J. Maxillofac. Oral Surg. (Jan–Mar 2020) 19(1):106–112
https://doi.org/10.1007/s12663-019-01230-z

ORIGINAL ARTICLE

An Assessment of the Osteogenic Potential of *Cissus quadrangularis* in Mandibular Fractures: A Pilot Study

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Received: 10 May 2018 / Accepted: 27 April 2019 / Published online: 6 May 2019

Abstract
Background A routine mandibular fracture matures in about 12 weeks and hence hinders normal function significantly. Alternatives are being researched to hasten the healing and allow early function. This prospective study aimed to assess the osteogenic potential of the drug *Cissus quadrangularis* (CQ) on the mandibular fracture healing.
Design Prospective randomized study.
Results The study between the groups revealed a statistically significant increase in the alkaline phosphatase levels in CQ group in comparison with control group. The radiographic findings (increment in density and rate of change of density), clinical findings (mobility, swelling, reduction in pain) and other biochemical findings (serum calcium, serum phosphorous) did not differ statistically between the CQ and control groups.
Conclusion Based on the lack of a statistically significant improvement in almost all parameters except for the alkaline phosphatase levels, we believe that a larger sample size is required to ascertain the absolute value of CQ before adding it to the mandibular fracture regimen.

Keywords Mandibular fracture · Fracture healing · *Cissus quadrangularis* · Ayurveda

Introduction

Mandibular fractures are among the most commonly encountered maxillofacial injuries [1]. These fractures, even after appropriate surgical interventions, are associated with postop discomfort and prolonged rehabilitation. Unconventional herbal remedies have thus been used in an attempt to hasten the healing and reduce the period of rehabilitation [2–5].

Cissus quadrangularis (CQ), a succulent plant of the Vitaceae family, is used frequently in Ayurveda for healing fractures [6]. The processed stem is ingested for early fracture union [7]. The efficacy of CQ on early ossification and remodelling of bones have been reported to be, due to its ability to stimulate metabolism and increase uptake of the minerals by the osteoblasts [3].

The current study was aimed to understand if CQ provides a quantifiably early healing and to understand the value of adding CQ to a regular regimen of mandibular fracture treatment. In this study, two similarly treated sets of mandibular fracture patients have been studied: one set that had been treated surgically with an addition of oral CQ and another set that had only been treated surgically.

Methodology

The study was conducted on 30 consecutive consenting patients who presented with isolated traumatic mandibular fractures who came to the Department of Maxillofacial Surgery in 2014–2015. Patients of either sex between the ages of 20–40 years, who presented within 24 h of the injury—isolated mandibular fractures: symphyseal, parasymphyseal or body fractures, were included. Patients who had mandibular condyle or angle fractures, other facial

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fractures, comminuted fractures, edentulous arches and metabolic or endocrine disorders were excluded. The patients in the study were randomly allocated to the CQ group and control group by using a computer generated software. The CQ group included 15 patients who were prescribed capsules of *C. quadrangularis* (250 mg each)—two capsules B.D. for 42 days post-trauma by an Ayurvedic practitioner. The control group was treated without the Ayurvedic drug. Over the counter, CQ capsules were used for this study (Himalaya Hadjod tablets 250 mg). Apart from CQ, all patients were given injectable antibiotics and analgesics for 5 days postoperatively.

The fractured mandible was treated intraorally with open reduction and internal fixation (ORIF) with miniplates under general anaesthesia. Surgical treatment was carried out within 24 h of the trauma. The following parameters were recorded preoperatively, on the 7th, the 21st and the 42nd day post-trauma: biochemical parameters—serum calcium, serum phosphorous, serum alkaline phosphatase, clinical parameters—pain, swelling, mobility and radiographic parameter on CBCT exposures: increment in density and rate of change of density.

