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POTENTIAL OF *CISSUS QUDRANGULARIS* TRANSDERMAL PATCH FOR FRACTURE HEALING

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Abstract

Critical-sized bone defects (CSDs) have a multi-factorial etiology including high-energy trauma, infection, and revision surgery. CSDs are a major clinical dilemma, as reliable, evidenced-based solutions do not exist. The purpose of this study is to develop a healing patch for the CSDs, investigating the best outcome of formulation developed from *Cissus qudrangularis* and to reduce the side effects of corticosteroids, chemotherapeutic agents, non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, and anticoagulants in the patient suffering from CSDs. The primary objective of the study was to estimate the potential of *Cissus qudrangularis* for fracture healing. The transdermal patch containing ethanolic extract of *Cissus qudrangularis* was developed by solvent casting method. The formulated patch was further studied for thickness of the patch, moisture content, moisture re-absorption, SEM analysis, drug content, *In-vitro* drug-release and *in-vivo* animal activity. Male Wister rats weighing 250-350 gm. was selected for the study. The fracture was induced by using triple point pressure method. The formulated patch then applied to the fractured bone and immobilized with silica cast. X-ray imaging was performed for assessment of Fracture healing capacity of *Cissus qudrangulris* with comparison to control group. The silica cast with herbal patch show better potential of fracture healing in comparison to control group. The results of this study led to the validation of the potential of *Cissus qudrangularis* as fracture healing agent that have been used for decades to treat the fracture traditionally in India.

Key words : *Cissus qudrangularis*, Transdermal Herbal patch, *In-vitro* drug release, fracture healing, wistar rat.

Introduction

Medicinal plants and herbs have been proved to be of great importance to the health of the individuals and communities. In recent years, many scientific investigations of traditional herbal remedies for several diseases have been carried out and this has lead in the development of alternative drug and therapeutic strategies. Since the consumption of medicinal plants is increasing, it is interesting to use these plants as a supplement in food taking into account that these plants can present a significant amount of trace elements (Alves *et al.,* 2000) and other nutrients. *Cissus quadrangularis* is one such plant which is been studied for its medicinal properties like its useful in bone fractures (Udupa *et al*., 1964, Singh *et al.*, 1962) obesity (Oben *et al.,* 2006) and neuropharmacological effects (Swamy *et al*., 2006). *Cissus quadrangularis* is the most common species, belonging to the family Vitaceae, commonly known as "Hadjod". It is an ancient medicinal plant native to the hotter parts of India and Ceylon. It is said to be also present in some parts of Srilanka, Malaya, Java and West Africa (Udupa *et al*., 1970). In Ayurveda, the plant has been documented for its medicinal uses in gout, syphilis, venereal diseases, piles, leucorrhoea (Yognarsimha, 2000) The Phytochemical studies of *Cissus quadrangularis* using different solvent extracts revealed that the plant contains a high amount of ascorbic acid, carotene, phytosterol substances, and calcium. There are very great important chemical compounds like β-sitosterol, δ- amyrin, and δ-amyrone (Mehta *et al.,* 2001). All of these components have potentially different metabolic and physiological effects.

The other properties from the extract of *Cissus quadrangularis* have been investigated such as the antibacterial activity (Kashikar *et al*., 2006) antioxidant activity (Chidambramurty *et al*., 2003) antiulcer activity, antiosteoporotic (Jainu *et al.,* 2006) analgesic and anti-

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inflammatory (Deka *et al.,* 1994), proteolytic (Balchandran *et al*., 1991) mutagenetic and genotoxic activity (Bah *et al*., 2006), which makes the plant a natural source of chemical compounds with medicinal value that can be used as a drug to cure diseases and making it a plant with greater commercial value.

