

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.606.347>

Evaluation of Fracture Healing Property of Ethanolic Extract of *Cissus quadrangularis* Using 3-Point Bending Model in Rats

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ABSTRACT

A study was undertaken to evaluate the fracture healing effect of ethanolic extracts of *Cissus quadrangularis* on experimentally fractured tibia of rats radiologically by using 3-point bending model. Closed transverse fracture of the mid – diaphysis of left tibia were created in rats after Phenobarbitone & Ketamine anaesthesia, by 3- point bending method. In the skiagram there was an evidence of union in *Cissus quadrangularis* treated group with extensive bony deposition compared to that of control [Normal saline] group. In our study, the findings assessed on the basis of radiological studies showed that ethanolic extract of *Cissus quadrangularis* had a definite fracture healing effect. The extracts can also be utilized as a new source of bone healer in the treatment of fracture.

Keywords

Closed transverse Fracture, Skiagram.

Article Info

Accepted:
26 May 2017
Available Online:
10 June 2017

Introduction

Bone is a very active tissue and that any injury to it will induce a series of biological and biochemical changes (Udupa *et al.*, 1964).

The chemical analysis of *Cissus quadrangularis* revealed a high concentration of vitamin C, carotene A, a substance like anabolic steroid and calcium.

It has been known for long time for its specific action on fracture healing (Udupa *et al.*, 1964; 1960), hence selected for observation of its actual effect on the process of fracture healing judged by radiological examination in experimental animals (rats).

Materials and Methods

The present study was conducted after the approval of research protocol by the Institutional Animal Ethical Committee, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha. *Cissus quadrangularis* purchased from local herbarium Nagpur”, identified and authenticated with the standard sample preserved as *Cissus quadrangularis*, by Botany Department, R. T. M. University Nagpur. The fleshy stems of CQ washed and cut into small pieces, air dried. Then 4 kg of chopped pieces of *Cissus quadrangularis* was soaked in 6 L of Ethyl Alcohol 99.9 % for 48 h, and the extract was filtered and allowed to air dry.

The total ethanol extract was concentrated in vacuo till a syrupy consistency is obtained. For administration, the extract was suspended in distilled water. Preliminary phytochemical studies of extracts done using standard procedure at Laboratory of Department of Pharmacognosy, R. T. M. Nagpur University, and Nagpur which shows the presence of Flavones, Saponins, Glycoloides, in the extract.

Experimental Animals

The study was conducted using 16 Wistar Albino Rats, of either sex weighing 150-200g obtained from Institute of Pharmaceutical Education and Research Bargaon (Meghe), Wardha.

Animals were acclimatized on standard nutritional and environmental condition in separate cages and fed with standard laboratory chow and provided with water *ad libitum*.

Before surgery every rat was subjected to clinical orthopedic evaluation and dorso-palmar radiographic views of the left tibia were obtained (40 kV/32mAs and 0.26 s) (Antti, 2002).

Animals were anaesthetized with Phenobarbitone 60mg/kg, IP and Ketamine [Anikate, 40 mg/kg, IP]. Then closed transverse fracture of the mid – diaphysis of left tibia were created in rats by three point bending method (Chuanyong *et al.*, 2006).

The bone was positioned horizontally with the anterior surface upwards. The pressing force was directed vertically to the midshaft of the bone. Each bone was compressed with a constant speed and compression force. These fractured limbs were stabilized with splints after reduction and animals were allowed to move freely after recovering from anesthesia.

Grouping of animals

Animals were divided into 2 groups of eight animals each.

Group I - Normal saline (NS) (2 ml/kg) orally for 30 days.

Group II - *Cissus quadrangularis* (CQ) orally (500mg/kg body weight) o. d, for 30 days.

Preparation of working solution

The required amount of syrupy extract of *Cissus quadrangularis* measured as per the dose/bodyweight (500mg/kg) of rats and daily fresh solution used to made with distilled water (2ml)

Radiological evaluation

The animals were evaluated clinically every day to determine general condition and lameness. Lameness was evaluated by observing each animal moving freely in the cage. Bone healing of NS group and drug treated groups was followed in each animal by radiographic evaluation immediately after surgery and every week thereafter for 4 weeks using the same technique. The left tibiae were placed in a standard lateral position over Kodak Lanex Single Screen. Radiographs were exposed on Kodak Min-R film with 40kV/ 32 mAs. The visibility of the fracture line was observed and scored as totally visible, partly visible, or absent.

Qualitative assessment included fracture line/margins, fracture gap, external callus appearance, bridging, and radiologic union done by radiographic evaluation. (Range of radiation maintained as per Potter who had demonstrated a relationship between exit dose and lethality in rats with 100 and 400kvp.)^[5]

Results and Discussion

Radiological findings taken at weekly intervals.