Pain was measured with a visual analogue scale, mobility was analysed by clinical manipulation of the fractured fragments, and the swelling was measured using a thread to measure the largest dimension vertical and horizontal dimensions. The CBCT scans in this study were acquired using a Kodak CBCT unit. The patients were positioned seated, and the head was secured with a chin support, a head support, and a forehead strap. The CBCT scans were saved as digital imaging and communications in medicine (DICOM) files. The radiographic images were set up for analysis similar to the study conducted by Bujtár et al. [8]; 1-mm axial sections along the fracture line were chosen. Twenty-five circular 4 mm^2 areas (a single pixel is 0.20 mm × 0.20 mm) were chosen on the Image J software to obtain a mean radiodensity along the fracture line. The areas chosen were devoid of metallic artefacts and the consequent noise. The increment in density and rate of change of density were calculated using the mean radiodensity values as per the formulae provided by Villarreal et al. [9] (Tables 1, 2).

Statistical Analysis

The approximation to normality of each quantitative variable was checked by means of the Shapiro–Wilk test. The multiple intergroup comparisons of each of the radiographic, biochemical and pain parameters were performed using a Greenhouse–Geisser analysis. Intergroup comparisons for mobility and swelling were analysed with Fisher's exact test and Chi-square tests, respectively. The statistical level of significance was set at $p < 0.05$.

Table 1 Formula for increment in density

Increment in density (ID)	
ID 7	RESULT 2 − RESULT 1/25
ID 21	RESULT 3 − RESULT 1/25
ID 42	RESULT 4 − RESULT 1/25

ID 7, 21 and 42 were the increments in density at 7th, 21st and 42nd days, respectively

RESULT 1: the mean radiodensity at the fracture site preoperatively, RESULT 2: the mean radiodensity value at the fracture site on the seventh day postoperatively, RESULT 3: the mean radiodensity value at the fracture site at 21 days, RESULT 4: the mean radiodensity value at the fracture site at 42 days

Table 2 Rate of change of density (RD)

Rate of change of density (RD)	
RD 7	RESULT 2 − RESULT 1/7
RD 21	RESULT 3 − RESULT 2/14
RD 42	RESULT 4 − RESULT 3/21

RD 7, 14, 21 and 42 were the rate of change in density during the first 7 days, between 7th and 21st days and between the 21st and 42nd days

RESULT 1: the mean radiodensity at the fracture site preoperatively, RESULT 2: the mean radiodensity value at the fracture site on the seventh day postoperatively, RESULT 3: the mean radiodensity value at the fracture site at 21 days, RESULT 4: the mean radiodensity value at the fracture site at 42 days

Results

In this prospective randomized trial, two groups of mandibular fracture patients were studied. Each group consisted of 13 male and 2 female patients. The control group consisted of 13 parasymphyseal and 2 symphyseal fractures. The CQ group consisted of 11 parasymphyseal, 3 symphyseal and 1 body fracture. Both the CQ group and the control group had similar treatment of mandibular fracture barring the intake of CQ capsules by the CQ group. About 13 cases in each group were treated with open reduction and internal fixation with titanium miniplates, and two cases in each group were treated with closed reduction and arch bar fixation. The results are described under the following headings—biochemical, clinical and radiographic parameters.

Biochemical findings In both the CQ and control groups, the serum calcium and serum phosphorous levels showed no statistically significant differences during the follow-up period. The alkaline phosphatase levels showed a statistically significant increase in CQ group in comparison with the control group at all the follow-up intervals ($p = 0.01$*) (Table 3; Figs. 1, 2, 3).

Table 3 Inter-comparison group of the various biochemical parameters

Biochemical parameters	Time period	Groups	Mean	SD	Level of significance
Serum calcium (SC)	SC preoperatively	CQ group	9.13	0.34	0.86
		Control group	9.21	0.61	
	SC 7th day	CQ group	8.66	1.35	
		Control group	8.92	0.53	
	SC 21st day	CQ group	9.04	0.54	
		Control group	9.15	0.45	
	SC 42nd day	CQ group	9.14	0.42	
		Control group	9.15	0.26	
Serum phosphorous (SP)	SP preoperatively	CQ group	3.47	0.79	0.806
		Control group	3.49	0.90	
	SP 7th day	CQ group	3.79	1.84	
		Control group	3.53	0.70	
	SP 21st day	CQ group	4.06	0.40	
		Control group	3.92	0.63	
	SP 42nd day	CQ group	4.20	0.38	
		Control group	4.06	0.55	
Serum alkaline phosphatase (SAP)	SAP preoperative	CQ group	65.80	15.58	0.01*
		Control group	81.33	25.71	
	SAP 7th day	CQ group	60.61	20.30	
		Control group	76.08	23.51	
	SAP 21st day	CQ group	91.13	19.49	
		Control group	87.83	19.86	
	SAP 42nd day	CQ group	80.60	18.88	
		Control group	98.25	21.14	

