

## Final report of ITK project

National Agriculture Technology Project  
Indian Council of Agriculture Research, New Delhi

# “Studies on the efficacy of Harjore (*Cissus quadrangularis*) on the fracture healing in animals”



*Submitted by*

**Dr. A. C. Varshney,**

**Principal Investigator**

**Dr. S. P Tyagi,**

**Co-Principal Investigator**

**Dr. S. K. Sharma,**

**Co-Principal Investigator**

**Dr. Adarsh Kumar,**

**Co-Principal Investigator**

**Department of Surgery and Radiology  
College of Veterinary and Animal Sciences  
CSKHPKV, Palampur, 176062 (H.P.)**

## Final progress report of the ITK project

**Code No. of ITK:**

**Title of the ITK:** “Studies on the efficacy of Harjore (*Cissus quadrangularis*) on the fracture healing in animals”

**Name of PI:** Dr. A. C. Varshney, Professor and Head, Department of Surgery and Radiology, COVAS, CSKHPKV, Palampur 176062

**Name of Co PI's:** Dr. S. P Tyagi, Assistant Professor, Dr. S. K. Sharma, Associate Professor, Dr. Adarsh Kumar, Assistant Professor, Department of Surgery and Radiology, COVAS, CSKHPKV, Palampur 176062

**Date of start of the sub project:** 1<sup>st</sup> March 2004

**Date of termination of the sub project:** 15 Sept 2004

**RA/SRF/JRF employed:** Nil

**Brief financial status:**

Original sanctioned amount: 1.49 lac

Revised sanctioned amount: 1.00 lac

Actual expenditure: 1.00 lac

**Introduction:** The role of herbal preparations in fracture healing has been documented extensively. A number of herbal preparations have been used as paste, oral decoction or even for injections in various experimental studies with variable effects. Harjore (*Cissus quadrangularis*) is one of such plant whose paste and has been shown to have positive influence on fracture healing process. The present study is proposed to verify the fracture-healing promotion activities of Harjore in a controlled scientific experimentation in animals.

**Objectives:**

- To study the efficacy of Harjore (*Cissus quadrangularis*) on the fracture healing in animals by clinical and haematological observations.
- To study the efficacy of Harjore (*Cissus quadrangularis*) on the fracture healing in animals by radiological and angiographic observations.

**Description of ITK:** Harjore is a perennial climber, which is used in treatment of bone fracture in animals as well as in humans. This practice is being used by the villagers of Samtoli village of Simdega district in Jharkhand for the last many years. Paste is preferred by crushing the Harjore plant and it is applied on the fracture part and then tied along with sticks. At every day interval it is replaced by fresh Harjore paste and this process is continued two to three times.

**Name and address of the discloser:**

Ms. Sushmita Khalkho, C/O Dr. Niva Bara, Department of Extension Education, Birsa Agriculture University, Ranchi (Jharkhand) 834006

**Location of the use of ITK:** Village Samtoli, block Simdega, Simdega (Jharkhand)

**Geographical indicators:** Harjore, a climber with stone fleshy quadrangular stems is found throughout the hotter parts of India and Sri Lanka

**Significant achievements:**

**Methodology:**

The experimental study was conducted in the Department of Surgery and Radiology, COVAS, CSKHPKV, Palampur (Himachal Pradesh). The necessary approval for conduct of animal experimentation was obtained from appropriate animal ethics committee prior to start of the study. The study was conducted on six adult healthy mongrel dogs of either sex weighing between 15-20 Kg divided into two equal groups. The first group (A) was kept as control group whereas the second group (B) served as test group.

Prior to the start of experiment, the animals were acclimatized in college kennels as per standard practice. The animals were dewormed with suspension albendazole (Albomar, Agrivet India Ltd) @ 10 mg/Kg body weight orally and vaccinated prophylactically against rabies by Inj. Rakhsharab (Indian Immunologicals) @ 1 ml per animal given subcutaneously. All the animals were maintained on standard and uniform diet during the entire course of study.

