www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(8): 591-594

www.thepharmajournal.com
Received: 19-06-2021
Accepted: 21-07-2021

Vishwakarma Raj Kumari
Ph.D. Scholar, (Department of Veterinary Surgery & Radiology, CVASC, GBPUA&T, Pantnagar), Uttarakhand, India

Roukuobinuo Houzha
Assistant Professor, (Veterinary Physiology & Biochemistry, CVASC, GBPUA&T, Pantnagar), Uttarakhand, India

Mridula Sharma
Assistant Professor, (Veterinary Gynaecology & Obstetrics, CVASC, GBPUA&T, Pantnagar), Uttarakhand, India

Manjul Kandpal
Professor, (Department of Veterinary Surgery & Radiology, CVASC, GBPUA&T, Pantnagar), Uttarakhand, India

SK Shukla
Professor (Veterinary Medicine, CVASC, GBPUA&T, Pantnagar), Uttarakhand, India

NS Jadon
Dean, (CVASC, GBPUA&T, Pantnagar), Uttarakhand, India

Arup Kumar Das
Professor & Head (Department of Veterinary Surgery & Radiology, CVASC, GBPUA&T, Pantnagar), Uttarakhand, India

Corresponding Author
Vishwakarma Raj Kumari
Ph.D. Scholar, (Department of Veterinary Surgery & Radiology, CVASC, GBPUA&T, Pantnagar), Uttarakhand, India

Study on haematobiochemical changes in induced segmental defect in rabbits

Vishwakarma Raj Kumari, Roukuobinuo Houzha, Mridula Sharma, Manjul Kandpal, SK Shukla, NS Jadon and Arup Kumar Das

Abstract
The present study was conducted in 24 healthy rabbits of either sex approximately 2.0 years of age, weighing 1.8 to 2.0 kg body weight. Prior to experiment each rabbit was examined to assess their health status by clinical, haematological and faecal examination. The surgical procedure was accomplished under premedication with inj. glycopyrrolate @ 0.01 mg/kg, SC (subcutaneously), later inj. xylazine @ 4 mg/kg IM (intramuscularly), and then induction of anaesthesia with inj. Ketamine @ 40 mg/kg IM. A five minutes interval was kept between each of these injections. All haematobiochemical parameters were falls in normal physiological reference range except few *viz*.; the value of haemoglobin showed a significant ($P<0.05$) decrease at day 15 of post-operative day and a significant ($P<0.05$) decrease in values of packed cell volume and total erythrocyte count were observed at day 7 and day 15 post-operative days as compared to its pre-experimental value. Among biochemical parameters serum alkaline phosphatase found significantly increased ($P<0.05$) from day 7 to day 60, a significant ($P<0.05$) increase in value of serum calcium was observed at day 7, 15, 30 and day 60, and a significant ($P<0.05$) increased was observed in value of serum phosphorus from day 7 to day 30 of the post-operative period as compared to its pre-experimental. The value of protein was reduced significantly ($P<0.05$) that observed at day-7, 15, 30 and day 60 and the blood glucose value showed a significant ($P<0.05$) increase at day 7 post-surgery as compared to its pre-experimental value. Overall both haematological and biochemical parameters were found within normal physiological reference range except few and these were also returning to pre-experimental values early in the post-operative period.

Keywords: rabbit, haematological, biochemical, segmental defects, glycopyrrolate

1. Introduction
Bone is a multifunctional tissue that provides structure to the body *viz*., it protects visceral organs, acts as a fulcrum for the muscles, a store house of calcium, phosphorous and a site of formation of blood and immune cells (Caplan, 1991 and Hing 2004) [2, 9]. In general a break or breach in structural continuity of bone is regarded as fracture. Traumatic injuries in rabbit usually result from vehicular injury, being stepped on, dropped, crushed by a piece of furniture, or bitten by a dog or cat (Rich, 2002) [16], probably results in large size segmental defects of long bones that commonly includes tibia, radius ulna and femur bone. Furthermore Clinical examination and radiological assessment are the most commonly used diagnostic aids in evaluation of fracture union. Other approaches for the clinical evaluation of bone status include study of bone mineral density, and haematobiochemical parmeters. While clinical examination and radiographic evaluation, provide information primarily about the bone macrostructure, integrity, quantity and ultimate outcome of healing, although haematobiochemical parmeters provide a dynamic picture about the underlying process of bone resorption including its turnover, pathogenesis and can differentiate between normal and delayed healing. Moreover they can be used to monitor short term effects of therapy and provide early indication of any impairment of healing process. Since haematological and biochemical attributes have important role for completion of all phases of fracture repair *viz*., inflammatory, reparative and remodeling phase. Hence, both these parameters play an important role for monitoring fracture healing and any deviation from normal physiological reference range is often an indication of an infection and it requires an immediate attention, failure to which would lead delayed union, non-union and malunion (kumar *et al*., 2016) [12]. Evaluation of haematological parameters can yield information about the red blood cell population and leukocyte response to stress and pathogens. Likewise monitoring of biochemical parameters *viz*., serum calcium level is one of important feature which give an early indication about the status of fracture healing in affected animals and detection of

