POTENTIAL OF CISSUS QUADRANGULARIS 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 quadrangularis 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 quadrangularis for fracture healing. The transdermal patch containing ethanolic extract of Cissus quadrangularis 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 quadrangulris 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 quadrangularis as fracture healing agent that have been used for decades to treat the fracture traditionally in India.

Key words : Cissus quadrangularis, 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 has 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 Sri Lanka, Malay, 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 (Chidambaramurty et al., 2003) antiulcer activity, antiosteoporotic (Jainu et al., 2006) analgesic and anti-
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, sulphur 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 Quadrangularis (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 osteolytic 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
Potential of *C. quadrangularis* Transdermal Patch for Fracture Healing

Methodology

**Formulation of transdermal patch**: Transdermal patches loaded with drug were prepared by solvent casting method. Required quantities 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 quadrangularis* was weighed and added to the above solution. Required quantity of dibutyl-n-phthalate 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 28.28 cm². 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 quadrangularis* 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 anesthetized 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.

<table>
<thead>
<tr>
<th>GP-1</th>
<th>GP-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Simple plaster</td>
<td>Plaster with <em>C. quadrangularis</em> formulation</td>
</tr>
<tr>
<td>06</td>
<td>06</td>
</tr>
</tbody>
</table>

**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 *Cissus quadrangularis* images.

**Discussion**

The transdermal patches of *Cissus quadrangularis* was developed successfully by solvent casting method. By the evaluation of physical parameter and drug content
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: PVP K 30 *i.e.* 2:1:1.

Table 1: Formula of Transdermal patch.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug (mg)</th>
<th>HPMC 100M (mg)</th>
<th>ERL 100 (mg)</th>
<th>PVP K30 (mg)</th>
<th>DMSO</th>
<th>n-DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.Q. #01</td>
<td>200</td>
<td>100</td>
<td>200</td>
<td>-</td>
<td>30%w/w</td>
<td>30%w/w</td>
</tr>
<tr>
<td>C.Q. #02</td>
<td>200</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>30%w/w</td>
<td>30%w/w</td>
</tr>
<tr>
<td>C.Q. #03</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>30%w/w</td>
<td>30%w/w</td>
</tr>
<tr>
<td>C.Q. #04</td>
<td>200</td>
<td>-</td>
<td>100</td>
<td>200</td>
<td>30%w/w</td>
<td>30%w/w</td>
</tr>
<tr>
<td>C.Q. #05</td>
<td>200</td>
<td>100</td>
<td>-</td>
<td>200</td>
<td>30%w/w</td>
<td>30%w/w</td>
</tr>
</tbody>
</table>

Table 2: Comparative account of the five formulated patches.

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

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.
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.

Acknowledgement

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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 plaster application in control and treated with herbal plaster, respectively.

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

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References