**Creation of fracture:**

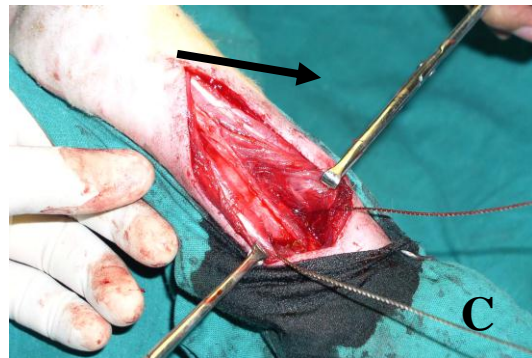
All the animals were prepared for aseptic surgery routinely. They were anaesthetized by Inj. xylazine and ketamine @ 2 and 10 mg/kg body weight respectively given intramuscularly 15 minutes after subcutaneous injection of atropine @ 0.045 mg/kg. The left ulnar bones of all the animals were subjected to diaphyseal fracture by osteotomy done with the help of giggly wire saw, chisel and hammer (Fig 1 B, C and D). After the creation of fractures, the surgical wounds were closed routinely (Fig 1E).

**Preparation of Harjore Paste/ Ointment:**

The Harjore (*Cissus quadrangularis*) plant stems were collected and completely dried in oven. These were then finely grounded in a grinder to form powder. Twenty grams of this powder was used for one application per animal of group I. Just before application the powder was mixed with sufficient quantity of liquid paraffin to form a paste.

**Application Technique:**

The paste thus prepared was applied on the skin around the fractured limb and covered with a layer of cotton bandage in the animals of group B. The fractured limbs were then stabilized suitably by means of a bi-valved plaster of Paris full limb cast and secured with adhesive tape and bandages. The Harjore paste was reapplied every at every 4 days interval till 20<sup>th</sup> postoperative day. No medication was used / applied on the fractured limb of animals of group A, however its fractured limbs were also supported by external cooptation as applied in group B.



**Fig 1**

- A Harjore (*Cissus quadrangularis*) plant
- B Incision site of operation
- C Osteotomy of ulna
- D Fracture line in ulna after osteotomy (arrow)
- E Closure of surgical site
- F Cannulation of brachial artery for angiography
- G External cooptation by POP cast



## **Evaluation of fracture healing:**

### **A) Clinical observations:**

All the animals were clinically examined regularly for the development and progress of inflammation and oedema at the fracture site. The extent of lameness, weight bearing capability and extent of pain was also recorded. Besides this, the routine clinical parameters such as rectal temperature, respiration rate and heart rate were also recorded at 0, 3, 7, 15, 30, 45 and 60 days postoperatively. The extent of lameness was evaluated during standing and locomotion phase as follows:

<b>S.N.</b>	<b>Signs</b>	<b>Extent of lameness</b>
1	Animal not bearing weight on affected limb	+++
2	Animal is occasionally touching the toes	++
3	Animal is frequently touching the toes	+
4	Animal is bearing full weight on affected limb	-

### **B) Haematological studies:**

5 ml blood was collected from cephalic/recurrent tarsal vein of all the dogs in heparinized syringes on days 0, 3, 7, 15, 30, 45 and 60 days postoperatively. A part of it was used for estimation of the levels of haemoglobin (Sahli's method), PCV (microhaematocrit method), TEC, TLC (Neubaur chamber) and DLC using wright's staining technique.

### **C) Biochemical Studies:**

The plasma was separated out from the remaining blood samples and the concentration of alkaline phosphatase, calcium and phosphorus were estimated using Semi-automatic Chemistry analyzer (RA-50, Bayer India Ltd).

### **D) Radiological Examinations:**

Plain medio-lateral radiographs of the fractured limb were taken at 0, 15, 30, 45 and 60 days post operatively in all the animals using standard radiographic exposure factors. These radiographs were studied for assessment of fracture healing process.