A phytogenic isolated steroid is believed to be the main constituent in *Cissus quadrangularis*. Studies on fracture healing suggest that this unidentified anabolic steroid may act on estrogenic receptors of the bone. Efficacy of *Cissus* quadrangularis on early ossification and remodeling of bones have been reported and it has been observed that *Cissus quadrangularis* acts by stimulation of metabolism and increased uptake of the minerals calcium, sulpher and strontium by the osteoblasts in fracture healing (Prasad *et al*., 1972)

Cissus quadrangularis is found to contain vitamins and steroids, which are found to have specific effect on bone fracture healing. The anabolic steroidal principles from *Cissus quadrangularis* showed a marked influence in the rate of fracture healing by influencing early regeneration of all connective tissues involved in the healing and quicker mineralization of callus. Systemic use of *Cissus quadrangularis* in rats caused complete restoration of normal composition of bone, after fracture in four weeks while the controls required six weeks. There was a shortening of about two weeks in the bone healing duration. The total weight of the fractured bone also came down towards normal much earlier than the controls indicating quickest bone remodeling. All the events namely fibroblastic phase (first week), collagen phase (second week) and osteochondroital phase (third and fourth weeks) were hastened by about 10 to 14 days in the treated group. (Udupa *et al*., 1964).

This hastening in the fracture healing was attributed to the stimulation of all the cells of mesenchyma origin, namely the fibroblasts, the chondroblasts and osteoblasts by *Cissus quadrangularis*. It has greater impact on osteoblastic proliferation than other cellular responses. In both the models the mucopolysaccharide and collagen levels of the bones in the treated group came down to normal at the end of only four weeks while the control required 6 weeks as confirmed with histological and histochemical observations (Singh *et al.*, 1962).

Petroleum ether extract of *Cissus Qudrangularis* (Linn) stimulates the growth of fetal bone during Intra Uterine Development Period; demonstrate that maternal treatment with Petroleum ether extract during gestation can dramatically influence the skeleton of the fetus.(Sen *et al*., 1966).

Bone healing is an extremely complex process which depends on the coordinated action of several cell lineages on a cascade of biological events, and has always been a major medical concern. Repair is typically characterized by four overlapping stages: the initial inflammatory response, soft callus formation, hard callus formation, initial bony union and bone remodeling. However, repair can also be seen to represent a juxtaposition of two distinct forces: anabolism or tissue formation, and catabolism or remodeling. These anabolic/catabolic concepts are useful for understanding bone repair without giving the false impression of temporally distinct stages that operate independently. The use of several drugs such as corticosteroids, chemotherapeutic agents, non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, anticoagulants and drugs which reduce osteoclastic activity have been shown to affect bone healing (Pountos *et al*., 2008). In numerous clinical situations enhanced bone formation and bone healing could lead to improved results of surgical procedures. *In vitro* investigations of growth factor effects on osteoblast chemotaxis and metabolism are described as well as *In vivo* studies with growth factor stimulation of fracture healing and bone healing to prosthetic-like implants. (Schinder *et al*., 2008).

Limitations of plasters

The affected limb is unreachable during treatment; the skin covered by plaster becomes dry and scaly because the affected outer skin cells are not washed or brushed off as it's the nature of the dressing in that. Skin complications would result in Plaster of Paris casts. That includes macerations, ulcerations, infections, rashes, itching, burns, and allergic contact dermatitis, which may also be due to the presence of formaldehyde within the plaster bandages. In hot weather, staphylococcal infection of the hair follicles and sweat glands can lead to severe and painful dermatitis (Darnell Kaigler *et al*., 2013).

Other limitations of plaster casts include their weight, which can be quite considerable, thus restricting movement, especially of a child. Removal of the cast requires destroying the cast itself. The process is often noisy, making use of a special oscillating saw that can easily cut the hard cast material but has difficulty cutting soft material like cast padding or skin. Although the removal is often painless, this can be distressing for the patient, especially children. It is possible to cut skin using a cast saw.(Benjamin *et al*., 2102). Additionally, plaster of Paris casts break down if patients get them wet.

The primary objective of the study was to formulate and develop herbal transdermal patch of *Cissus*

qudrangularis. And to estimate the potential of *Cissus qudrangularis* transdermal patch for fracture healing.

Methodology

Formulation of transdermal patch : Transdermal patches loaded with drug were prepared by solvent casting method. Required quantites of polymers were weighed and dissolved in a mixture of methanol and chloroform in the ratio of 1:1 and stirred for 3-4 hours. The ethanolic extract of *Cissus qudrangularis* was weighed and added to the above solution. Required quantity of dibutyl-n-pthalate as plasticizer and DMSO as penetration enhancer were added to the above solution and stirred until a cleared solution is obtained. The resulted uniform solution was poured within a glass bangle of area 28.28cm^2. An inverted funnel was placed over the ring to prevent the fast evaporation of the solvent and kept for 24 hrs to dry the patch. Evaluation of Transdermal patch were performed to confirm the best formulated patch. The Best formulated patch was selected among the five formulations on the basis of physical appearance, Drug release, In-vitro drug release study and SEM analysis. The best selected patch was used for further animal study.

