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Research Article

TO EVALUATE EFFICACY OF *ASTHISHRUNKHALA* (*CISSUS QUADRANGULARIS*) ON ACCELERATION OF BONE HEALING IN FRACTURED BONE

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ABSTRACT

The healing of a fracture is one of the most remarkable of all the repair processes in the body since results not in a scar but in the actual reconstitution of the injured tissue in something very like its original form. It is not to be expected therefore that the mechanisms controlling such a process will be easily elucidated and indeed they involve problems of cellular homeostasis which are among the most fundamental in biology. If it is not quite thou cunning'st pattern of excelling nature, then it is something quite close to it and a great deal of that pattern.

Present study shows experimental evaluation of effect of *Asthishrunkhala* (*Cissus quadrangularis*) on bone healing in fractured bone by histo-pathological study. During study concerned literature was reviewed critically. The study was divided into phytochemical, pharmacological and histopathological study. Drug was identified and authenticated from authorized institute and phytochemical screening done after successive extraction of extract. Two groups were made. First Group A (Treatment group) was further divided into six subgroups containing five animals each received extract of *Cissus quadrangularis* 500mg/kg body weight, Group B is control group. After completing the study it was observed that mature bone or bone repair process seen weakly at 2nd – 3rd week proving the acceleration of bone healing process with gradual re-modeling. Statistical analysis shows that histopathological study in group A found to be highly significant $p<0.001$ from 4th week onwards. Overall the bone healing process shows 10% faster healing period in group A than group B.

KEYWORDS: *Asthishrunkhala, Cissus quadrangularis,* Biological bone healing factor, Histopathological study.

INTRODUCTION

The management of orthopedic trauma has always been presented a challenge to surgeons. Man's desire to wage has ensured a steady supply of musculoskeletal injuries over the year and many advances in orthopedic trauma management have come about as a result of having to treat progressively more serious injuries.

Patients want rapid and modern treatment of their injuries as well as reconstruction of their worn out joints in order to preserve their physical life styles well into their senior years. Governments concerned about dwindling birth rates also want the same people to work longer to maintain economics of scale. Thus the orthopedic surgeons need to adopt the techniques which allow effective treatment and rapid rehabilitation.

In Ayurveda, *Bhavprakash* stated various osteogenetic drugs like *Asthishrunkhala* (*Cissus quadrangularis*) accelerate the process of bone healing.[6]

Recent studies have also suggested that *Asthishrunkhala* (*Cissus quadrangularis*) stimulates osteoblastogenesis and can be used as preventive/ alternative natural medicine for bone diseases such as osteoporosis.[1]

By exerting an anabolic anti-glucocorticoid effect cisssus helps to preserve muscle tissue by increasing strength.[2] Cissus exerts anti-glucocorticoid properties and is suggested by a number of studies.[3] Cissus also possesses anti-microbial antioxidant property.[4]

Three months sub-chronic toxicity studies conducted showed no toxic effect on experimental animals. Studies show that it is safe lethal dose LD 50 is over 2000mg/kg on experimental animals.[5]

Hence it was decided to undertake a series of study for the experimental evaluation of effect of *Asthishrunkhala* (*Cissus quadrangularis*) on bone healing in fractured bone by histopathological study. During study concerned literature was reviewed critically. The study was divided into phytochemical, pharmacological and histopathological study. Drug was identified and authenticated from authorized institute and phyto chemical screening done after successive extraction of extract. Two groups were made. First Group A (Treatment group) was further divided into six subgroups containing five animals each received extract, Group B (Control group). After anaesthetized, the animals were fractured and immobilized with POP and histopathological study was carried out weekly. The observations were observed and evaluated statistically.

AIMS AND OBJECTIVES

1. To review literature regarding the *Asthi* (bone) and principles of management of fracture with its types from Ayurvedic as well as modern text.
2. To correlate the principles regarding fracture in Ayurveda with modern principles.
3. To evaluate the effect of *Asthishrunkhala* (*Cissus quadrangularis*) on acceleration of the bone healing at fractured fragments of bone.

MATERIALS AND METHODS

Plan of work

Study was divided into phytochemical pharmacological and histopathological study.

Phytochemical work

Collection of plant material

The plant material (stem of *Cissus quadrangularis*) was collected from local herbarium.