specific biochemical markers of bone formation in serum, such as alkaline phosphatase activity can be clinically useful in evaluating the progress of the healing process and serves as an additional tool in predicting fractures at risk of developing a non-union (Komnenou *et al.*, 2005) [11]. Though, there is little information available on the use of blood parameters tests as diagnostic and prognostic indicators of fracture healing in rabbits. The objective of this study is to observe changes in haematobiochemical parameters during pre and post-surgical period of inducing segmental defect in rabbits.

2. Materials and Methods

2.1. Animals

The present study was conducted in 24 healthy rabbits of approximately 2.0 years of age of either sex weighing 1.8 to 2.0 kg body weight. Prior to experiment each rabbit was examined to access their health status by clinical, haematological and faecal examination. They were fed with 80–100 g overnight soaked gram and dalia in the morning and succulent green grasses in the evening in their daily diet and water *ad libitum*. One week before experimentation rabbits were acclimatize.

2.2. Anaesthesia and surgical intervention.

Animals were kept off-fed and off-water for four to five hours before induction of anaesthesia. included inj. Glycopyrrolate [1], @ 0.01 mg/kg SC, injection xylazine [2] @ 4 mg/kg subcutaneously SC, inj. Ketamine [3] @ 40mg/kg SC with an interval of 5 minutes followed by maintenance of anaesthesia with sevoflurane [4] @ 1-1.5% concentration with 100% O_2 saturation. The left tibial region (spanning from stifle to mid metatarsus) was aseptically prepared before surgery. For the surgical maneuver the rabbits were restrained, on the operation table, in left lateral recumbency and draped adopting stringent sterile fashion for orthopaedic procedure. Then 1.0 cm segmental defect was induced using orthopedic saw under continual flow of normal saline. After completion of surgical procedure the incised wound was closed. Muscles were placed in apposition using 3-0 polyglactin 910 sutures [5] in continuous pattern and skin was sutured with 2-0 polyglactin 910 sutures in horizontal mattress pattern.

2.3. Parameters studied

For haematologicalstudy intravenous blood samples (0.5 ml) were taken aseptically from marginal ear vein of rabbits at day 0, 7, 15, 30, 60 and day 90 and were analyzed for haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) that included lymphocyte, monocyte, neutrophils, eiosinophils and monocyte.

For biochemical studies (at day 0, 7th, 15th, 30th and at day 90th) one milliliter of blood from ear vein was aseptically drawn in disposable insulin-syringe and a drop of blood from the bevel tip of the syringe was touched to the reaction zone of test strip of glucometer [6] (Gluco One Blood Glucose monitoring system) and the strip was allowed to fill automatically through capillary action. The digital reading of the machine was recorded. Rest of the collected blood was poured in vial containing clot activator. Thereafter, serum was harvested and subsequently analyzed for: Alkaline phosphatase, calcium, phosphorus, and total protein. All these parameters were analysed using ERBA kit [7].

3. Statistical Analysis

Data were analyzed using "IBM SPSS Statistics Version 20" and expressed as Mean ± SE and 'Paired-samples T test' was used for comparison from base value. Significance of differences was determined by method given by Snedecor and Cochran (1994) and values of $P < 0.05$ were considered statistically significant.

4. Results and Discussion

In the present study the mean ± SE values of haematological parameters at different time intervals are given in table 1.

Table 1: Mean ± SE values of haematological parameters in two groups at different time intervals.

