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Haemato-biochemical Response to Kirschner Pin and Improvised Chrome Vanadium Long Screw Used for the Stabilization of Femoral Fracture in Goats

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ABSTRACT

The stainless-steel orthopaedic implants used for the management of various types of fracture internal fixations are very expensive; this makes their utilization in food animals relatively noneconomical. There is a need to have an improvised implant that is cheaper and available for use in food animals to manage complicated fractures requiring open reduction and internal fixation. The objective of this study is to evaluate the haematological and biochemical changes following the reduction of stable femoral fractures in goats using conventional (Kirschner pin) and improvised chrome vanadium-coated long crews. Twelve apparently healthy Red Sokoto bucks were randomly divided into two groups of six (n=6) were used for this study. A transverse femoral diaphyseal fracture was created using orthopaedic wire in both groups. In group A, a conventional Kirschner pin size 4.0x125 mm^2, single trocar, non-threaded, was used to immobilize the fracture, while in group B, an improvised test chrome vanadium (long screw) size 2.3 mm was used for the fracture immobilizations. Blood samples were collected before fracture induction at the base line, serving as a control. The haematological and biochemical assessments were performed at 0 weeks (immediate postoperative) and subsequently at 2, 4, 6, 8, 10 and 12 weeks postoperatively. Packed cell volume (PCV%), haemoglobin concentration (Hb), red blood cell count (RBCs), white blood cell count (WBCs) and differential leukocytic count (neutrophils, lymphocytes and monocytes) were evaluated. Also, serum activity of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) was detected. Besides, serum calcium, phosphorous and creatinine levels were evaluated. There were no significant differences between the two groups in PCV, haemoglobin, RBCs, WBCs and neutrophils, and the values were within the normal range. However, lymphocytes and monocytes were significantly ($p<0.05$) different at weeks 2 and 6, respectively. Biochemical parameters revealed significant ($p<0.05$) changes in serum ALT (weeks 0, 2, 4), ALP (week 6), creatinine (weeks 10 and 12), and calcium (week 2) at some postoperative intervals. However, no variations were observed in serum AST and phosphorous, which were within the normal range. The improvised chrome vanadium (Long screw) can be used safely in goats for the management of stable femoral fractures without significant adverse changes to hemato-biochemical profiles within twelve weeks.

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INTRODUCTION

Small ruminant production plays a very important role in the economies of many developing countries and the world because of its ease of management, low cost of production, quick maturity, and acceptability. This economic importance is primarily associated with their small size, as it favours low investment, a small risk of loss, and their high reproductive efficiency **(Jemberu *et al.*, 2022).** However, these animals are prone to many types of trauma, majorly associated with road traffic accidents

(RTA), most especially in developing countries where they are being managed under a semi-extensive system. In Nigeria, the increase in vehicular and motorcycle flow on roads has also led to an increase in the number of fracture cases in the roaming sheep and goat population **(FRSC, 2000).**

Rigid fixation of fractures involving femoral diaphysis requires open reduction and internal fixation, which is not sustainable in goats reared by low- and medium-income earners, mostly living in rural communities. Therefore, a trial of other affordable materials, such as chrome vanadium-coated long screws, could be another option. Evaluation of haematological and biochemical parameters may play a key role in monitoring fracture healing; it may also predict a possible systemic response to implants used for the management of the fracture. Haematological and biochemical deviation from the normal physiological reference range could be an indication of an abnormal healing process and may require immediate attention, failure of which may lead to delayed union, non-union, or malunion **(Umeshwori *et al*. 2015; Patil *et al*., 2017).**

Haematological assessment is a mirror of what is happening in the body, and it particularly guides and directs clinicians towards the health status of the animal. Cellular activities involving red blood cells (RBCs), lymphocytes, monocytes, segmented neutrophils, eosinophils and basophils are important in wound and bone healing **(Gabriel *et al*. 2014).** The detection of specific biochemical markers of bone formation in serum, such as alkaline phosphatase, calcium and phosphorus activity, can be clinically useful tools in predicting fracture healing and the risk of developing complications. It also aids the clinician in intervening properly **(Komnenou *et al*., 2005).** Therefore, the aim of this study was to evaluate the effects of the test implants in chrome vanadium (long screw) on haematological and biochemical parameters as a guide for evaluating the proper healing or complication occurrence during the femoral fracture healing process.