SC serum calcium, *SP* serum phosphorous, *SAP* serum alkaline phosphatase

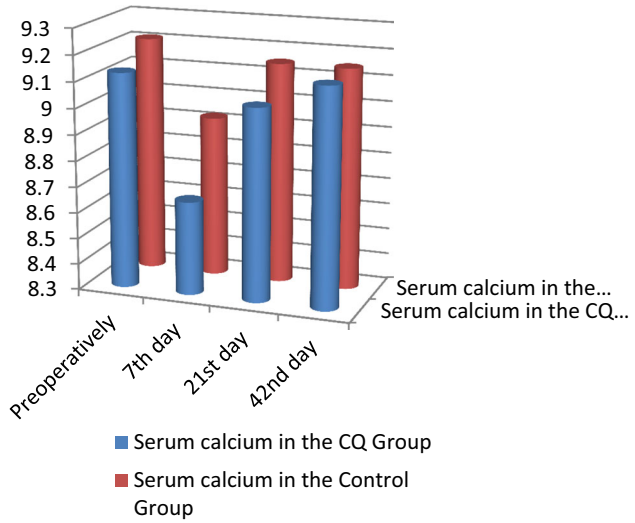

Fig. 1 Serum calcium comparison between the CQ and control groups

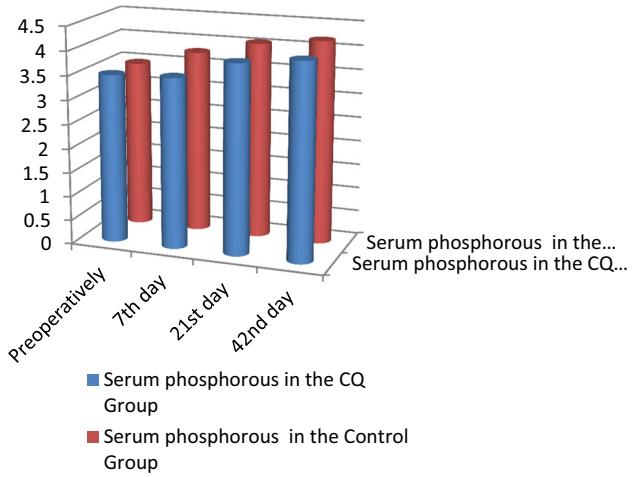

Fig. 2 Serum phosphorous comparison between the CQ and control groups

Clinical findings and radiographic findings There was no statistically significant difference between the two groups across all clinical parameters and radiographic parameters. No side effects to the CQ drug were recorded (Tables 4, 5; Figs. 4, 5, 6, 7, 8).