### **E) Angiographic studies:**

Angiograms of the fractured limb were obtained at 0, 15, 30, 45 and 60 postoperatively. Arteriography was carried out in all the animals under general anaesthesia as described earlier taking routine aseptic precautions. Their brachial artery of the fractured limb was exteriorized and cannulated using 20 G intravenous canula (Fig 1F). The contrast agent containing Diatrizoate acid and Meglumine (Contrastin-76%, Dabur India Ltd) was injected @ 20 ml per animal rapidly in to the brachial artery. The mediolateral radiographs were taken immediately after injection using standard radiographic exposure factors. The cannula was removed and the brachial artery was sutured using 6-0 Vicryl (Ethicon). The surgical wound was closed routinely. The arteriograms



were evaluated and compared and the course, number, contour and caliber of the vessels supplying the fractured area to assess the amount of blood supply at the site.

**F) Statistical analysis:** Statistical analysis of the data was carried out using student's t test and comparison between the treatments and days was done at 5 % level of significance.

### Results and discussion:

#### Clinical observations:

The rectal temperature, respiration rate and heart rate in the animals of both the groups remained within the normal range and did not show any significant change during entire course of study (Table 1, 2 & 3). Feed intake was also normal in all the animals of both groups throughout the period of study. The animals of both the therapeutic groups showed moderate pain and lameness in the affected limb up to day 3 postoperatively. The pain gradually reduced in all the animals subsequently. The rate of decline of the pain was however greater in the animals of group B when compared to control group (A). Accordingly in general the animals of group B started early complete weight bearing on the affected limb from 25<sup>th</sup> day onwards as compared to 38<sup>th</sup> day in control group (Table4).

**Table-1:** Effect of fracture healing using Harjore (*Cissus quadrangularis*) paste on rectal temperature in dogs.

Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	101.93± 0.13	101.86± 0.13	101.8± 0.2	101.93± 0.13	102.06± 0.18	101.6± 0.23	102± 0.12
Group-B (N=3)	100.86± 0.24	101.06± 0.44	101.13± 0.24	101.73± 0.27	101.73± 0.18	101.26± 0.48	101.53± 0.29

\*= Day at which treatment was instituted.  
P> 0.05 between days as well as groups.

N= Number of animals in each group.  
Group A – Untreated control  
Group B- Animals treated with Harjore paste

**Table: 2-** Effect of fracture healing using Harjore (*Cissus quadrangularis*) paste on respiration rate of animals.

Group No.	Postoperative days						
	0	3	7	15	30	45	60
Group-A (N=3)	39 ± 2.0	41 ± 3.0	42± 3.0	41 ± 2.0	42 ± 4.0	40 ± 2.0	38 ± 1.0
Group-B (N=3)	38 ± 3.0	36 ± 4.0	38 ± 3.0	44 ± 5	39 ± 4.0	40 ± 6.0	40 ± 3.0

**Table-3** Effect of fracture healing using Harjore (*Cissus quadrangularis*) paste on heart rate of animals.

Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	92 ±3.0	93 ±3.0	95 ±4.0	95 ±3.0	94±2.0	91±3.0	93± 2.0
Group-B (N=3)	86 ± 1.0	86 ± 2.0	92 ± 4.0	86 ± 3.0	75 ± 4.0	86 ± 2.0	86 ± 2.0

**Table 4:** Extent of Lameness in animals in control and test groups

Group No.	Position of animal	Postoperative days						
		0	3	7	15	30	45	60
Group A (Control)	Standing	+++	+++	+++	++	+	-	-
	Moving	+++	+++	+++	++	++	+	
Group B (Test)	Standing	+++	+++	++	+	-	-	-
	Moving	+++	+++	+++	++	+		-

**Haematological observations:**

All the animals showed an insignificant rise in neutrophils count in the immediate postoperative period which gradually fell back to normal by 14<sup>th</sup> day in most of the animals. No significant change was observed in any of the other haematological parameters throughout the period of study in all the animals of both the groups when compared in between and also within the groups (Table 5, 6, 7, & 8).