Animal activity of herbal plaster : The activity has been approved by CPCSEA with reg. no. 490-01/Q/CPCSEA and protocol no. KUDOPS/52.

F# 02 of *Cissus qudrangularis* was found the best formulation amongst respective five formulations which was further studied on Rat model. The fracture was induced with the help of triple point pressure method. In this method we have developed a wooden instrument by which animal would feel minimum pain. The animal had been anesthesized previously with the help of Inj. Ketamine 100mg/Kg of B.W. as general anesthetic. That had been procured from M/s Chugh Medicine traders Roorkee. Experiment on animal was performed in presence of veterinary Doctor. There were two groups developed for the study. That is following.

GP-1	GP-2
Control Simple plaster	Plaster with *C. qudrangularis* formulation
06	06

Locomotors activity of animals

By this activity we can count the movements of animals per minute which could be correlated with the pain feeling in animal model. Locomotors activity was determined by Actophotometer.

Results

Results of animal activity showing with the help of Images.

Fig. 1 : Natural Image of *Cissus Qudrangularis*.

Fig. 2 : Dried stem of *Cissus Qudrangularis* plant.

Discussion

The transdermal patches of *Cisssus qudrangualris* was developed successfully by solvent casting method. By the evaluation of physical parameter and drug content

Fig. 3 : Image of best selected patch.

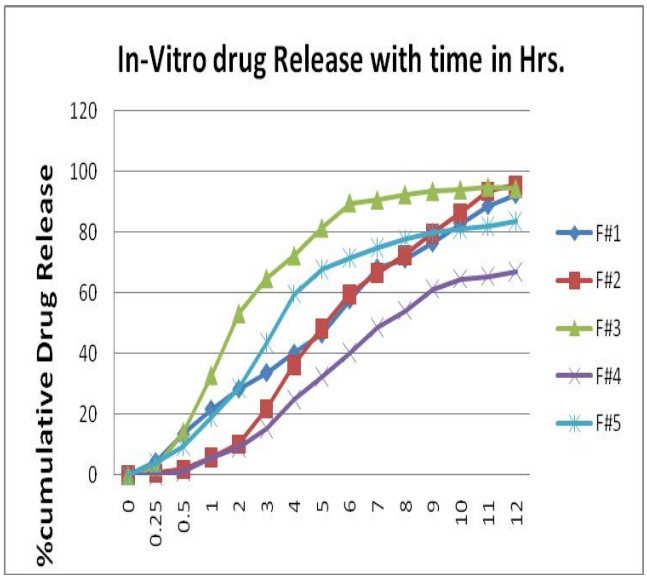


Fig. 4 : Graph showing *In-vitro* % cumulative drug release of various formulations.

Table 1 : Formula of Transdermal patch.

Formulation	Drug (mg)	HPMC 100M (mg)	ERL 100(mg)	PVP K30(mg)	DMSO	n-DBP
C.Q. #01	200	100	200	-	30%w/w	30%w/w
C.Q. #02	200	200	100	100	30%w/w	30%w/w
C.Q. #03	200	100	100	100	30%w/w	30%w/w
C.Q. #04	200	-	100	200	30%w/w	30%w/w
C.Q. #05	200	100	-	200	30%w/w	30%w/w

Table 2 : Comparative account of the five formulated patches.

Formulation	Thickness (mm)	Appearance	Moisture content	% Drug content	Flatness	Folding endurance
C.Q. #01	0.64	Thick, Flexible, Translucent, Smooth	15.40	95.34	100%	470
C.Q. #02	0.42	Thin, Flexible, Translucent, Smooth	9.27	96.42	100%	365
C.Q. #03	0.28	Thin, Flexible, Translucent, Rough	10.53	93.28	95%	370
C.Q. #04	0.25	Thin, Brittle, Translucent, Rough	5.23	70.45	70%	40
C.Q. #05	0.29	Thin, Flexible, Translucent Smooth	10.85	92.37	100%	430

it is found that F#1,F#2, F#3 and F#5 complies with the standard. *In-Vitro* drug release study follow and is best fitted to Zero–order and Higuchi kinetics. F#2 has been selected as best formulation. By the physical appearance of F#4 did not develop in proper patch due to the composition of ERS 100 and PVP K30. F#2 has the best ratio of HPMC K 100: ERL 100: PVPK 30 *i.e.* 2:1:1.