MATERIALS AND METHODS

Experimental animals and grouping

The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria. Twelve apparently healthy Red Sokoto bucks aged between 1 and 1.5 years, weighing between 12 and 17kg, were used for this experiment. All the bucks were clinically examined and determined to be healthy. They were kept in an experimental animal pen and fed on wheat bran and bean husk for three weeks before the commencement of the experiment. The goats were allowed to have access to feed and water *ad libitum* throughout the experimental period.

The goats were randomly grouped into two experimental groups; A and B, those treated using conventional Kirschner pin (1170-4500 Ø4.0mm Schanz Screw, CE 0434, 20160312) as positive control (group A) and those treated with improvised chrome vanadium long screw (J-S FOCUM(R) Screwdriver with 11 Inch Chrome Vanadium steel shaft for Repair of Home Improvement and Craft) as experimental test group (group B) **(Fig.1).** Operated limbs were randomly selected in each group. Feed and water were withheld for 12 and 6 hours respectively before the surgery in order to avoid regurgitation and aspiration pneumonia due to the effect of anesthetic agents.

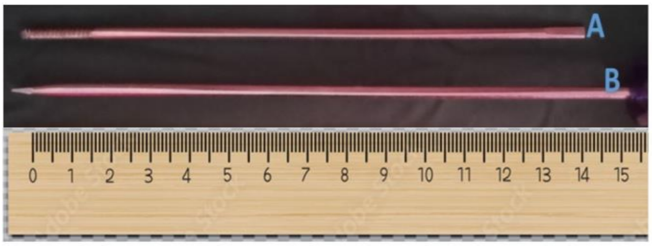

Fig.1: The conventional intramedullary pin Kirshner pin (A) and the improvised long screwdriver pin coated with chrome vanadium (B).

Fracture Model

The goats were pre-medicated with diclofenac sodium (Anhui Chengshi Pharmaceutical Co., Ltd., Anhui, China.) intramuscularly at a dose rate of 3 mg per kg, followed by sedation with Xylazine hydrochloride (VMD, Arendonk, Belgium) at a dose rate of 0.05mg per kg. Induction and maintenance of anaesthesia were achieved with ketamine hydrochloride (Swiss Parenterals PVT. LTD., Gujarat, India) at 5mg per kg body weight intravenously. The surgical site was prepared in a standard manner for aseptic surgery using chlorhexidine (Sarolifecare Limited, Ibadan, Nigeria) and povidone iodine solution. The femoral diaphysis was exposed, as described by **Piermattei and Greeley (2013).**

Using orthopaedic wire a simple transverse fracture was created at the mid-shaft of the femoral diaphysis. The fractures were reduced with traction and countertraction using retrograde insertion of a Kirschner pin for group A and a chrome vanadium long screw for group B. An immediate post-surgical radiograph was taken to ascertain the internal fixation and confirm the alignment of the fractured segment. All surgical procedures were conducted by the same surgeon, and each procedure finished within 30 minutes.

Haematology and Biochemical Analysis

The blood samples were collected for haematological and biochemical evaluation from the indwelling pre-placed catheter at the jugular vein. The serum was separated using a centrifuge (Model SH120, New Life Medical Instrument, England) at 4000 rpm for five minutes. The evaluated haematological parameters were packed cell volume (PCV%), haemoglobin concentration, red blood cell count (RBC), white blood cell count (WBC), and differential leukocyte count (DLC). The evaluation was done at a two-week interval, i.e., from 0-week to 12-week postoperative. While the determined serum biochemical parameters were calcium, phosphorous, activity of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzymes, and creatinine, which were evaluated at the same timing as in haematological parameters (from 0-week to the 12th week postoperative), Haemoglobin estimation (Hb) was carried out using the Cyanmethaemoglobin method.

