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# Evaluation of the osteogenic potential of Cissus quadrangularis on titanium discs and assessment of its clinical efficacy by analysing biomarkers in dental implant patients

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**Conflicts of Interest:** Nil

## Abstract

**Aims**: To evaluate the osteogenic potential of cissus quadrangularis over titanium implants and its clinical efficacy.

**Settings and Design**: Observational study

**Methods and Material**: Titanium discs were coated in Chitosan- Cissus quadrangularis- Hyaluronic acid solution and osteoblastic proliferation and viability were assessed by MTT assay and scanning electron microscopy.

In the vivo part, after impant placement Control group was given amoxicillin and clavulanic acid 625mg and analgesic diclofenac sodium for 3 days and Study group was given regular antibiotics followed by Cissus Quadrangularis capsules 250 mg/ B.D for 50 days post-surgery. Serum alkaline phosphatase and serum calcium level was assessed preoperatively to set up baseline values and post operatively at 28$^{th}$ and 56$^{th}$ day.

**Statistical analysis used**: Levene's Test for Equality of Variances Paired sample t test ANOVA

**Results**: Titanium discs with cissus quadrangularis showed increased confluence of osteogenic MG-63 cells compared to pure titanium discs and it showed increased adherence of MG-63 cells (p value $\leq$0.05). The serum alkaline phosphatase and calcium values were statistically analyzed by ANOVA and highest value was observed in 8$^{th}$ week postoperative of study group.

**Conclusions**: From this study it can be inferred that Cissus quadrangularis is a potential material to promote osseointegration and to shorten the treatment time between tooth implant placement and prosthetic loading.

**Keywords**: Alkaline phosphatase, Cissus quadrangularis, Osseointegration, Implant

## Introduction

The introduction of Osseo integrated implants in dentistry symbolizes a turning point in clinical dental practice. In the last two decades, it became clear that clinical implantology had advanced to a great extent and this treatment represents a predictable approach to the replacement of lost teeth. [1] Brane mark et al in Sweden introduced the concept of osseointegration whereby predictable long-term implant function could be achieved following a strict protocol.[2]

Mesenchymal stem cells give rise to osteoblasts which help in bone formation. These mentioned mesenchymal cells have the ability to undergo differentiation and proliferation to pre-osteoblasts, then they will mature to actively functioning osteoblasts. Implant Stability and success mainly depends on osseointegration. The great majority of clinicians and patients are interested in shortening the treatment time between tooth implant placement and prosthetic loading.

Cissus quadrangularis, a perennial climber widely used in traditional medicinal systems of India has been reported to possess bone fracture healing, analgesic properties and can increase osteoblastic activity.[3] Cissus quadrangularis can promote the formation of osteoblasts thereby helping the repair of fractured bones.[4,5]

There is very less literature currently available regarding the potential of cissus quadrangularis in osseointegration.

The clinical success of dental implants depends on successful osseointegration. Osseointegration is a crucial factor that has to be thought before establishing long time treatment plans.[6,7] Getting the knowledge of biological events and healing stage events which happens after implant placement is necessary for understanding different factors acting on bone-implant interface.[8]

The success and stability of implant is mainly attributed to osseointegration. Dental professionals are mainly focusing to reduce the treatment span between extraction of tooth and placement of implant. [9] This current study intends to assess the osteogenic potential of Cissus quadrangularis over titanium implants and its clinical efficacy.

## Materials and methodology

**Number of samples:** The study was planned in 2 parts: (i) In-vitro part where evaluation was done to check the osteogenic potential of Cissus quadrangularis over titanium discs, and asses the local effect of Cissus quadrangularis over the implant surface. (ii) In-vivo part where titanium implants were placed in patients selected through inclusion and exclusion criteria; followed by evaluation of bio markers to check the clinical efficacy and systemic effect of Cissus quadrangularis in osseointegration when administered as an oral drug/ supplement during healing (osseointegration) phase of implant.

Using ‘N’ master app, with the power of 80% and alpha error of 5% based on sample, mean 4 with standard deviation 1, sample size of 16 per group for in vitro part and 11 per group for in vivo part was calculated. In the in-vitro part of the study 32 samples were included. Group A includes 16 samples which are pure titanium discs and group 2 includes 16 samples which are titanium discs with Cissus quadrangularis.