The best formulation was further evaluated for the potential of fracture healing in Rat model.

The *In-vivo* result shows fast healing in the group treated with herbal transdermal patch in comparison with control group.

X-ray Image showing Different stages rejoining of fracture at different day's interval.

Before fracture Fig. 1 : (C)

Fig. 2 : Rat I

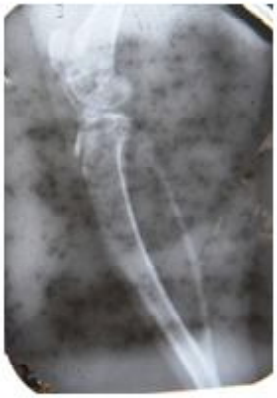


Fig. 3 : Rat II

After fracture Fig. 1 : (C)

Fig. 2 : Rat I

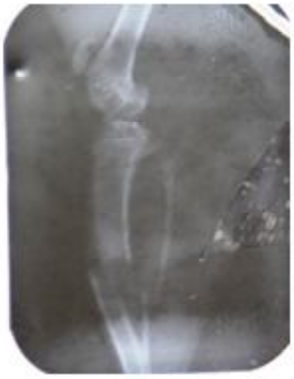
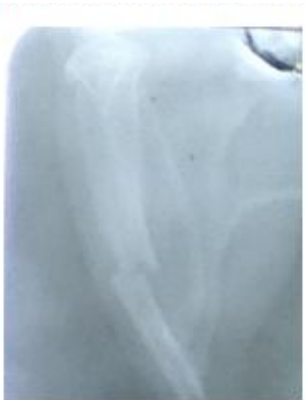


Fig. 3 : Rat II



Rejoining after 15 days Fig. 1 : (C)

Fig. 2 : Rat I

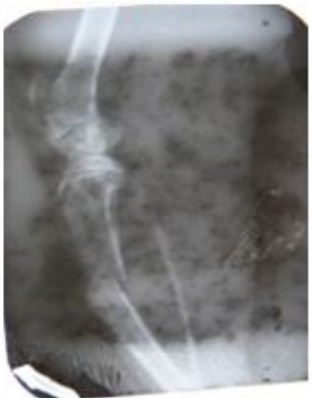


Fig. 3 : Rat II

Conclusion

The tablet form of *Cissus quadrangularis* is also available in the market by the brand name "Hadjod" that is more effective rather than other corticosteroids and NSAIDs used for the treatment of fractured patients. Therefore *Cissus quadrangularis* was incorporated in the transdermal patch which would penetrate to the fractured area and improve healing process. The pain and time would be reduced in the healing process of fractured bone when we combine the transdermal patch with silica cast. The result of X-ray Imaging show fast healing in the group treated with the herbal transdermal patch+ Silica cast in comparison to simple silica cast. The animal movements were very fast than the control group that shows less pain in the treated group.