**Table 5:** Effect of fracture healing using 'Harjore' on haemoglobin (gm %) of animals.

Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	9.8±0.4	9.6±0.8	10.2±0.6	10.8±0.6	10.0±0.4	9.8±0.40	10.06±0.40
Group-B (N=3)	10.4±0.10	10.6±0.20	10.2±0.80	10.2±0.30	10.4±0.4	10.2±0.10	10.2±0.50

**Table 6:** Effect of fracture healing using 'Harjore' paste on PCV (%) of animals.

Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	41.4±1.6	42.6±2.0	45.2±2.4	44±2.4	44±2.0	45.2±1.4	44.6±2.6
Group-B (N=3)	44.2±2.6	42.6±3.2	44±2.4	42±1.2	44±2.0	44.8±0.6	44.6±1.6

**Table 7:** Effect of fracture healing using 'Harjore' paste on TLC (thousand/cumm) of animals

Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	14.41±2.52	14.44±0.92	15.02±2.49	15.49±3.99	14.51±4.22	14.44±3.42	14.56±4.68
Group-B (N=3)	13.21±1.81	14.2±1.82	14.56±1.6	14.91±4.95	14.15±4.90	14.31±5.5	14.81±2.2

**Table 8:** Effect of fracture healing using ‘Harjore’ paste on DLC of animals.

Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	N 77.5±3.0 L 21.5±2.5	78.5±2.0 21±2.0	84.5±4.0 22.5±3.0	76±3.0 22±4.0	75.5±4.5 23.5±4.0	78.5±3.5 20.5±4.5	78±3.0 22±3.0
Group-B (N=3)	N 72±2.0 L 26±2.0	76±1.0 26±3.0	78.5±2.5 24.5±1.5	71.5±1.5 25.5±3.5	73.5±1.5 24.5±2.5	73±2.5 26±2.5	74.5±1.0 24±1.5

**Biochemical observations:**

Calcium levels in blood slightly increased in group B and slightly decreased from 0 day to 3<sup>rd</sup> day however, the variation in these values were insignificant. The blood calcium levels remained at a slightly higher but insignificant levels in both the groups at all subsequent observation intervals except on day 7<sup>th</sup> (Table 9). The blood phosphorous values also remained at slightly higher levels on most of the observation intervals except on day 3<sup>rd</sup> in group B; whereas, in group A the phosphorous levels were higher continuously after 7<sup>th</sup> postoperative day. The change was however not statistically significant between the days as well as between the groups (Table 10) The alkaline phosphatase levels in the blood of the animals of both the groups was significantly higher on 3<sup>rd</sup> and 7<sup>th</sup> day and became normal subsequently by 14<sup>th</sup> day (Table 11).

**Table 9:** Effect of fracture healing using ‘Harjore’ paste on calcium conc. (mg/dl) of animals

Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	9.9±1.23	9.1±1.53	9.6±1.16	10.5±0.64	10.3±1.52	10.8±0.15	10.4±0.60
Group-B (N=3)	7.9±0.76	8.5±1.58	7.5±1.21	9.2±0.36	9.76±0.88	9.4±2.15	9.26±1.29

