23	4.82±0.24
Serum Alkaline Phosphatase (U/L)	0	102.00±3.72	98.20±3.52	125.50±5.61	105.50±5.42	114.28±6.84	107.42±5.73
	15	138.00±6.40	136.00±6.50	167.00±6.29	127.00±4.28	151.83±5.46	131.83±6.56
	45	83.34±6.02	93.64±6.09	116.16±4.74	96.26±4.76	108.33±4.82	98.33±4.99
	60	76.26±4.65	84.17±3.64	82.83±3.97	70.83±2.92	86.16±3.64	83.16±4.63

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