Authentication of plant

The plant was identified and authenticated with the standard sample preserved as *Cissus quadrangularis* by Botany Department in authorized institute.

Preparation of plant material in powdered form

Drying of *Cissus quadrangularis* – The fleshy stems were fresh, they were washed and cut into small pieces, air dried for 7 days and again chopped into fine pieces and then subjected to grinding to get powder.

Preliminary screening of phytochemicals

General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them (Trease & Evan -1983). Preliminary Phytochemical Screening included the following steps.

Inclusion criteria

The study was conducted using 36 Wistar Albino rats of either sex weighting 150–300gm.

Experimental animals were divided firstly in 2 groups.

Group A (Treatment group)

Further divided into 6 subgroups 1st (T_1 to T_5), 2nd (T_6 to T_{10}), 3rd (T_{10} to T_{15}), 4th (T_{16} to T_{20}), 5th (T_{21} to T_{25}), 6th (T_{26} to T_{30}) each containing 5 albino rats and received extract orally 500mg/kg of body weight OD for 7 days, 14 days, 21 days, 28 days, 35 days, 42 days, respectively from the day of fracture. For well identification animal ID were given by numbering from T_1 to T_{30}.

Group B (Control group)

Contains 6 albino rats (C_1 to C_6) and after fractured the fracture limb was stabilized with complete pop.

Treatment protocol for fracture healing

Dose: *Cissus quadrangularis*- 500mg/kg body weight OD

Preparation of working solution

The required amount of soft sticky extract of *Cissus quadrangularis* was weighed and mixed with adequate lactose and then dispersed in water and volume was measured as per the dose /body weight of Albino rats and daily fresh solutions were used.

Acclimatization

Animals were acclimatized for 8 days in the laboratory before dosing. Animals were kept on standard nutritional and environment condition in separate cages. They were housed under standard condition of light, temperature and humidity. They were fed with standard laboratory food and provided with water.

Creating fracture

Animals were anaesthetized with Xylasazine 10mg/kg and Ketamine 40mg/kg with the help of insulin syringe. Practically it was very difficult to stabilize the fractured femur by POP, hence it was decided to operate the tibia fracture instead of femur. After anesthetization closed transverse fracture of the mid diaphysis of Rt. tibia was created in each animal of all groups on 1st day by three point bending method. The bone was positioned horizontally with the anterior surface upwards. The pressure was directed vertically to the mid shaft of the bone. Each bone was compressed with a constant speed and compression force. The fractured limb and alignment of fracture was immediately confirmed by X-ray

(Radiological evaluation). After radiological evaluation fractured limbs were stabilized with complete POP after proper reduction and animals were allowed to move freely after recovering from anesthesia.

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.

Histopatological Study

Specimens cut for histopathological examination were decalcified and studied for fracture union. They were finally stained with heamatooxyline and eosin. Fracture callus was assessed for

1. Degree of cellularity
2. Amount of callus
3. Cartilage
4. Bone matrix formation
5. Woven bone and mature bone formation
6. Medullary repairs

Cortical repair and was scored as

1= average (upto 25%)
2= good (upto 25-50%)
3= very good (50-90%)
4= excellent (90-100%)

Therefore histopathological grades (HPG) were awarded as follows

Grade 4:- Complete bony union.

Grade 3:- Less than complete bony union as evidenced by the presence of a small amount of cartilage in fracture callus.

Grade 2:- Complete cartilage union.

Grade 1:- Incomplete cartilaginous union evidenced by the retention of fibrous elements in the plate.

Grade 0:- Psedoarthrosis formation or nonunion seen as an inconvertible cavity within the cartilage plate between fracture fragments containing blood or other fluid.

OBSERVATION AND RESULTS

Following observations were noted during study.

Table 1: Week wise study of overall healing process in treatment Group A at Glance

	1 Week	2 Week	3 Week	4 Week	5 Week	6 Week
Callus Amount	35%	65%	80%	85%	80%	80%
Cell	80%	80%	75%	55%	50%	35%
Vascular	35%	80%	85%	65%	45%	45%
Cartilage	35%	55%	65%	55%	50%	50%
Haversian System	5%	25%	35%	45%	65%	80%
Bonematrix	5%	30%	35%	50%	60%	80%
Med.Rep	5%	30%	55%	60%	50%	50%
Cortical Rep	5%	30%	30%	45%	60%	55%

Statistical Analysis

For present study 36 Wistar albino rats were taken and divided into two groups. In Group A i.e., experimental group was subdivided into six groups each containing 5 animals. In Group B i.e., control group contain six animals. Weekly histopathological studies were carried out in both group and observations were noted weekly.