**Table 10:** Effect of fracture healing using ‘Harjore’ paste on phosphorus conc. (mg/dl) of animals

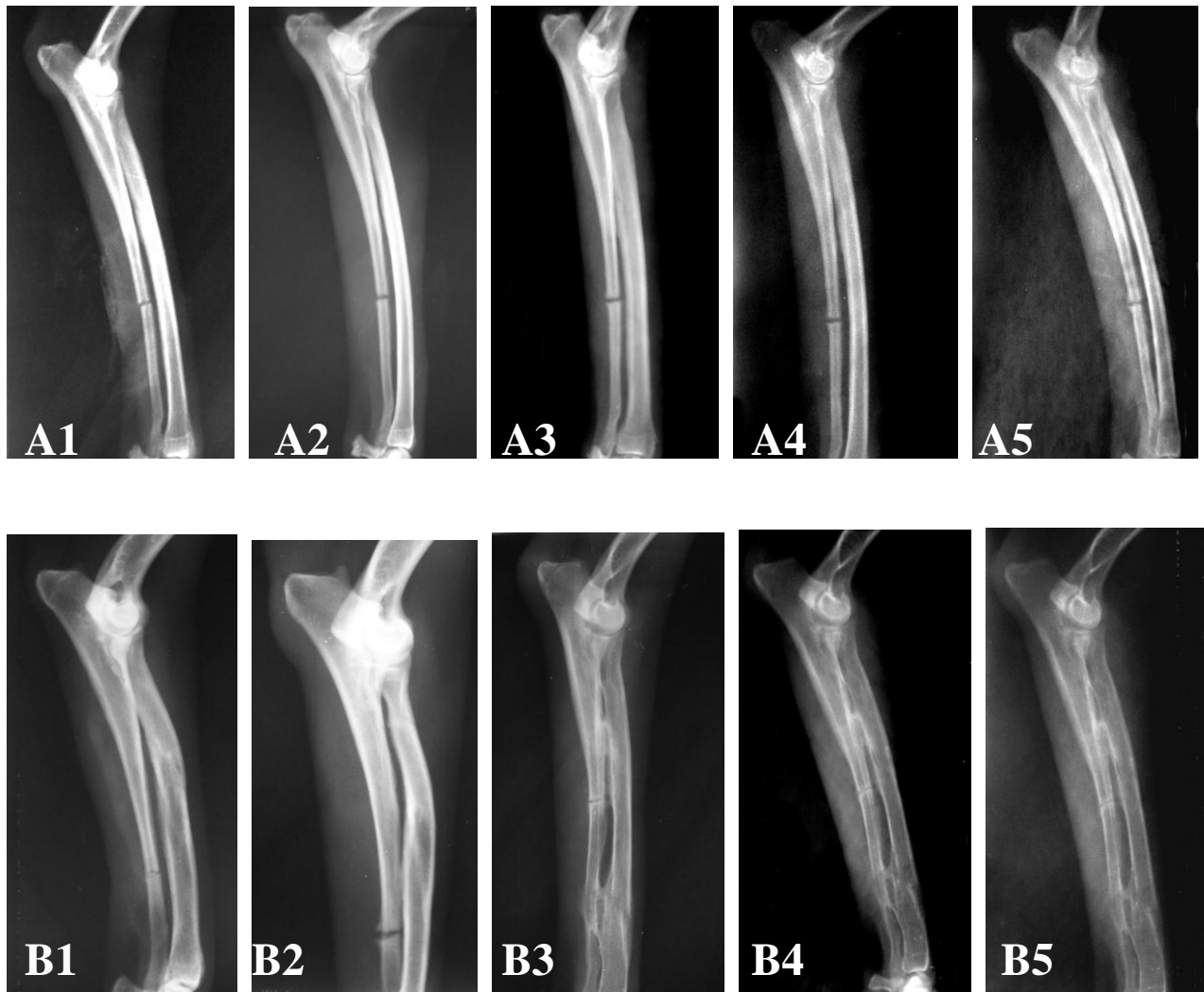
Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	5.13±0.17	5.0±0.34	5.1±0.70	5.7±0.28	5.8±0.11	6.36±0.68	6.10±0.52
Group-B (N=3)	3.90±0.26	3.66±0.48	4.23±0.23	5.0±0.46	5.4±0.88	5.9±0.81	4.5±0.32