In the in vivo part of study, 22 individuals needing implant placement to replace the missing teeth in the mandibular posterior region was selected. Group A includes participants who were receiving clavulanic acid 625mg, amoxicillin and analgesic diclofenac sodium for 3 days. Group B includes participants who were receiving clavulanic acid 625mg, amoxicillin and analgesic diclofenac sodium for 3 days followed by

Cissus Quadrangularis capsules 250 mg/B.D. for 50 days.

Ethical clearance was obtained from The Institutional Review Board & Institutional Ethical Committee.

IRB APPROVAL NUMBER: SRMDC/ IRB/ 2019/ MDS/ No.204

**Sample preparation for in-vivo part**

**Titanium sample preparation**

From commercially available titanium rods (Ti6Al4V) (ISO 5832-3:2021) 32-disc samples of 10mm diameter and 1mm height were milled manually (Zip titan cutting discs, Indmas Engineering Works). For cutting, a semi-synthetic liquid coolant (KYOCERA) was used to avoid overheating which may alter the physicochemical properties of the titanium metal rod. The 32 samples obtained were finished by using 4.0 paper (1200 grit aluminium oxide).

**MTT assay**

Chitosan (CS) and hyaluronic acid (HA) were dissolved in phosphate-buffered saline (PBS) separately at a concentration of 20 mg/mL. These two components are polysaccharides and can act as scaffolds for carrying hydrogels. Chitosan and hyaluronic acid were mixed at a volume ratio of 5/5 at room temperature. Cissus quadrangularis (CQ) extract was prepared by the maceration technique. The solution was prepared by mixing both the above mentioned in 50–50 by weight. The titanium discs were coated in Chitosan- Cissus quadrangularis- Hyaluronic acid solution for 24 hours at 37ºC in a hot air oven and it was then transferred to in vitro culture analysis. Osteoblastic proliferation and viability were assessed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl—tetrazolium bromide (MTT) assay and scanning electron microscopy (SEM).

**Osteoblastic cell culture**

Mg-63 cells resembling human osteoblasts (Sasham Biologicals Pvt Ltd) were cultured in minimum essential medium (MEM) at 37° C with the specific humidified atmosphere of 5% carbon dioxide.

In a six well plate, Mg- 63 cells were plated and incubated for 37° C. Once the cell lines reach the confluence, the discs were added and incubated for 24 hours. After incubation, along with the sample 100µl/ well (5mg/ ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for four hours (Figure 1). After incubation, one ml of Dimethyl sulfoxide (DMSO) was added in all wells. The absorbance at 570 nm using UV Spectrophotometer was measured by using DMSO as the blank. Measurements were performed and the concentration obligatory for fifty percentage inhibition (IC50) was determined graphically. The number of viable cells was evaluated in percentage by means of the below formula: (Mosmann,1983)[10] % Cell viability = $A_{570}$ of treated cells / $A_{570}$ of control cells × 100

The cellular attachment and cell growth on the disc were evaluated by plate reading UV – Spectrophotometer to check their osteogenic potential using MTT assay. Viable cells converted MTT agent into a purple-coloured formazan product with an absorbance maximum near 570 nm. The cell proliferation rate was evaluated after 24 hours and 72 hours for the study group and control group (Figure 2).