Test

Name of test applied for Group A was 'One-way analysis of variance with Turkey's Multiple Comparison Test'. It was not possible to apply test for Group B as sample size was one and SD was 0.

Statistical analysis of treatment group shows following results by One-way analysis of variance with Turkey's multiple comparison test.

Table 2: Week wise statistical analysis of Callus amount in treatment Group A

Callus Amount						
	1week	2week	3week	4week	5week	6week
Mean	1.4	2.6	3.2	3.4	3.2	3.2
Median	1	3	3	3	3	3
SD	0.5477	0.5477	0.4472	0.5477	0.4472	0.4472
Std. Error	0.2449	0.2449	0.2	0.2449	0.2	0.2
P value summary		*	***	***	***	***
P Value		P < 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Table 3: Week wise statistical analysis of Cellularity in treatment Group A

Cellularity						
	1week	2week	3week	4week	5week	6week
Mean	3.2	3.2	3	2.2	2	1.4
Median	3	3	2	2	2	2
SD	0.4472	0.5477	0	0.4472	0	0
Std. Error	0.2	0.2449	0	0.2	0	0
P value summary		NS	NS	*	**	***
P Value		P > 0.05	P > 0.05	P < 0.05	P < 0.01	P<0.001

Table 4: Week wise statistical analysis of Vascularity in treatment Group A

Vascularity						
	1week	2week	3week	4week	5week	6week
Mean	1.4	3.2	3.4	2.6	1.8	1.8
Median	1	3	3	2	2	2
SD	0.5477	0.4472	0.5477	0.5477	0.4472	0.4472
Std. Error	0.2449	0.2	0.2449	0.2449	0.2	0.2
P value summary		***	***	*	NS	NS
P Value		P < 0.001	P < 0.001	P < 0.05	P > 0.05	P > 0.05

Table 5: Week wise statistical analysis of Cartilage in treatment Group A

Cartilage						
	1week	2week	3week	4week	5week	6week
Mean	1.4	2.2	2.6	2.2	2	2
Median	1	3	2	2	1	1
SD	0.4472	0.4472	0.5477	0.4472	0	0
Std. Error	0.2	0.2	0.2449	0.2	0	0
P value summary		**	***	**	*	*
P Value		P < 0.01	P < 0.001	P < 0.01	P < 0.05	P < 0.05

Table 6: Week wise statistical analysis of Haversian system formation in treatment Group B

Haversian System						
	1week	2week	3week	4week	5week	6week
Mean	0.2	1	1.4	1.8	2.6	3.2
Median	0	1	1	2	3	3
SD	0.4472	0.7071	0.5477	0.4472	0.5477	0.4472
Std. Error	0.2	0.3162	0..2449	0.2	0.2449	0.2
P value summary		NS	*	***	***	***
P Value		P > 0.05	P < 0.05	P < 0.001	P < 0.001	P < 0.001

Table 7: Week wise statistical analysis of Bone matrix formation in Group B

Bone matrix						
	1 Week	2 Week	3 Week	4 Week	5 Week	6 Week
Mean	0.2	1.2	1.4	2	2.4	3.2
Median	0	1	2	2	2	3
SD	0.4472	0.4472	0.5477	0	0.5477	0.4472

Std. Error	0.2	0.2	0.2449	0	0.2449	0.2
P value summary		*	**	***	***	***
P Value		P < 0.05	P < 0.01	P < 0.001	P < 0.001	P < 0.001

Table 8: Week wise statistical analysis of Medullary repair process in treatment Group A

Medullary Repair						
	1 Week	**2 Week**	**3 Week**	**4 Week**	**5 Week**	**6 Week**
Mean	0.2	1.2	2.2	2.4	2	2
Median	0	1	2	2	2	2
SD	0.4472	0.4472	0.4472	0.5477	0.7071	0.7071
Std. Error	0.2	0.2	0.2	0.2449	0.3162	0.3162
P value summary		NS	***	***	***	***
P Value		P > 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Table 9: Week wise statistical analysis of Cortical repair in treatment Group A