The red blood cell count (RBC) and white blood cell count (WBC) were carried out using Neubauer's slide method. The packed cell volume (PCV) was determined using Microhaematocrit method as described by **Bull *et al*. (2000).** The differential leukocyte count was carried out according to the standard procedure described by **Rosenzweig and Fleisher, (2014).**

Serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST) activity were determined using kinetic UV method (IFCC), Manufactured by Randox laboratories Ltd. Serum alkaline phosphatase was determined using PNPP-AMP Kinetic Assay Method, Manufactured by Randox laboratories Ltd. The creatinine level was determined using colorimetric Assay Method, Manufactured by Randox laboratories Ltd. Serum Calcium was determined using atomic absorption spectrometry and phosphorous was determined using spectrophotometry method.

Statistical Analysis

The Data collected were presented as mean±SEM. Data analysis was performed using two way repeated measure mixed model ANOVA, with treatment factor group and repeated factor weeks. Multiple comparisons were performed using Benferroni's Post-hoc test. Probability value of < 0.05 was considered statistically significant at 95% confidence interval using statistical software InVivoStat (V3.0).

RESULTS

Haematological Changes

PCV values were within the normal range; there was no significant difference (P > 0.05) at different postoperative intervals. Even though a continuous, gradual increase in the PCV was observed, it peaked at the 10th week (26.50±1.33) and 12th week (26.83±2.03) in groups A and B, respectively .

There was an increase in the value of Hb postoperatively from the 2nd week onward for both groups and subsequently fluctuating, but the values were not significantly different at any interval. The RBC count decreases on the 2nd week postoperatively in group A but increases in group B, but not significantly **(Table 1).**

The total WBC count decreased on the 2nd week postoperatively in both groups. However, the values were not significantly different at any postoperative healing interval **(Table 1).** The neutrophil percentage increased in group A and decreased in group B on the second week post-operatively, but there was no significant difference observed at any postoperative healing interval (**Table 1**).Lymphocytes peak value (53.50±1.80) was recorded in group A at the 6thpostoperative week, whereas in group B the peak value (63.00±6.89) was recorded on the 4th week postoperative. There was significant difference (P<0.05) among the lymphocytic values at the second week but all the values were within normal physiological range **(Table 1).**

The mean monocytes value was higher on the 8th week postoperatively when compared with preoperative days in all the groups. However, it fluctuated within the normal physiological limits in all the groups with a significant difference (P<0.05) recorded on the 6th postoperative week **(Table 1).**

Biochemical Changes

Serum ALT differed significantly (P<0.05) at various stages of the postoperative period. The highest value (43.22±14.05) was observed on the 2nd week for group A, while 40.26±15.58 was recorded for group B at the 4th week postoperatively **(Table 2).** The serum AST values did not show any significant variation in both groups throughout the post-operative period (12 weeks) **(Table 2).**

The preoperative serum ALP values were higher when compared to postoperative intervals in both groups. Although the values did not show any significant difference (P<0.05) except on the 6th week postoperatively **(Table 2).**

The highest value of creatinine was recorded at the 6th and 4th postoperative weeks (38.22±10.32 and 38.41±4.11) for group A and B respectively. A significant difference (P<0.05) was observed at the 10th and 12th week in groups A and B. The serum calcium values showed a significant difference (P<0.05) at the second week postoperative only . The mean value of the serum phosphorous showed no significant variation during different period postoperatively **(Table 2).**

Table 1: Haematological changes at different post-operative periods in group A and B:

Parameters	Weeks in Intervals						
	0	2	4	6	8	10	12
PCV % (A)	23.67 ± 1.48	24.33 ± 1.52	25.00 ± 1.07	25.00 ± 1.16	25.17 ± 1.49	26.50 ± 1.33	26.17 ± 1.32
PCV % (B)	22.83 ± 0.70	23.50 ± 0.77	24.50 ± 1.48	24.17 ± 0.88	23.83 ± 1.01	25.00 ± 1.03	26.83 ± 2.03
Hb g/dl (A)	7.70 ± 0.49	8.12 ± 0.52	8.19 ± 0.38	8.49 ± 0.53	8.29 ± 0.29	8.93 ± 0.32	8.33 ± 0.45
Hb g/dl (B)	7.52 ± 0.23	7.93 ± 0.28	7.72 ± 0.51	7.93 ± 0.28	7.70 ± 0.31	7.47 ± 0.34	8.82 ± 0.68
RBCx10^6/mm^3 (A)	14.67 ± 0.89	13.42 ± 1.32	16.33 ± 0.98	14.71 ± 1.15	15.58 ± 0.82	15.85 ± 1.13	16.14 ± 1.05
RBCx10^6/mm^3 (B)	14.18 ± 0.64	14.79 ± 0.71	14.44 ± 1.12	14.61 ± 1.10	14.41 ± 0.78	14.27 ± 0.83	17.28 ± 1.26
WBCx10^3/mm^3 (A)	12.95 ± 1.65	11.93 ± 0.67	15.24 ± 0.87	12.93 ± 1.09	14.59 ± 0.68	13.37 ± 0.81	13.41 ± 1.89
WBCx10^3/mm^3 (B)	12.30 ± 0.75	11.75 ± 1.32	12.74 ± 1.16	13.24 ± 0.87	13.80 ± 1.18	11.32 ± 1.00	13.25 ± 1.19
Neutrophils % (A)	49.00 ± 3.71	54.33 ± 4.03	56.00 ± 2.94	44.67 ± 2.09	49.50 ± 2.10	50.83 ± 3.49	49.00 ± 4.97
Neutrophils % (B)	47.67 ± 2.67	44.33 ± 1.98	36.00 ± 6.12	46.83 ± 4.90	47.50 ± 7.98	42.67 ± 4.60	42.67 ± 4.34
Lymphocytes % (A)	48.33 ± 3.48	45.17 ± 4.12*	40.83 ± 2.86	53.50 ± 1.80	46.17 ± 2.05	45.67 ± 3.78	46.83 ± 4.30
Lymphocytes % (B)	51.33 ± 2.61	54.67 ± 2.12*	63.00 ± 6.89	49.67 ± 4.73	48.17 ± 7.33	53.00 ± 4.08	57.33 ± 4.84
Monocytes % (A)	1.50 ± 0.42	0.50 ± 0.50	1.67 ± 0.49	1.00 ± 0.26*	3.17 ± 0.88	2.50 ± 0.42	1.00 ± 0.26
Monocytes % (B)	1.00 ± 0.26	0.83 ± 0.30	1.83 ± 0.88	2.00 ± 0.63*	2.50 ± 0.67	2.50 ± 0.80	1.17 ± 0.40

Means with * shows a significant difference ($p < 0.05$) between the two experimental groups.
Group A: Femoral diaphyseal fracture reduction using Kirschner pin.
Group B: Femoral diaphyseal fracture reduction using long screw coated with chrome Vanadium.

Table 2: Serum biochemical values in pre and postoperative weeks (A and B):