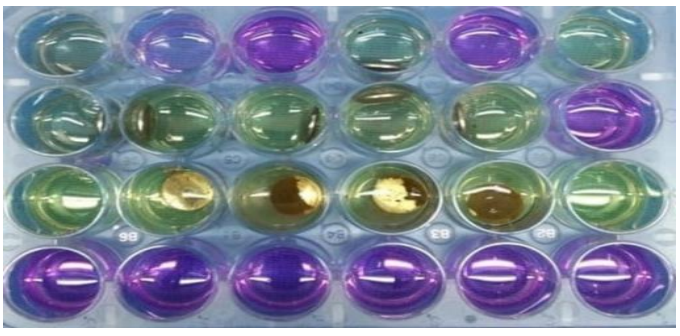


Figure 1: Showing addition of MTT reagent

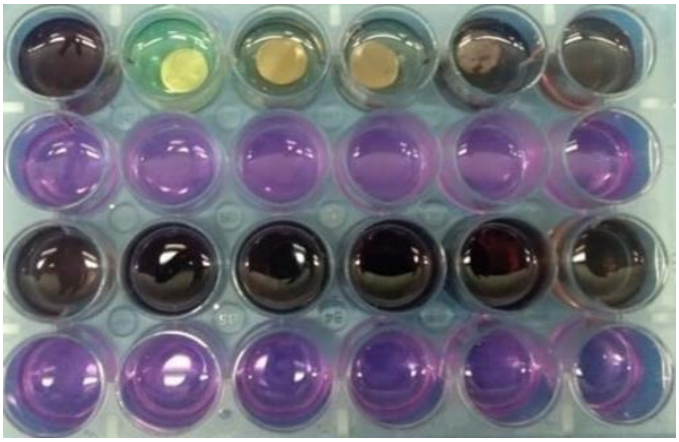


Figure 2: Formation purple colour

After 72 hours one sample in each group was fixed and then studied for cell migration and osseointegration, under a scanning electron microscope (SEM) in a magnification range of 20 to 2,00,000 x after the evaluation of osteoblastic like MG-63 cells. (Figure 3)

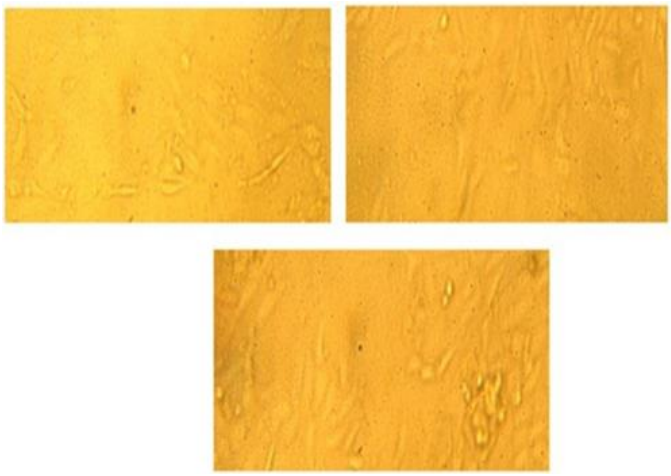


Figure 3: MG-63 cells after 24 hours and 72 hours.

Examining under SEM was utilized to contemplate the type of cell attachment. Following 3 days of culture, samples were splashed with “PBS solution” and secured in 2.5% “glutaraldehyde”, and post-fixation is accomplished with 0.1% osmium tetroxide. The samples are then dried out with 75%, 95%, and 100% of evaluated ethanol arrangement. Dried out samples were covered with platinum and imaged utilizing Scanning electron microscopy.

Serum alkaline phosphatase and serum calcium assay (In-vivo part)

In this study, 22 individuals needing implant placement to replace the missing teeth in mandibular posterior region was selected. The participants were divided into two groups (eleven participants in each group). Viewing the CBCT images which have the cross-sectional reformatted images of the bone quantity and quality at each desired implant site allowed to develop the surgical plan. All patients underwent an adequate pre-surgical preparation consisting of detailed case history, consenting of patient, blood investigations and radiographic examination. All patients met inclusion and **exclusion criteria**

**Inclusion criteria**

1. Patients with missing teeth and wants replacement with implants.
2. Age group: 25-45 years.
3. South Asian population with equal gender distribution.
4. BMI Index within the range of 18.5-29.9 kg/m².
5. Individuals with serum alkaline phosphatase level within the range of 20-140 IU/L and serum calcium level:8.5-10.5 mg/dl.
6. Capable of and freely willing to provide informed consent prior to participating in the study.
7. Individuals medically fit for implant placement.

**Exclusion criteria**

1. Immuno compressive patients
2. Patients of calcium disorder.
3. Pregnant / Lactating women.
4. Alcoholics and/or drug abusers.
5. Patient on steroids, oral contraceptive pills.
6. Patients suffering from major systemic illness necessitating long term drug treatment (Rheumatoid arthritis, psycho-neuro-endocrinal disorders, cardio

vascular, disorders, Musculo skeletal disorders, connective tissue disorders, metabolic disorders etc.)