**Table 11:** Effect of fracture healing using ‘Harjore’ paste on alkaline Phosphatase (U/L) of animals.

Group No.	Postoperative days						
	0*	3	7	15	30	45	60
Group-A (N=3)	124±8.0	150±9.0	143±6.4	125±9.0	124±10	133±5.8	118±4.2
Group-B (N=3)	118±18	150±13	150±17	117±7.2	108±12	126±21	112±8.0



### Radiological Evaluation:



**Fig 2**

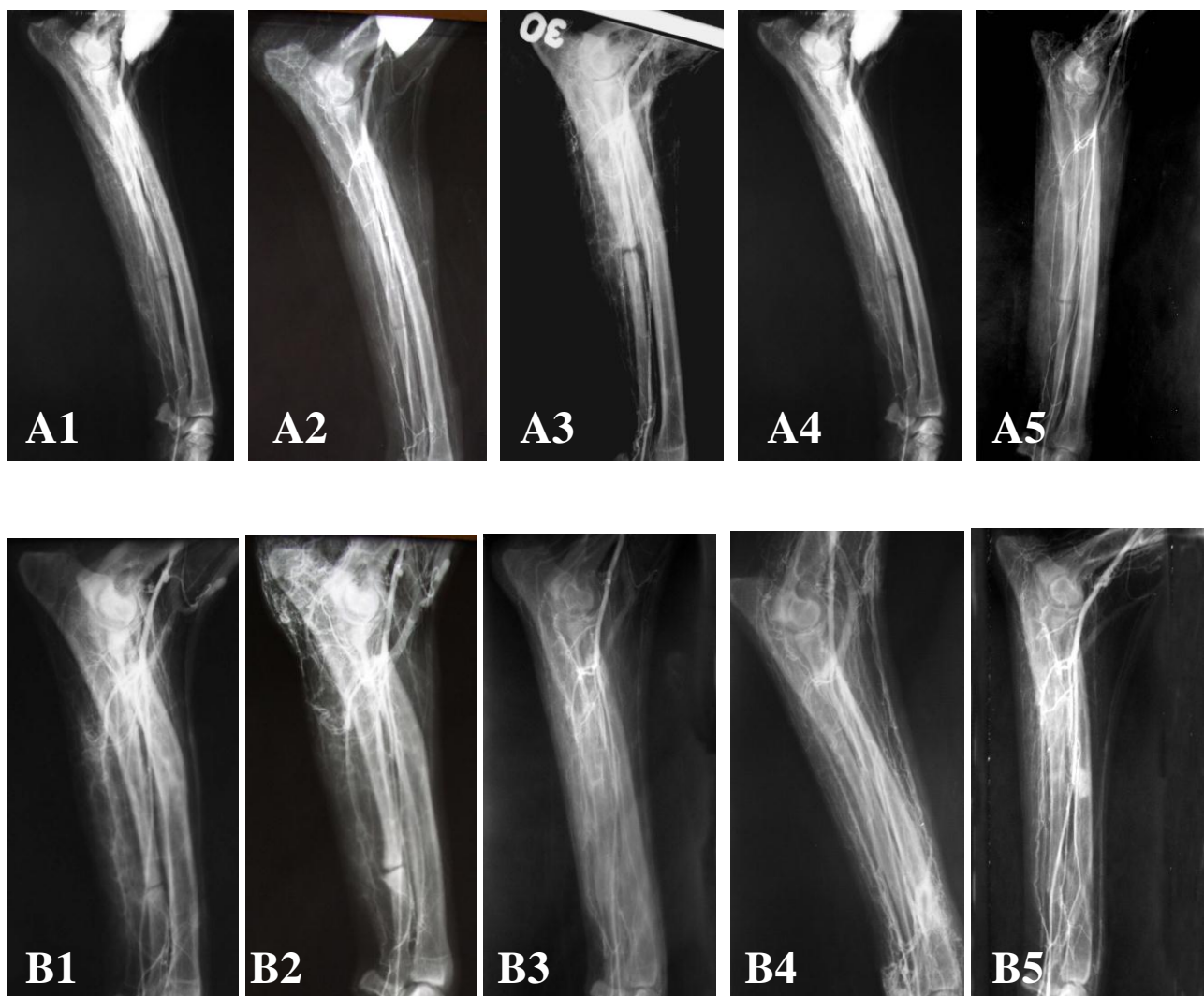
**A** Radiographs of control limb at day 0 (A1), 15 (A2), 30 (A3), 45 (A4) and 60 (A5) after fracture

