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Effect of *Terminalia arjuna* in accelerating healing process of experimentally fractured tibia of rats: A preliminary study

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ABSTRACT

A study was undertaken to evaluate the effect of ethanolic extract of *Terminalia arjuna* Linn (AT) on the healing process of experimentally fractured tibia of rats. AT treated animals' revealed faster initiation of healing process than the control animals on radiological examination. Healing was almost complete within 4 weeks of fracture in the treated animals & remained incomplete in the control animals.

Keywords: *Terminalia arjuna*, fracture healing, tibia, Radiological examination

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INTRODUCTION

The ancient Indian science of Ayurveda is significant as a preventive & curative medical system. Ayurvedic medicines are mainly of plant origin. *Terminalia arjuna* (Roxb.) Wight & Arnot is one such plant widely used in the preparation of important ayurvedic formulations like *arjuna ishtam*, *Laksagugula*. [1] Different Sanskrit Names of *Arjuna* are Dhaval - because of its whitish or grey bark. Kakubha - due to its being a large tree. Indradu - due to its covering a large area. Viravriksha - due to its hard woody nature. Nadisarja - because it is commonly found by the side of rivers and water bodies. [2]

It belongs to the family Combretaceae, which contains 250 species of *Terminalia*. [1] In India, *Terminalia chebula*, *T. bellrica* and *T. ciliata* are major related species. [3] Botanical Description: This is a large tree attaining a height of 20 to 26 meters. Trunk- straight, pale white and smooth externally and tender, thick and reddish internally. Leaves – sub opposite, similar to guava leaves, 10-15 pairs of leaflets at the end. Leaflets – tongue shaped 10-15 cm in length and 8-10 cm in breadth. There are small nodes at the top of the petioles immediately below the leaves. Flowers – occur all round white or yellow stalks. Fruit – similar to kamarah fruit (*Averrhoa cavambola* Linn.). Flowering stage is in summer whereas fruiting stage is in winter. The bark is shed like snake skin once a year. Habitat: This plant invariably found near rivers & streams. In 'Terai' region at the foot of Himalaya, Bengal, Bihar, Madhya, Pradesh and Brahma Pradesh. [4]

Chemical composition: The bark contains calcium carbonate 34%, other calcium salts 9% and tannin 16%. Besides, it also contains aluminium, magnesium, organic acid, colouring matter and sugar. [4] Other active constituents of *Terminalia Arjuna* include tannins, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, and arjunglycosides), flavonoids (arjunone, arjunolone, and luteolin), Gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, zinc, and copper. [5]

Terminalia arjuna holds a reputed position in both Ayurvedic and Unani Systems of medicine. Charaka assigns the *Arjuna* tree to the udardaprasamanani group of anti-urticarial drugs. Vagbhata places it in the vellantaradi & nyagrodhadi groups indicated in urinary disorders & broken bones, respectively. According to the sarangadhara Samhita, AT belongs to the nyagrodhadi group specific for fractures, ulcers, uterine & urological complaints, skin diseases etc. [1]

The present work was undertaken because the *Arjuna* tree is an important plant in the Indian materia medica with poorly explored fracture healing potential.

MATERIALS & METHODS

The study was conducted in Department of pharmacology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha. The research protocol was approved by the Institutional

Animal Ethical Committee, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha. The plant was identified & authenticated with the standard sample preserved as *Terminalia Arjuna* by Botany Department, R. T. M. Nagpur University and Nagpur.

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

The preliminary phytochemical studies did under the guidance of Dr.N.R.Itankar, at Laboratory of Department of Pharmacognosy, R.T.M.Nagpur University and Nagpur. Extracts was tested for preliminary phytochemical studies using standard procedure.

It shows the following results:

Terminalia arjuna shows the presence of Tannins, Saponins and Carbohydrates in the extracts.

Ethanolic extract of Bark of *Terminalia arjuna*

Dried bark of *Arjuna* were coarsely powdered.4 kg of coarse powered of *Terminalia arjuna* was soaked in 6 L of ethanol for 48 h, & the extract was filtered & allowed to dry under fan. The last traces of solvent were removed under vacuum drier & the brown powdered mass obtained which was pulverized & sieved for further use. For administration, the extract was suspended in sugar water. Solution of *Arjuna* prepared fresh daily. (6)

Animals were acclimatized for 8 days in the laboratory before experiment. Animals were kept on standard nutritional & environmental condition in separate cages. They were housed under standard condition of light, temperature & humidity. They were fed with standard laboratory chow & provided with water ad libitum.

Grouping of animals: Animals were randomly selected into 2 groups of eight animals each.

Group I (Control) received normal saline (2 ml/kg) orally for 30 days. They served as control animals.

Group II treated with *Terminalia arjuna* .This group received extracts of *Terminalia arjuna* with sugar water orally (500 mg/kg body weight) bid, for 30 days.

Health status of the animals was monitored during treatment period.

Pre-surgical evaluation

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). (7)

Surgery

Creating tibia fracture –

Animals were anaesthetized with Phenobarbitone 60mg/kg, IP & Ketamine [Anikate, 40 mg/kg, IP]. Then closed transverse fracture of the mid – diaphysis of left tibia were created in all four groups by three point bending method (8). The bone was positioned horizontally with the anterior surface upwards. The pressing force was directed vertically to the mid-shaft of the bone. Each bone was compressed with a constant speed & compression force. These fractured limbs were stabilized with splints after reduction & animals were allowed to move freely after recovering from anesthesia.