1st week

X-ray did not reveal any marked difference in the various groups [CQ, NS]. The fragments were freely mobile. Broken ends were still visible on x-ray. This is a fibroblastic phase [Plate 1 (1.I, 1.II)].

2nd week

The skiagram showed beginning of callus formation and bridging of gap in CQ treated group but no such results observed in NS treated group, there the gap was still visible. Mobility of fragments still present to certain extent [Plate 2 (2.I, 2.II)].

3rd week

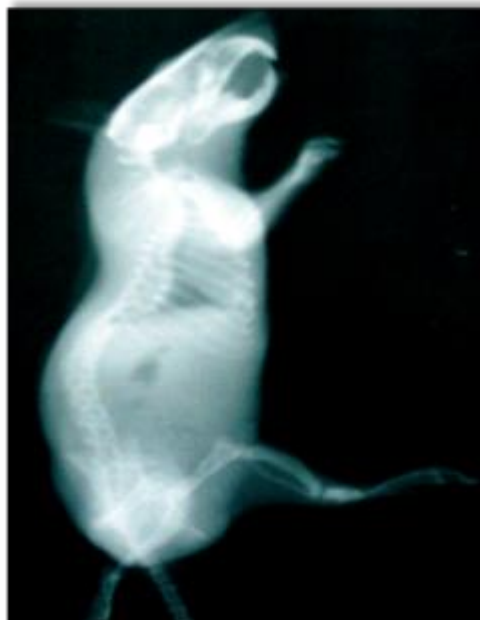
The x-ray showed greater amount of calcification of callus in CQ treated group in which one could hardly see a gap at the fracture site, fragments could not be elicited whereas in NS group some gap was still visible. This is an osteogenic phase [Plate 3 (3.I, 3.II)].

4th week

In the skiagram there was an evidence of union in CQ group. Almost complete bridging of the fracture ends with extensive bony deposition compared to that of control [Normal saline] group is seen in CQ treated group. This is also an osteogenic phase [Plate 4 (4.I, 4.II)].

Radiological findings 1st week after fracture healing

(Plate 1.I) *Cissus quadrangularis*



(Plate 1.II) Normal saline



Radiological findings 2nd week after fracture healing

(Plate 2.I) *Cissus quadrangularis*



(Plate 2.II) Normal saline



Radiological findings 3rd week after fracture healing

(Plate 3.I) *Cissus quadrangularis*



(Plate 3.II) Normal saline



Radiological findings 4th week after fracture healing

(Plate 4.I) *Cissus quadrangularis*



(Plate 4.II) Normal saline



It is well known since many centuries to the indigenous medical practitioners and also to the people working in jungles that a paste prepared from the herb CQ applied to the injured limbs greatly helps in the healing of fractures. Further it was tried for internal use by way of drinking decoction prepared by boiling the fresh herb in milk. This indicated that the active principle might be a fat soluble substance. Hence we prepared alcoholic extract for use in animals.

Radiography is an established method for evaluating fracture healing both in clinical use and in animal studies. Radiographs are able to visualize callus formation after mineralization (Aronson *et al.*, 1994; Kato *et al.*, 1998; Reichel *et al.*, 1998).

Radiological findings in the present study demonstrated that, in both the groups during first week there was no evidence of callus formation and the fragments were freely mobile.

During 3rd and 4th weeks the callus progressively became harder and the movement between fragments could not be elicited in the herb treated group. Our results are in conformity to those found in the study

of Guru Charan Prasad and Udupa, 1963. At fourth week of fracture radiograph of the CQ treated group revealed almost complete bridging of the fracture ends with extensive bony deposition and periosteal reaction in contrast to that of normal saline treated group. Ham, 1974 also demonstrated similar radiographic evidence of early periosteal reaction and bony dissolution in the *Cissus quadrangularis* treated animals (Bos *et al.*, 1983).

Our study indicates that daily administration of an alcoholic extract of *Cissus quadrangularis* orally in the dosage of 500mg/kg seems to have definite action on the rate of healing of fractures. The active principles responsible for the fracture healing property of *Cissus quadrangularis* need to be investigated further to elucidated the precise modus operandus of this herb. Proposition to administer *Cissus quadrangularis* deserved to be explored in depth for defining its place in the overall management of fracture healing.

Acknowledgement

Heartfelt gratitude to Mr. Rajaram A. Hire for his kind support in performing experiment and throughout research work.

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How to cite this article:

Reena R. Giri, Kiran R. Giri and Kamlesh Palandurkar. 2017. Evaluation of Fracture Healing Property of Ethanolic Extract of *Cissus quadrangularis* Using 3-Point Bending Model in Rats. *Int.J.Curr.Microbiol.App.Sci.* 6(6): 2924-2931. doi: <https://doi.org/10.20546/ijcmas.2017.606.347>