**B** Radiographs of test limb (Harjore treated) at day 0 (B1), 15 (B2), 30 (B3), 45 (B4) and 60 (B5) after fracture

The radiographs taken immediately after creation of fracture and coaptation of limb at day '0' showed the presence of a clear radiolucent line of fracture in the mid diaphysis of ulna in all the animals of both groups (Fig 2 A1 and B1). In both the groups this fracture line appeared widened in the radiographs taken on day 15<sup>th</sup> postoperatively (Fig 2 A2 and B2). However in group B this widening was restricted more or less only in the outer third portion of the fracture line. Moreover, development of periosteal callus from both ends of fracture fragments was clearly visible in group B whereas no callus activity was identifiable in group A at this stage. The bridging of periosteal callus was complete at day 30<sup>th</sup> postoperatively in the animals of group B whose radiolucent fracture line also turned hazy toward transcortical aspect of ulna at fracture site indicating active osteogenesis (Fig 2 B3). In the animals of group A, the development of periosteal callus was also clear as small radioopaque areas extending beyond the cis and trans cortical margins of ulna at the fracture site in the radiographs taken on day 30<sup>th</sup> postoperatively, however the callus was still at its

preliminary stage and was not of bridging nature (Fig 2 A3). In the radiographs taken at day 45, remodeling of periosteal callus as evident by reduction in its volume was very well evident in all the animals of group B (Fig 2 B4). In this group, the endosteal callus activity in the form of increased radiodensity on endosteal site of the ulnar bone was also clear in all the animals. The fracture line turned hazy but still visible in group B at this stage. Whereas in animals of group A, the bridging of callus was still not complete radiographically even at this stage and the radiolucent fracture line was clearly visible (Fig 2 A4). The radiographs taken on day 60<sup>th</sup> postoperatively revealed further progress in fracture healing in both the groups. However, on comparative basis the fracture union was far more advanced in all the animals of group B then group A. In group B, the medullary continuity was restored, further reduction in the amount of endosteal and periosteal callus was seen and the fracture line was barely visible at this stage (Fig 2 B5). Whereas, in group A, the fracture line was clearly visible, amount of periosteal callus still moderate and the medullary continuity not restored at this stage.

### Angiographic Evaluation



**Fig 3**

**A** Angiograms of the fracture site of control limb at day 0 (A1), 15 (A2), 30 (A3), 45 (A4) and 60 (A5) after fracture

**B** Angiograms of the fracture site of test limb (Harjore treated) at day 0 (B1), 15 (B2), 30 (B3), 45 (B4) and 60 (B5) after fracture