Parameters/Time intervals		O day	7 day	15 day	30 day	60 day	90 day
Hb (g/dL)		11.64±0.55	11.44±0.56	11.18*±0.48	11.67±0.61	12.29±0.71	12.50±0.80
PCV (%)		37.72±0.67	34.59*±0.84	29.68*±0.39	37.31±0.57	37.13±1.00	36.36±0.62
TEC (10^6 cells /µL)		5.75±0.14	5.40*±0.14	5.32*±0.11	5.73±0.13	5.87±0.14	5.66±0.11
TLC (10^3 cells /µL)		8.47±8.47	8.57±0.09	8.54±0.09	8.53±0.09	8.56±0.08	8.62±0.10
DLC (%)	Lymphocyte	45.66±0.42	45.50±0.34	45.27±0.31	45.27±0.45	45.33±0.34	45.00±0.38
	Monocyte	6.05±0.15	5.72±0.31	5.88±0.30	6.33±0.18	5.55±0.28	6.11±0.22
	Neutrophils	44.55±0.59	44.72±0.27	44.72±0.41	43.83±0.37	45.27±0.36	45.61±0.35
	Eiosinophils	3.30±0.30	3.00±0.37	3.45±0.43	3.29±0.30	3.24±0.37	3.60±0.43
	Basophils	1.90± 0.41	1.90±0.23	1.80±0.29	1.79± 0.41	1.84±0.23	1.94±0.29

*$P<0.05$, Means differ significantly from the pre-experimental value with in a row.

The haematological parameters were evaluated in the present study to study any changes during and in post-operative days of segmental bone defect repair of tibia bone. In agreement to present findings Umeshwori *et al.* (2015) [18] also reported that haematological parameters like TLC, neutrophil count were assessed to study the presence of any infection in the post-operative period of fracture stabilization. In the present study all haematological parameters values of rabbit were found within normal physiological reference range and no significant ($P<0.05$) changes were observed in them except few *viz.*, the value of haemoglobin showed a significant ($P<0.05$) decrease at day 15 of post-operative day as compared to its pre-experimental value. A significant ($P<0.05$) decrease in values of packed cell volume and total erythrocyte count were observed at day 7 and day 15 post-operative days as compared to its pre-experimental value. The value of total leucocyte count showed non-significant changes as compared to its pre-experimental value throughout the course of experiment. Among differential leucocyte parameters none of the parameters showed any significant changes as compared to its pre-experimental values. Similar findings were also reported by Kilic, (2004) [10] who reported that pooling of circulating blood cells in the spleen and other reservoirs secondary to decreased sympathetic activity could be the reason for a decrease in hemoglobin concentration, packed cell volume and total erythrocyte count**.** Or it might be due to shifting of fluid from extravascular compartment to intravascular compartment in order to maintain normal cardiac output (Wagner *et al.*, 2001) [19].

In the present study the mean ± SE values of biochemical parameters at different time intervals are given in table 2.

Table 2: Mean ± SE values of biochemical parametersin two groups at different time intervals.

Parameters-Time intervals	O day	7 day	15 day	30 day	60 day	90 day
Serum ALP (IU/L)	42.91^{a}±0.69	73.46^{a*}±2.92	76.67^{a*}±2.84	64.27^{a*}±2.33	49.54^{a*}±0.67	41.91^{a}±0.47
Serum Calcium (mg/dL)	12.42^{a}± 0.27	13.69^{a*}±0.43	18.71^{b*}±0.25	16.02^{a*}±0.39	15.73^{b*}±0.28	13.07^{a}±0.34
Serum Phosphorus (mg/dL)	4.51^{a}±0.14	6.48^{b*}±0.27	7.43^{a*}±0.27	6.53^{a*}±0.20	4.83^{a}±0.19	4.67^{a}±0.13
Total Serum Protein (g/dL)	6.30^{a}±0.17	5.77^{a*}±0.16	5.52^{b*}±0.14	4.90^{b*}±0.07	5.30^{a*}±0.20	5.70^{a}±0.32
Blood Glucose (mg/dL)	110.50^{a}± 0.54	124.00^{a*}±2.83	104.38^{a}±5.75	109.37^{a}±5.41	111.13^{a}±4.39	115.00^{a}±4.47

*P<0.05, Means differ significantly from the pre-experimental value with in a row.