Parameters	Week in Intervals						
	0	2	4	6	8	10	12
ALT U/l (A)	41.02 ± 9.81*	43.22 ± 14.05*	18.32 ± 2.92*	17.64 ± 2.28	15.78 ± 1.60	16.74 ± 2.44	15.07 ± 1.88
ALT U/l (B)	19.17 ± 2.00*	27.93 ± 6.36*	40.26 ± 15.58*	29.61 ± 13.16	17.81 ± 2.40	15.80 ± 1.72	15.45 ± 2.02
AST U/l (A)	44.31 ± 2.30	45.98 ± 2.54	38.39 ± 3.34	43.89 ± 3.31	34.72 ± 2.42	36.20 ± 2.95	39.52 ± 3.27
AST U/l (B)	40.39 ± 4.73	36.64 ± 4.87	37.42 ± 4.79	45.80 ± 2.79	38.39 ± 2.84	39.00 ± 3.91	39.69 ± 2.16
ALP U/l (A)	282.90 ± 64.09	259.90 ± 48.09	209.30 ± 68.28	143.98 ± 62.50*	152.24 ± 38.58	109.02 ± 43.21	99.76 ± 29.99
ALP U/l (B)	330.30 ± 53.39	242.39 ± 54.98	113.40 ± 34.58	91.77 ± 24.87*	104.42 ± 28.81	178.48 ± 52.69	159.16 ± 37.54
CRE **µ**mol/l(A)	28.80 ± 5.07	35.65 ± 8.57	32.77 ± 2.83	38.22 ± 10.32	33.82 ± 10.83	26.84 ± 3.69*	32.57 ± 2.49*
CRE **µ**mol/l (B)	20.41 ± 2.91	26.60 ± 5.06	38.41 ± 4.11	33.19 ± 3.62	36.34 ± 7.91	36.18 ± 0.81*	35.89 ± 1.11*
CALmg/l (A)	0.07 ± 0.00	0.07 ± 0.00*	0.06 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	0.07 ± 0.00
CAL mg/l (B)	0.07 ± 0.00	0.08 ± 0.00*	0.07 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.08 ± 0.00
PHO mg/l (A)	0.64 ± 0.09	0.56 ± 0.02	0.59 ± 00	0.60 ± 00	0.58 ± 00	0.58 ± 00	0.54 ± 0.03
PHO mg/l (B)	0.70 ± 0.07	0.61 ± 0.04	0.58 ± 00	0.61 ± 00	0.59 ± 00	0.61 ± 00	0.54 ± 0.03

Means with * shows a significant difference at ($p< 0.05$) between the two experimental groups.
Group A: Femoral diaphyseal fracture reduction using Kirschner pin.
Group B: Femoral diaphyseal fracture reduction using chrome vanadium long screw.

DISCUSSION

In the present study, a non-significant increase in the values of haemoglobin concentration and PCV was observed at the 2nd post-operative week in both groups. While the RBC count decreased in the Kirschner pin group (group A) and increased in the chrome vanadium long screw group (group B) on the 2nd week postoperatively, this was followed by a transient fluctuation of the values within the normal physiological range. The increase in PCV, haemoglobin concentration and erythrocyte count on postoperative days could be an indication of erythropoiesis, as observed in related studies by **Gabriel *et al*. (2014); Dharmendra *et al.*, (2016) in goats, Singh *et al.* (2008);Tembhurne *et al.* (2010), and Patil *et al*., (2017)** in dogs. They observed non-significant variation in the values of these parameters from baseline to postoperative values within the normal physiological limit. The decline in the value of the RBC count in group A on the second week may be attributed to the blood loss during the surgical procedure. Total leucocyte count showed an insignificant decrease on the 2nd week in both groups and subsequently, the values increased on the 4th week in both groups A and B and later fluctuated onwards.

These findings were contrary to the findings of **Gabriel *et al.,* (2014); Dharmendra *et al.,* (2016)** in goats and **De'Souza (2012)** in canines, where a slight increase in WBC count post-operatively was reported. The increase in WBCs on the 4th week postoperatively could be attributed to a normal response to trauma or stress of confinement **(Binuramesh *et al*., 2005; Rau *et al*., 2023).**

An insignificant increase in neutrophils was observed in group A on the second and fourth weeks postoperatively, which subsequently fluctuated onwards, while a non-significant decrease was recorded in group B on the 2ndand and 4th weeks postoperatively, and the values fluctuated onwards within the normal range. The increase in the value of neutrophils after surgery could be attributed to the immediate onset of an inflammatory response to the surgical trauma inflicted, as reported by **Tembhurne *et al.,* (2010); De'Souza (2012); Dharmendra *et al*., (2016); Alimi *et al*., 2020** in various species of animals. The increase in lymphocyte count observed in group B could be due to tissue injury during surgical interventions or inflammation resulting in the production of immuno-regulatory cytokines (lymphokines and monokines) by macrophages and monocytes **(Hsing and Wang, 2015).**

These cytokines are responsible for activating pituitary-adrenal axes to release glucocorticoids, which in turn cause lysis of the lymphoid tissues and a reduction in circulating lymphocytes **(Alimi *et al*., 2022).** The variation observed in the monocyte counts in both could be due to inflammatory conditions at the surgical site as well as stress during fracture healing **(Shema *et al*., 2024).** These findings were contrary to the observations made by **Dharmendra *et al*., (2016)** in goats, where non-significant variation in the values of monocyte count at different post-operative intervals during fracture healing was observed.