At day 0 after creation of fracture, the angiograms were obtained immediately after injection of contrast agent into the brachial artery at the distal third humeral region. They revealed complete opacification of the brachial artery and its distal branches throughout the distal limb in all the animals of both the groups. The relatively wider brachial artery branched into thinner collateral ulnar artery, superficial brachial artery and median artery at the proximal metaphyseal level of radius bone. The main trunks of these branches were clearly visible and well defined throughout their course alongside radius and ulna. The superficial brachial and median arteries after originating from main brachial artery, immediately gave rise to many prominent smaller arterioles which coursed proximally, distally and caudally in the adjoining area. The superficial brachial artery once again branched into prominent medial and lateral divisions at about proximal third diaphyseal aspect of radius and ulna. The diameter of the main trunks of these vessels and median artery gradually decreased distally, however their entire course along side radius and ulnas well as carpal joint was very clear. The collateral ulnar artery coursed mainly on caudal aspect of ulna and tapered off quickly by the distal third diaphyseal aspect of ulna. All along their course, all these main arteries gave rise to numerous minute branches which supplied the bones and soft tissues of the area. The density of arterioles and other vessels was relatively more in proximal as well as in distal metaphyseal area of radius and ulna, whereas at rest of the places the penetration of these smaller vessels was almost uniform. At dorsal aspect of radius, the intensity of vascular network appeared further less due to presence of less quantity of soft tissue as such (Fig 3 A1 and B1).

Angiograms of the limb treated with Harjore paste (Group B) at day 15<sup>th</sup> postoperatively revealed extensive vascular proliferation in the proximal ulnar region. The radiodensity was profound in the developing callus surrounding fracture site indicating its extensive vascularization (Fig 3 B2). The angiograms in the control group at this stage revealed only slight increase in overall vascular proliferative activity as compared to treated group. No increase in radiodensity just around the fracture fragments were noticed in this group indicating little healing activity at the site (Fig 3 A2).

At day 30, the increased vascularity was seen in both the groups. However the minute vascular network was less prominent in group B and relatively more pronounced in group A when compared to their respective appearances at day 15<sup>th</sup> (Fig 3 A3 and B3). The fracture line was totally masked by radiodense vessels coursing around the fracture site in group B. Whereas, the fracture line was clearly visible in group A indicating lesser vascularization compared to group B.

The opacification of the site due to increased vascularity was reduced in both the groups at day 45<sup>th</sup>. The angiograms of the control group at this stage revealed increased radiodensity at fracture line indicating greater healing tissue progression at the site compared to its day 30<sup>th</sup> appearance. However, the increase in local vascularity was relatively less compared to test group (Fig 3 A4 and B4). In test group the fracture line was still totally obscured by radiodense vessels.

The vascular pattern in test group resembled to normal 0 day angiogram except with a slight increased vascular network opacity at day 60<sup>th</sup> postoperatively. Whereas, in control group the fracture line was still slightly visible indicating incomplete union at the fracture site (Fig 3 A5 and B5).

On the basis of above clinical, haematological, biochemical, radiological and angiographic studies, it can therefore be inferred, that the use of 'Harjore (*Cissus quadrangularis*)' paste over fractured area help in fracture healing process of bone without any apparent adverse effects. The application of 'Harjore' paste increases the local blood vascularity which in turn brings in more nutrients, phagocytes and osteoblasts at the fracture site and thereby results in greater osteogenic turnover. This facilitates the fracture-healing process by way of early bridging of fracture gap, rapid gain in structural strength of bone and early remodeling of fracture callus.

**Conclusion:**

The application of 'Harjore (*Cissus quadrangularis*)' in paste form over fractured area hasten the fracture-healing process.

**Publications:** Nil

**Patents:** Nil

**Technology developed:** The claim of the discloser about the ITK has been validated through systematic experimental research study on animals. The ways to use the paste of 'Harjore' were established and recommended for supporting fracture healing process in animals.

**Infrastructure developed:** Nil

**Civil works:** Nil

**Linkage developed:** Nil

**Impact:** Nil

**Human resource development:** Nil

**Technology dissemination:** Nil

**Monitoring and evaluation:** Nil

**Honours/awards:** Nil

**How have process changes helped to bring about:** NA

**Utilization of the results of the sub-project after termination of the sub-project-** The initial results indicate the usefulness of 'Harjore' paste in treatment of fracture cases in animals, however a follow-up study targeting larger data size encompassing many other animals species as well is desired before envisaging any potential commercial use of the plant in this direction. NATP financial support for these follow-up studies is desired.