The values of serum alkaline phosphatase found significantly increased (*P*<0.05) from day 7 to day 60 of the present study. Alkaline phosphatase is an important indicator for bone formation as it is secreted by osteoblasts. This enzyme triggers the mineralization of the osteoid by increasing the local concentration of calcium phosphate. Anaraki *et al.*, (2021) [1] also found a remarkable increase in levels of alkaline phosphatase after orthopaedic surgery in rabbits. In agreement with the present result few researches also reported similar findings in value of ALP enzyme during fracture healing (Singh *et al.*, 2013; Komnenan *et al.*, 2005) [17, 11] that the initial weekly elevation and reduction afterwards has a positive correlation with osteoblast cells activation, differentiate with new bone formation and starting of the remodeling phase. Monitoring of serum calcium level is one of important feature which give an early indication about the status of fracture healing in affected animals. In the present study a significant (*P*<0.05) increase in value of serum calcium was observed at day 7, 15, 30 and day 60 of post-operative period as compared to its pre-experimental value. This might be due to deposition of calcium to the site of segmental defect in response to stimulation of the thyroid gland to secrete calcitonin which is a normal physiological phenomenon during fracture healing. In agreements with the present study Giri *et al.* (2015) [7] also reported that high levels of calcium and phosphorus in the blood and extracellular fluids trigger the deposition of calcium phosphate crystals in the osteoid and make it harder. In the present study a significant (*P*<0.05) increased was observed in value of serum phosphorus from day 7 to day 30 of the post-operative period as compared to its pre-experimental value and thereafter it returns near to its pre-experimental value. In accordance to the present results it was reported that the plasma phosphorus increased slowly, reached a peak level after 2-3 weeks, and then slowly returned to initial value (Nilsson and Westlin, 1972) [13]. This initial increase in serum phosphorus might be due to necrosis and destruction of bone cells at the fracture site following creation of long bone segmental defect where the stored phosphorus is released into the blood. In the present study the value of protein was reduced significantly (*P*<0.05) that observed at day-7, 15, 30 and day 60. However it returns near to its pre-experimental value by 90 days post-surgery. This might be due to damage or trauma associated with creation of segmental defect in tibia bone lead to negative nitrogen balance and resulted in decrease serum protein level. Or it might be due to increased utilization and inhibition of protein synthesis and amino acids uptake during post-surgical period. Prasad (2008) [15] also reported reduction in serum protein during acute and chronic diseases. Desborough (2000) [4] had also reported that the increased level of catecholamine in injured animals inhibited the protein synthesis and also reduces the concentration of amino acids in the circulation. The blood glucose value in the present study showed a significant (*P*<0.05) increase at day 7 post- surgery as compared to its pre-experimental value. Thereafter it showed a decline and reached near to its base line value by day 15 post-surgery. Though all values of serum glucose was found within normal physiological reference range for rabbit in this study. This increment in its value at one week post-operatively might be due trauma from surgical procedure itself and the effects of anesthesia induces stress on the body lead to higher levels of stress hormones mainly cortisol, that will slowly raise blood glucose levels. Or this kind of stress hyperglycemia following traumatic injury or surgical interventions is largely mediated by glucagon hormone which induces stress hormone secretion. As this traumatic injuries due to surgical interventions induce stress hormone secretion (Harp *et al.*, 2016) [8] in turn promoting oxidative stress and insulin resistance, finally resulting in hyperglycemia (Esposito *et al.*, 2002; Chen *et al.*, 2013) [6, 3]. By two weeks post-operatively BG value returns near to its pre-experimental value in the present study might be due to decreased cortisol level in response to decreased in intensity of stress on animal's body. Similar findings were also reported by O'Neill and Co-workers (1991) [14] and he also suggested that intensity of increment in blood glucose imitated the stress response. Similarly Di Luzio *et al.* (2020) [5] also defined stress hyperglycemia related with orthopedic surgery in human patients.

5. Conclusion

Haematological and biochemical parameters were found within normal physiological reference range except few and these were also returning to pre-experimental values early in the post-operative period. The present study also showed that haematological and biochemical parameters are found good indicators for evaluation of fracture healing of segmental defect of tibia in rabbit. More over both these parameters along with clinical and radiographical examination could provide detailed information of segmental fracture healing.