Cortical Repair						
	1week	**2week**	**3week**	**4week**	**5week**	**6week**
Mean	0.4	1.2	1.2	1.8	2.4	2.2
Median	0	1	2	2	2	2
SD	0.4472	0.4472	0.4472	0.4472	0.5477	0.4472
Std. Error	0.2	0.2	0.2	0.2	0.2449	0.2
P value summary		*	**	***	***	***
P Value		P < 0.05	P < 0.01	P < 0.001	P < 0.001	P < 0.001

Table 10: Week wise statistical analysis of Bone Healing Process (HPG) in treatment Group A

HPG						
	1 Week	**2 Week**	**3 Week**	**4 Week**	**5 Week**	**6 Week**
Mean	0.4	1.2	1.6	2.4	2.6	3.2
Mean%	10%	30%	40%	60%	65%	80%
Median	0	1	2	2	2	3
SD	0.5477	0.4472	0.5477	0.5477	0.8944	0.8367
Std. Error	0.2449	0.2	0.2449	0.2449	0.4	0.3742
P value summary		NS	NS	***	***	***
P Value		P > 0.05	P > 0.05	P<0.001	P<0.001	P< 0.001

After starting the treatment total effect of therapy assessed by histopathological study in treatment group was not significant on 2nd and 3rd week but then from 4th week onwards found to be highly significant p<0.001***. But in the first to third week when compared, control group was significant in callus amount, cellularity, vascularity, haversian system, Medullary repair, cortical repair and bone matrix formation.

DICUSSION

During weekly bone matrix study it was observed 5% at 1st week in Group A but was 0% in group B. It was observed that bone matrix formation reveals slowly increased from 1st week to 6th week. Group A showed mean 10% more bone matrix formation than Group B.

Gradual increase in medullary repair was observed upto 4th week but was decreased onwards upto normal level. 4% more medullary repair was seen in Group A than Group B.

Week wise cortical repair study shows earlier beginning of repair process from 1st week in Group A than Group B with steady increase upto 6 week. Mean 9% more cortical repair process was more in Group A than Group B.

During the study it was observed that during administration of extract orally there was local irritation at mouth externally where the diluted extract came into contact with the skin or mucosa.

Also Group A animals shows early mobility as compared to Group B which may be due to analgesic, anti inflammatory activity of *Asthishrunkhala* (*Cissus quadrangularis*) and early bone maturation. As previous research study had proved. Thus it was concluded that the early progressive increase in callus formation and progressive decrease the early bone maturation process thus woven bone which also forms the initial fracture repair tissue and later is replaced by lamellar bone as the fracture remodels.

Madhura rasa of the drug which is *Sthairyakar, Sandhankar* and *Sarva Dhatuvivardhana* might have acted upon each *Dhatu (Dhatvagni)* level there by strengthening the base for quality *Asthi Dhatu nirman* to have *Sandhankar* property; while *Kashaya Rasa, Laghu, Ruksha Guna* have *Shoshana, Lekhana, Ropana* properties thus act as a *Shothahara,* stopped the haemorrhage, absorbed haematoma, promotes healing process and ultimately unites the fracture bone. According to *Ashraya Ashrayi bhava*[7] described by *Vagbhata Shamana* of *Vata Dosha* leads to *Asthi Vriddhi. Madhura vipaka* and *Ushna virya* of *Asthishrunkhala* leads to *Shamana* (alleviation) of *Vata dosha. Madhura rasa, Madhura vipaka, Saara guna* and *Raktashodhana* property of *Asthi-shrunkhala*[6,8] might act on local circulation with increasing cellularity and vascularity thus the promoting process of healing of fracture faster and bone union.

During study, it was not possible to take same number of albino rats in control group as the bone healing process is an establish fact and also ethically it was not allowed to take as they all were have to sacrifice.