7. History of hypersensitivity to any of the trial drugs or their ingredients.

8. Patients who have completed participation in any other clinical trial during the past six months.

Following comprehensive clinical and radiographic examination of the patient, the original Brane mark protocol requiring a vestibular flap with a two-stage approach was followed. The underlying bone and osteotomy site was exposed for implant osteotomy preparation and insertion. The implant (ADIN Dental Implant Systems Ltd.) was placed and buried under the soft tissue. Group 1(Control Group) was given regular antibiotics and analgesics regimen. Antibiotics was amoxicillin and clavulanic acid 625mg and analgesic diclofenac sodium for 3 days after implant placement. Group 2 (Study Group) was given amoxicillin and clavulanic acid 625mg and analgesic diclofenac sodium for 3 days followed by Cissus Quadrangularis capsules FSSAI and FDA number: ISO 9001:2008) 250 mg/ B.D for 50 days post-surgery. Serum alkaline phosphatase and serum calcium level was assessed preoperatively to set up baseline values and post operatively at 28th and 56th day. Samples was collected morning after overnight fasting.

**Results**

The mean cell proliferation of the MG -63 cell line after 24 hours was evaluated in two groups. The highest mean value (79.69) was observed in group B while the lowest mean value (49.83) was found in group A. The standard deviation was 2.888 in group A, 0.970 in group B with standard errors of 1.444 and 0.485 respectively.

Cell proliferation of the MG- 63 cell line in 72 hours was found to be 79.69 in group B which was high when compared to groups. Standard deviation was found to be 3.506 in group A, 2.123 in group B with standard errors of 1.753 and 1.062 respectively (Table 1).

Table 1: Standard deviation and standard error (24 hrs and 72 hrs).

| | Sample | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| % Cell Viability - 24 Hours | Titanium Discs | 4 | 49.83 | 2.888 | 1.444 |
| | Titanium Discs+ CQ | 4 | 76.40 | .970 | .485 |
| % Cell Viability - 72 Hours | Titanium Discs | | 58.50 | 3.506 | 1.753 |
| | Titanium Discs+ CQ | | 79.69 | 2.123 | 1.062 |

Table 2 shows Levene's Test for Equality of Variances which was used to assess the equality of variances, F value of 2.923 was obtained for 24 hours and 0.823 for 72 hours respectively and the value of P was lower than 0.05 which is relevant as per the statistics. The test shows that there is a significant difference in cell proliferation between the groups. Group B has a higher value of osteoblastic cell proliferation as compared with Group A in 24 hours and 72 hours.

Table 2: Levene's Test for Equality of Variances (24 hrs &72 hrs)

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| % Cell Viability - 24 Hours | Equal variances assumed | 2.923 | .138 | -17.443 | 6 | .000 | -26.567 | 1.523 | -30.294 | -22.841 |
| | Equal variances not assumed | | | -17.443 | 3.668 | .000 | -26.567 | 1.523 | -30.952 | -22.183 |
| % Cell Viability - 72 Hours | Equal variances assumed | .823 | .399 | -10.341 | 6 | .000 | -21.192 | 2.049 | -26.207 | -16.178 |
| | Equal variances not assumed | | | -10.341 | 4.940 | .000 | -21.192 | 2.049 | -26.480 | -15.905 |

Mean serum alkaline phosphatase values (pre-op,4th week,8th week) were observed and the highest mean value (106.664) was found in the 8th week postoperative study group while the lowest mean value (97.091) was found in the preoperative of control group (Table 3).

Table 3: Mean serum alkaline phosphatase values (pre-op, 4thweek, 8thweek)

| | Group | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Serum alkaline phosphatase- Preoperative | Control Group | 11 | 97.091 | 4.1582 | 1.2538 |
| | Study Group | 11 | 97.136 | 4.8266 | 1.4553 |
| Serum alkaline phosphatase - 4th week postoperative | Control Group | 11 | 98.982 | 4.4441 | 1.3399 |
| | Study Group | 11 | 104.327 | 4.6407 | 1.3992 |
| Serum alkaline phosphatase - 8th week postoperative | Control Group | 11 | 99.782 | 4.3993 | 1.3264 |
| | Study Group | 11 | 106.664 | 4.6932 | 1.4151 |

F value of 0.307,0.33 and 0.024 was obtained for preoperative, 4th-week post-operative and 8th week postoperative values respectively using Levene's Test for Equality of Variances and the value of P was lower than 0.05 which is relevant as per the statistics (Table 4).