6. References

1. Anaraki N, Beyraghi AH, Raisi A *et al.* The effect of aqueous extract of *Prunus dulcis* on tibial bone healing in the rabbit. J Orthop Surg Res 2021;16:362. https://doi.org/10.1186/s13018-021-02498-z.
2. Caplan AI.Mesenchymal stem cells. Journal of Orthopaedic Research 1991;9(5):641-650.
3. Chen Y, Yang X, Meng K *et al.* Stress-induced hyperglycemia after hip fracture and the increased risk of acute myocardial infarction in non-diabetic patients. Diabetes Care 2013;36:3328-3332.

[https://doi:10.2337/dc13-0119].
4. Desborough JP. The stress response to trauma and surgery. BJA: British Journal of Anaesthesia 2000;85(1):109-117. [https://doi.org/10.1093/bja/85.1.109].
5. Di Luzio R, Dusi R, Mazzotti A, Petroni ML, Marchesini G, Bianchi G. Stress Hyperglycemia and Complications Following Traumatic Injuries in Individuals With/Without Diabetes: The Case of Orthopedic Surgery. Diabetes Metab Syndr Obes 2020;13:9-17. [https://doi.org/10.2147/DMSO.S225796].
6. Esposito K, Nappo F, Marfella R, *et al.* Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation 2002;106:2067-2072. [https://doi:10.1161/01.CIR.0000034509.14906.AE].
7. Giri R, Giri K, Palandurkar M. Use of biochemical parameters in radiologically proved fracture healing property of Arjuna Terminalia, in rats. Res J Pharm, BiolChem Sci 2015;6(4):336-43.
8. Harp JB, Yancopoulos GD, Gromada J. Glucagon orchestrates stress-induced hyperglycaemia. Diabetes ObesMetab2016;18:648-653. [https://doi:10.1111/dom.12668].
9. Hing KA. Bone repair in the twenty-first century: Biology, chemistry or engineering. Philosophical Transactions: A Mathematics Physics English and Science Journa 2004;362(1825):2821-2850.
10. Kilic N. Physiological and hematological changes in ketamine and diazepam anesthesia in horse. Indian Journal of Veterinary 2004;81:396-398.
11. Komnenou A, Karayannopoulou M, Polizopoulou ZS, Constantinidis TC, Dessiris A. Correlation of serum alkaline phosphatase activity with the healing process of long bone fractures in dogs. Vet Clin Pathol 2005;34(1):35-8. [https://doi: 10.1111/j.1939-165x.2005.tb00006.x.].
12. Kumar Dharmendra, Bhargava MK, Singh Randhir, Shahi Apra, Aparajita J, Swami Madhu *et al.* Haematological Changes during Fracture Healing in Goats. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 2016;9(9):01-03. [https://doi: 10.9790/2380-0909010103].
13. Nilsson Bo E, Westlin Nils E. The Plasma Concentration of Alkaline Phosphatase, Phosphorus and Calcium Following Femoral Neck Fracture, Acta Orthopaedica Scandinavica 1972;43:6, 504-510. [https://doi: 10.3109/17453677208991272].
14. O'neill PA, Davies I, Fullerton KJ, Bennett D. Stress hormone and blood glucose response following acute stroke in the elderly. Stroke 1991;22:842-847.
15. Prasad K. Serum biochemical changes in rabbits on a regular diet with and without flax lignan complex following a high-cholesterol diet. The International journal of angiology: official publication of the International College of Angiology. Inc 2008;17(1):27-32. [https://doi.org/10.1055/s-0031-1278276].
16. Rich GA. Rabbit orthopedic surgery. Vet Clin North Am Exot Anim Pract 2002;5(1):157-68. [https://doi: 10.1016/s1094-9194(03)00051-3.].
17. Singh Ajai, Mahdi AA, Srivastava RN. Evaluation of serum alkaline phosphatase as a biomarker of healing process progression of simple diaphyseal fractures in adult patients. Int Res J Biol Sci 2013;2:40-43.
18. Umeshwori N, Kumar A, Saini NS, Singh S. Evaluation of closed and open intramedullary pinning for repair of tibial fractures in dogs. Indian Journal of Veterinary surgery 2015;36(1):33-36.
19. Wagner A, Muir W, Hinchcli K. Cardiovascular effects of xylazine and detomidine in horse. American Journal of Veterinary Research 2001;52:651-657.