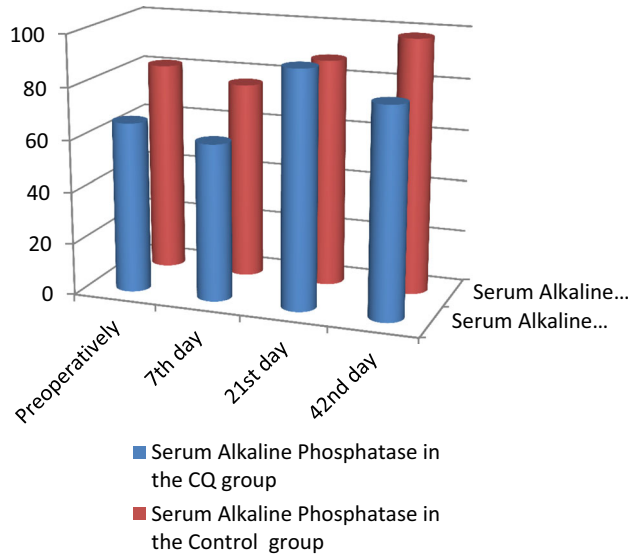

Fig. 3 Serum alkaline phosphatase comparison between the CQ and control groups

Discussion

The healing in mandibular fractures is often prolonged and leads to loss of productivity in young adults in whom this fracture is commonly seen [1]. The fracture stabilizes in 4–6 weeks and matures at 12 weeks [10]. This study aimed to evaluate the efficacy of CQ in hastening mandibular fracture healing and gauge the possibility of adding the drug as an adjuvant to regular mandibular fracture treatment.

The value of *C. quadrangularis* on early ossification and remodelling of bones have been reported by various authors in animal models [7, 11–13] and human patients [2, 3, 14]. Pharmacologically, *C. quadrangularis* contains high amount of carotene A, anabolic steroidal substances and calcium. It also contains ascorbic acid and calcium oxalate, two asymmetric tetracyclic triterpenoids, onocer-7-ene-3α,21β-diol (C30H52O2 m.p. 200–202 °C) and onocer-7-ene-3β,21α-diol (C30H52O2, m.p. 233–234 °C).

Table 4 Intergroup comparisons of clinical parameters

Clinical parameters	Time period	Groups	Mean	SD	Level of significance
Visual analogue scale (VAS)	VAS preoperatively	CQ group	7.40	1.35	0.471
		Control group	8.58	1.31	
	VAS 7th day	CQ group	6.67	2.32	
		Control group	7.42	1.62	
	VAS 21st day	CQ group	1.47	1.46	
		Control group	1.83	1.03	
	VAS 42nd day	CQ group	0.20	0.41	
		Control group	0.33	0.89	

Parameter	Time period	Groups	Present (in %)	Absent (in % of cases)	Level of significance
Mobility	Mobility preoperatively	CQ group	100.00	0.00	1.000
		Control group	80.00	20.00	
	Mobility 7th day	CQ group	13.30	86.70	0.224
		Control group	20.00	80.00	
	Mobility 21st day	CQ group	0.00	100.00	–
		Control group	0.00	100.00	
	Mobility 42nd day	CQ group	0.00	100.00	–
		Control group	0.00	100.00	
Swelling	Swelling preoperatively	CQ group	73.30	26.70	0.330
		Control group	93.30	6.70	
	Swelling 7th day	CQ group	6.70	93.30	0.785
		Control group	0.00	100.00	
	Swelling at 21st day	CQ group	0.00	100.00	0.147
		Control group	0.00	100.00	
	Swelling at 42nd day	CQ group	0.00	100.00	0.343
		Control group	0.00	100.00	

Table 5 Intergroup comparisons of radiographic parameters

Clinical parameters	Time period	Groups	Mean	SD	Level of significance
Increment in density	ID 7	CQ group	0.15	0.75	0.667
		Control group	− 0.09	0.66	
	ID 21	CQ group	0.37	0.74	
		Control group	− 0.04	0.67	
	ID 6	CQ group	0.37	0.71	
		Control group	− 0.06	0.79	
Rate of change of density	RD 7	CQ group	0.45	2.70	0.591
		Control group	− 0.38	2.30	
	RD 21	CQ group	0.35	0.93	
		Control group	0.21	1.07	
	RD 42	CQ group	− 0.07	0.83	
		Control group	− 0.07	0.82	

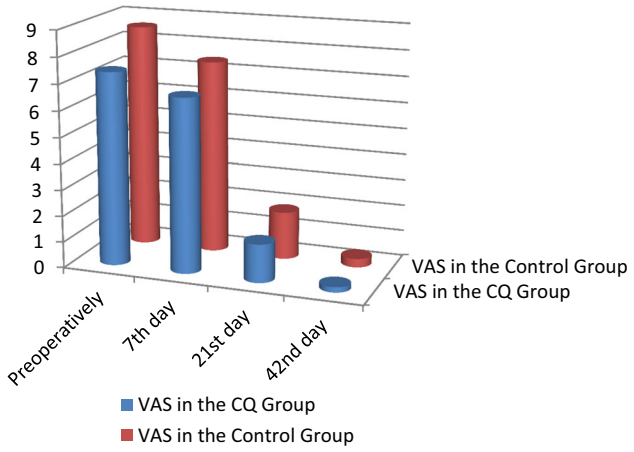

Fig. 4 Comparison of visual analogue scale (VAS) between the CQ and control groups