Treatment protocol for fracture healing

Determination of doses

Terminalia arjuna - 500mg/kg body weight

Preparation of working solution

The required amount of powdered extract of *Terminalia arjuna* measured as per the dose/bodyweight (500mg/kg) of rats used as fresh solution prepared with sugar & water (2ml)

Pre and Post-operative evaluation

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 control group & drug treated group 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. [7] [Fig.4.12] The visibility of the fracture line was observed and scored as totally visible, partly visible, or absent. The angulations' degree of the healing callus was observed and scored: significant angulations' (more than 5 degrees) or no angulations'. [7] 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 & lethality in rats with 100 & 400kvp.) [9]

RESULTS

The radiological pictures taken at weekly intervals.

1st week

X-ray did not reveal any marked difference in both the groups [AT & NS]. The fragments were freely mobile .Broken ends were still visible on x-ray.

2nd week

The skiagram showed beginning of callus formation & bridging of gap in AT treated groups but no such results observed in NS treated groups, there the gap was still visible. Mobility of fragments still present to certain extent.

3rd week

The x-ray showed greater amount of calcification of callus in AT treated group in which one could hardly see a gap at the fracture site, fragments could not be elicited whereas in other group [NS] some gap was still visible.

4th week

In the skiagram there was an evidence of union in AT treated groups. Almost complete bridging of the fracture ends with extensive bony deposition compared to that of control [Normal saline] group is seen in AT treated groups.

The above findings convincingly proved that the herb *Terminalia arjuna* have definite influence on the rate of fracture healing.

1st Week					
Groups	Fracture line	Fracture gap	Callus appearance	Bridging	Mobility of fragment
Normal Saline	Totally visible	++++	No	Absent	Freely mobile
<i>Terminalia arjuna</i>	Totally visible	++++	No	Absent	Freely mobile
2nd Week					
Normal Saline	Totally visible	++++	No	Absent	Freely mobile
<i>Terminalia arjuna</i>	Partly visible	+++	+	Seen	Mobility present to certain extent
3rd Week					
Normal Saline	Totally visible	+++	No	Absent	Mobility present
<i>Terminalia arjuna</i>	Partly visible	+	++	Seen	Fragments couldn't be elicited
4th Week					
Normal Saline	Totally visible	+++	No	Absent	Mobility present
<i>Terminalia arjuna</i>	Absent	Not seen	+++	Seen	Fragments couldn't be elicited

Fig. 1 Radiological findings 1st week after fracture



Normal saline Treated



***Terminalia arjuna* Treated**

Fig. 2 Radiological findings 2nd week after fracture



Normal saline Treated



***Terminalia arjuna* Treated**

Fig. 3 Radiological findings 3st week after fracture



Normal saline Treated



Terminalia arjuna Treated

Fig. 4 Radiological findings 4st week after fracture



Normal saline Treated



Terminalia arjuna Treated

DISCUSSION

Instances are not rare in the past to illustrate that the use of certain medicinal herbs in some particular diseases by the indigenous medical practitioners of some area of the world has

had rationality. Many a time's detailed investigation had revealed not only useful information but had led to the findings of many newer synthetic substances of more potent action. [10]

The callus of 4th week in the rats which received *Terminalia arjuna* was larger & denser than that of Normal saline treated group in which no callus was seen even at 4th week.

The beneficial effects of the herb *Terminalia arjuna* seems to be beyond doubt as a promoter of the bone healing in Toto. [2]

Terminalia arjuna had also been tried for internal use by way of drinking bark decoction prepared by boiling the herb in milk for sometime or with honey or sugar. [2] This indicated that the active principle might be a fat soluble substance. Though these claims have been made since long to our knowledge, no scientific studies have been conducted so far to confirm or refute these long standing claims. [11]

Radiography is one basic method for evaluating fracture healing both in clinical use & in animal studies. Radiographs are able to visualize callus formation after mineralization [12-14]. Radiographs are usually taken immediately after surgery to examine the location of the fracture & the quality of fixation. For long bone fractures, the healing parameters, such as callus formation, quality of union & bone remodeling can be quantified on radiographs by different scoring systems. [15, 16] In the present investigation, the radiographic evidence of early periosteal reaction & bony dissolution in the *Terminalia arjuna* treated groups indicates a faster healing process.

Our study indicates that daily administration of an ethanolic extract of *Terminalia arjuna* 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 *Terminalia arjuna* need to be investigated further to elucidated the precise modus operandus of this herb. There is much scope for detailed investigation on the use of various chemical substances in the biological phenomenon of fracture healing. Thence this field of fracture which is a common problem in surgery & which has not been hitherto explored thoroughly by surgeons would be one of the most exciting subjects for further study.

CONCLUSION

1. The present results indicate that the ethanolic extract of *Terminalia arjuna* produce beneficial effect in fracture healing & support the claims of its traditional wages as traditional healer.
2. The observed effect could be due to presence of tannin & saponins & tripenoid contents of TA which have definite action on bone regeneration & calcium, phosphorus & alkaline phosphatase metabolism which also plays important role in osteoblastic activity.
3. Detailed studies on the active constituents are needed which might provide new insight in fracture healing promoter drugs.



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