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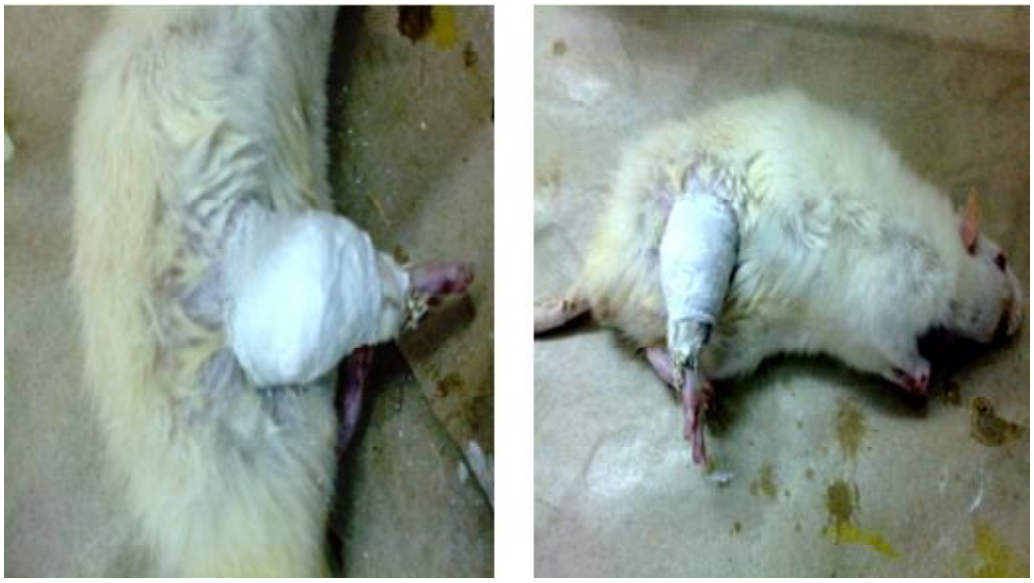
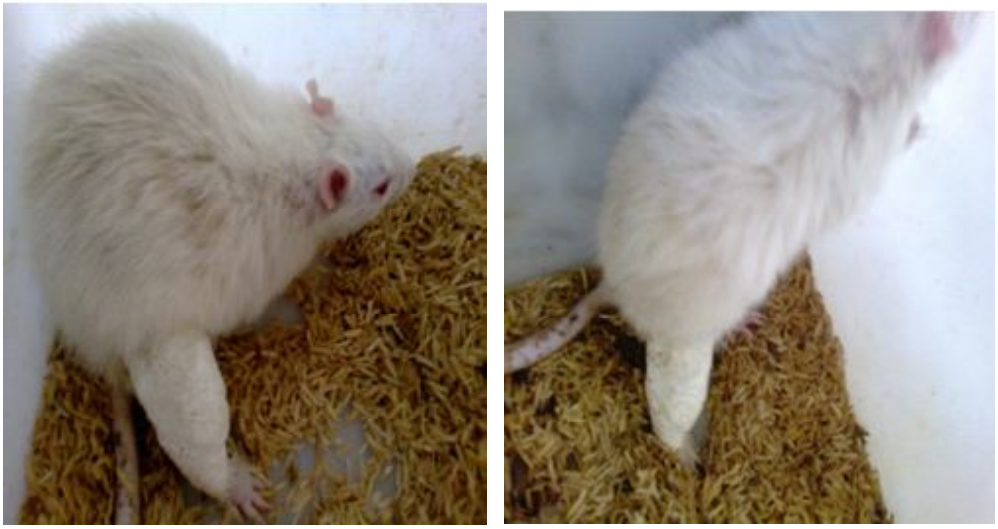


Figs. 5, 6 : Animal was anesthetized and plaster was applied on control and treated group, respectively.



Figs. 7, 8 : Movement of animals after 2 days of plasters application in control and treated with herbal plaster, respectively.

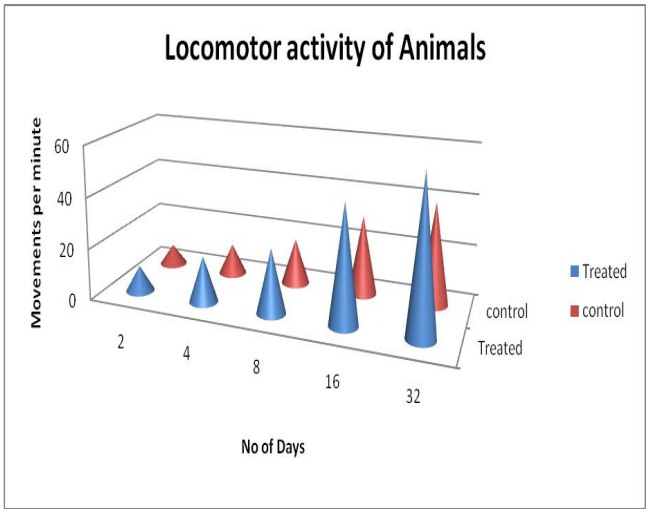


Fig. 9 : Showing locomotors activity of animals during the treatment.

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