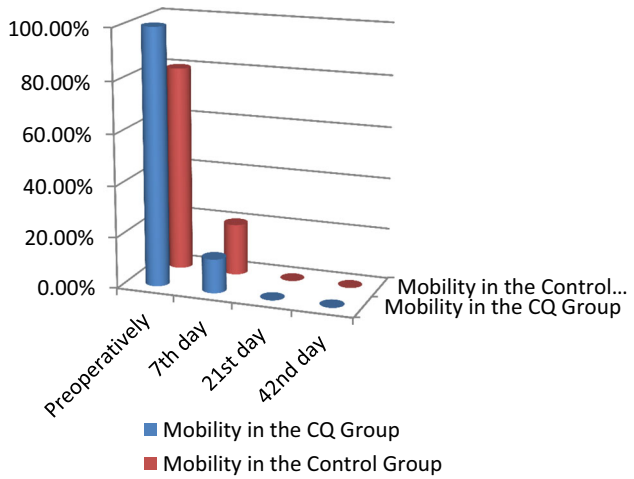

Fig. 5 Comparison of the fracture mobility between the CQ and control groups

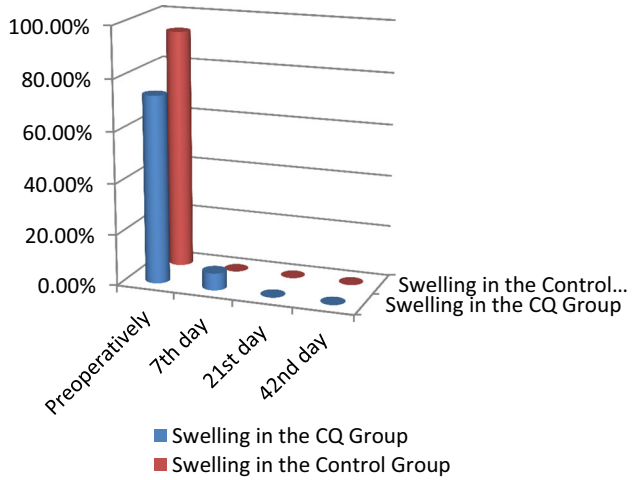

Fig. 6 Comparison of the swelling between the CQ and control groups

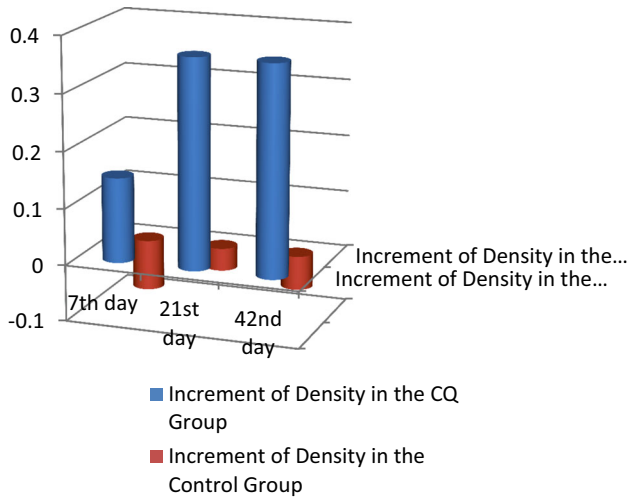

Fig. 7 Comparison of the increment in density between the CQ and control groups

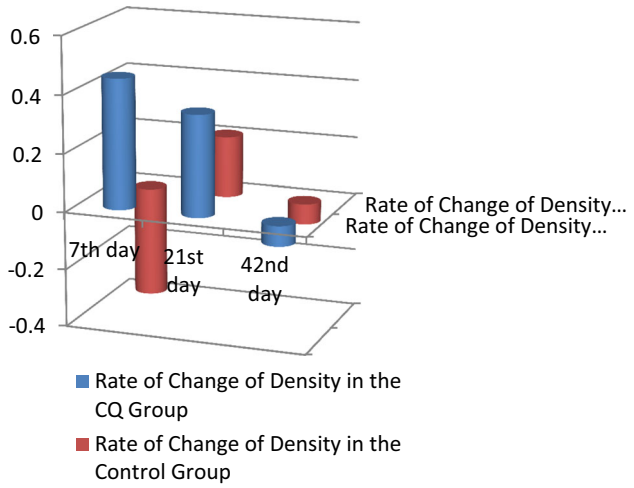

Fig. 8 Comparison of the rate of change of density between the CQ and control groups

The presence of β-sitosterol, δ-amyrin and δ-amyrone has also been reported. The aerial parts of *C. quadrangularis* is found to contain a new asymmetric tetracyclic triterpenoid, 7-oxo-onocer-8-ene-3β-21α-diol (C30H50 O3, m.p. 235–237 °C), 4-hydroxy-2-methyl-tricos-2-ene-22-one, 9-methyloctadec-9-ene, heptadecyl-octadecanoate, icosanylicosanoate, 31-methyl tritiacontan-1-ol, 7-hydroxy20-oxo-docosanyl cyclohexane and 31-methyl tritiacontanoic acid. It also conatins small amounts of taraxeryl acetate, friedelan-3-one, taraxerol and isopentacosanoic acid [15]. Though the exact mechanism of action has not been identified, a radioactive study conducted with calcium (Ca45) indicated that CQ stimulated cells of mesenchymal origin—the fibroblasts, the chondroblasts and osteoblasts at early stage and hastened the healing at the fracture site by about 10–14 days in the treated group [15]. Other studies have also found that a phytogenic steroid isolated from CQ stimulates osteoblasts and leads to early fracture healing [4, 6]. The other purported cause is that *C. quadrangularis* builds up the chemical composition of the fractured bone, namely its mucopolysaccharides, collagen, calcium, phosphorus and others as well as its functional efficiency [4]. During fracture healing, alkaline phosphatase—an enzyme secreted by the osteoblast, increases, indicating callus formation [6]. The volume and mineralization of the callus are proportional to the increase in the levels of alkaline phosphatase [16]. In the current study, we found a statistically increased level of alkaline phosphatase in the CQ group in comparison with the control