The values of alanine aminotransferase (ALT) were found to be statistically significant in both groups at the preoperative week and subsequently at 2nd and 4th postoperative weeks. Although, the values were within the normal range, a marked elevation is usually accompanied by hepatocellular injury or hyperactivation of hepatocytes in the production of healing-associated molecules such as fibronectin and IGF **(Ndrepepa and Kastrati, 2019).**

The values of aspartate aminotransferase (AST) fluctuated at different post-operative intervals in both groups, and comparisons between the groups showed no significant changes. Similar observations were also reported by **Tembhurne *et al*., (2010**) in canines during the period of fracture healing. The serum alkaline phosphatase level was significantly higher in the pre-operative week in both the Kirschner pin group and the chrome vanadium long screw group. The elevation of the alkaline phosphatase level is usually associated with the proliferation of osteogenic cells from the periosteum of a destructed bone, which is a rich source of alkaline phosphatase **(Komnenou *et al*., 2005).** These results are in agreement with the earlier workers; **Hegade *et al*., (2007); Phaneedra *et al.,* (2016)** in canines. The decrease in serum alkaline phosphatase values observed at different post-operative intervals in both groups was contrary to the observations reported by **Umarani and Ganesh (2003); Ghosh *et al*., (2003).** They reported increased serum alkaline phosphatase activity throughout the study period, which was attributed to muscle, skin trauma and the early stages of bone repair.

A major increase in the activity of the biochemical parameters ALP and S-bone ALP in the first two weeks indicates inadequate fracture fixation, delayed bone healing and the formation of a visible and significant callus **(Muljacic *et al*., 2013).** The overall pattern of ALP expression during fracture healing includes an initial drop, followed by a gradual increase until reaching a peak, and finally returning to near-baseline levels once healing is complete **(Laurer *et al*., 2000; Nakagawa *et al*., 2006**). Although, the serum creatinine values were within the normal range but the significant differences observed could be due to stress during fracture healing **(Fermandez and Kidney, 2007).** On the contrary, **Tembhurne *et al.,* (2010)** reported no significant changes in serum cretinine levels at different postoperative periods in canines during fracture healing.

The variation in serum calcium level at the initial stage may be a result of a rise in osteoclastic activity, leading to the resorption of dead bone cells **(Vinit, 2018).** The decrease in serum calcium levels at the 4th week could be due to the deposition of excess calcium at the fracture site. **Nagaraja *et al*., (2003); Umarani and Ganesh (2003)** reported similar observations following internal fixation of femoral fractures in goats. The serum phosphorous showed no significant variation postoperatively, and the values were within the normal range. Similar observations were reported by **Chandy (2000).**

CONCLUSION

The study showed that the use of improvised long screws coated with chrome vanadium as internal fixatives has little or no detrimental effect on haematological and biochemical parameters during fracture healing in goats. The long screw coated with chrome vanadium is readily affordable and accessible, and if properly sterilized could serve as an alternative to conventional intermedullary implants for stabilization of stable femoral diaphyseal fractures in goats. It is recommended that further studies be conducted to evaluate the histopathological changes and to determine the immunogenic and carcinogenic effects of the long screw coated with chrome vanadium as an implant for fracture reduction in goats.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author's contribution missing

AUS and BS conceptualized the study and designed the experiment. AAA, YAS and MPC conducted the surgery while AMS conducted the blood sampling, hematological and data analysis. All authors participated in the manuscript draft.

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