During study, no adverse effects were noted with *Asthishrunkhala* (*Cissus quadrangularis*). Hence it is safe for administration. On the basis of these above observation it can be said that *Asthishrunkhala* (*Cissus quadrangularis*) can be used in acceleration of bone healing in fractured bone as an adjuvant therapy. During study it was not possible to measure Bone Tensile strength, Bone Mineral Density and elasticity of bone due to lack of availability of instrumentation, so further study may be needed to carry for more specification. As sample size was small in control group this might have affected the results so it is the need to carry same study with equal and large sample size in both the groups to get more precise and specific results.

Summary

- 13% more average mean of callus amount formation seen Group A than Group B.
- Averagely 5% more cellularity was seen in group A than Group B.
- Peak vascularity was observed on 3rd week in both group but was 10% more in Group A (85%) than Group B (75%)
- 10% more formation in Haversian system upto six week was observed in Group A than Group B
- Bone matrix formation was seen increase from 1st week to 6th week in both the group.
- In Group A earlier accelerated medullary repair was observed, peak at the 4th week and 5th week in Group A and Group B respectively.
- Overall the bone healing process showed 10% faster healing period in Group A than Group B.

CONCLUSIONS

- *Asthishrunkhala* (*Cissus quadrangularis*) is the biological factor for bone healing.
- *Asthishrunkhala* (*Cissus quadrangularis*) showed a marked influence in the rate of fracture healing by influencing early regeneration.
- Early completion of calcification process and earlier remodeling phenomenon lead to early recovery of Cissus treated animals.
- Matured bone or bone repair process seen at peak level during 2nd-3rd week proving the acceleration of bone healing process.
- *Asthishrunkhala* (*Cissus quadrangularis*) helps in achieving normal tensile strength in minimal period.
- *Asthishrunkhala* (*Cissus quadrangularis*) also act as anti-inflammatory and analgesic so it reduces use of analgesic, thus promote healing and early mobility also proved by previous researches.
- In immobilized limb depletion of calcium occur in bones it prevent depletion.
- *Asthishrunkhala* (*Cissus quadrangularis*) can act as best drug because is easily available economical effective without any side effects and it may be used in general practice in fracture patients.
- The study shows that *Asthishrunkhala* can be used for accelerating bone healing in fractured bone as an adjuvant therapy.
- The present study was conducted on low sample size due to ethical problem also work has to be carried to study role of *Asthishrunkhala* (*Cissus quadrangularis*) at molecular level to make more precise and to strengthen our principles.

- As sample size was small in control group this might have affect the results so it is needed to carry same study with equal and large sample size in both the group to get more precise and specific result.

REFERENCES

1. Potu BK, Bhat KMR, Rao MS, Nampuraith GK, Chamallamudi MR, Nayak SR Petroleum ether extract of Cissus quadrangularis enhances bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis. Pubmed 2009, 64 (10) -993 -8
2. Combaret L, Taillandier Dwaardevetn, Bechet D Rallier C, clasustre A, Guzard J Bcochem J Lysosomal proteolysis in skeletal muscle. Pubmed. 2004 feb. 15.378,239-46
3. Prasad GC, udupa KN. Indian J med Res Chemical and histochemical studies on the organic constituents in fracture repair in rats. The journal of bone and joint surgery. 1963 British vol. 45(4), 770-779.
4. Chidambara murthy KN, Vanitha mahadeva swamy M, Ravishankara GA. Antioxidant and antimicrobial activity of Cissus quadrangularis. Journal of medicinal food. 2003/7/1 vol.6 99-105.
5. Aimmanas Attawish, Pranee chavalittumrong, Songpol chivapat, Anchalee chuthaputti, Sadudee rattanjarasroj and Somkiat Punyamong Songklanakarin journal of science and technology. Subchronic toxicity of Cissus quadrangularis. 2002 vol. 24(1) 1-176 Jan-march, 39.
6. Vd. Gangasahay pandey, Bhavprakash Nighantu with commentary, Chaukhambha Bharati academy, Varanasi, Reprint 2013, page 403-404
7. Dr Brahmanand Tripathi Ashtanga Hrudayam of Vagbhat with the commentaries Sarvanga-sundar by Arundatta and Ayurved Rasayana by Hemadri. 2003 Chaukhamba Surbharti Prakashan, Varansi. page 165.
8. Vd. Bapalal Nighantu Aadarsh Purvardha 2nd edition Chaukhamba Bharati Prakashan, Varanasi. Page 304-305.

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