group at all the intervals but no statistically significant difference across all the radiographic, clinical and other biochemical parameters between the two groups. These findings were in contrast to the findings of Deka et al. [7], who showed an early reduction in serum calcium and increased alkaline phosphatase indicating a faster callus formation in the CQ treated sample. Singh et al. [2, 3], on the other hand, showed an increase in the levels of calcium, phosphorous and alkaline phosphatase and a faster rate of reduction in pain, mobility and swelling in the CQ treated group.

Though there have been efforts to evaluate the efficacy of CQ, no absolute conclusion has been derived due a lack of adequate number of human studies and systematic reviews or meta-analysis of the medicinal use of this plant [17]. The other deterrent is the possibility of toxicity which has been associated with ingestion of various Ayurvedic drugs [18]. The study population had no side effects to the drug similar to the findings of all the previously mentioned studies.

Conclusion

Herbal remedies like *C. quadrangularis* have been propagated to hasten the fracture healing process. To be used as an adjunct, we feel that the healing needs to be clinically significant and radiographically quantifiable. In this study, we found biochemical findings which inferred a faster callus formation in patients who had consumed CQ; however, there was no significant radiographic or clinical improvement when compared to the control group. Based on this pilot study, we believe adding CQ as an adjunct to the regular regimen of mandibular fracture treatment may not have much merit and recommend that a larger population be tested to generalize its value.

Acknowledgements We would like to thank Dr. Lalitha BR, Professor, Government Ayurveda College, Bangalore, who provided insight and expertise that greatly assisted in the current research.

Compliance with Ethical Standards

Ethical Clearance All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Reference No. 02_D010_43915.

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