Table 4: Levene's Test for Equality of Variances (pre-op,4th week, 8th week).

| t-test for Equality of Means | | Levene's Test for Equality of Variances | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Serum alkaline phosphatase-Preoperative | Equal variances assumed | .307 | .586 | -.024 | 20 | .981 | -.0455 | 1.9209 | -4.0523 | 3.9614 |
| | Equal variances not assumed | | | -.024 | 19.571 | .981 | -.0455 | 1.9209 | -4.0580 | 3.9671 |
| Serum alkaline phosphatase - 4th week postoperative | Equal variances assumed | .033 | .859 | -2.759 | 20 | .012 | -5.3455 | 1.9373 | -9.3867 | -1.3043 |
| | Equal variances not assumed | | | -2.759 | 19.963 | .012 | -5.3455 | 1.9373 | -9.3871 | -1.3038 |
| Serum alkaline phosphatase - 8th week postoperative | Equal variances assumed | .024 | .880 | -3.548 | 20 | .002 | -6.8818 | 1.9395 | -10.9276 | -2.8360 |
| | Equal variances not assumed | | | -3.548 | 19.917 | .002 | -6.8818 | 1.9395 | -10.9287 | -2.8349 |

The test shows that there is a significant difference in serum alkaline phosphatase level between the groups. The 4th and 8th week have higher value of serum alkaline phosphatase level as compared with preoperative values.

Table 5 represents the mean serum calcium values in three groups. It was observed that there was the highest mean value in the (10.355) 8th week postoperative of study group while the lowest mean value (8.891) was found in the preoperative of control group.

Table 5: Mean serum calcium values (pre-op,4th week,8th week).

| | Group | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Calcium - Preoperative | Control Group | 11 | 8.891 | .2700 | .0814 |
| | Study Group | 11 | 9.055 | .5681 | .1713 |
| Calcium - 4th week postoperative | Control Group | 11 | 9.736 | .4249 | .1281 |
| | Study Group | 11 | 10.073 | .3228 | .0973 |
| Calcium - 8th week postoperative | Control Group | 11 | 9.945 | .3503 | .1056 |
| | Study Group | 11 | 10.355 | .1809 | .0545 |

F value of 2.958, 0.348, and 5.680 was obtained for preoperative, 4th-week post-operative and 8th week postoperative respectively using Levene's Test for Equality of Variances and the value of P was lower than 0.05 which is relevant as per the statistics (Table 6).

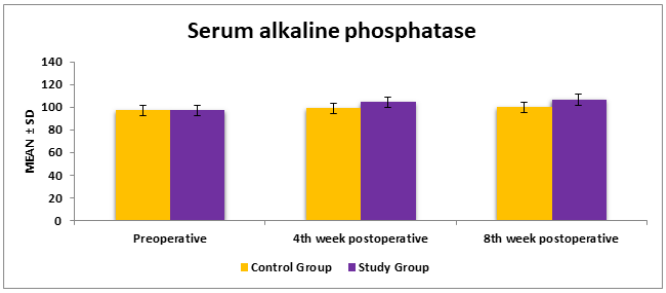
Table 6: Levene's Test for Equality of Variances (pre-op,4th week,8th week)

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Calcium - Preoperative | Equal variances assumed | 2.958 | .101 | -.863 | 20 | .398 | -.1636 | .1896 | -.5592 | .2320 |
| | Equal variances not assumed | | | -.863 | 14.299 | .402 | -.1636 | .1896 | -.5696 | .2423 |
| Calcium - 4th week postoperative | Equal variances assumed | .348 | .562 | -2.091 | 20 | .050 | -.3364 | .1609 | -.6720 | -.0008 |
| | Equal variances not assumed | | | -2.091 | 18.658 | .050 | -.3364 | .1609 | -.6735 | .0008 |
| Calcium - 8th week postoperative | Equal variances assumed | 5.680 | .027 | -3.441 | 20 | .003 | -.4091 | .1189 | -.6571 | -.1611 |
| | Equal variances not assumed | | | -3.441 | 14.979 | .004 | -.4091 | .1189 | -.6625 | -.1557 |

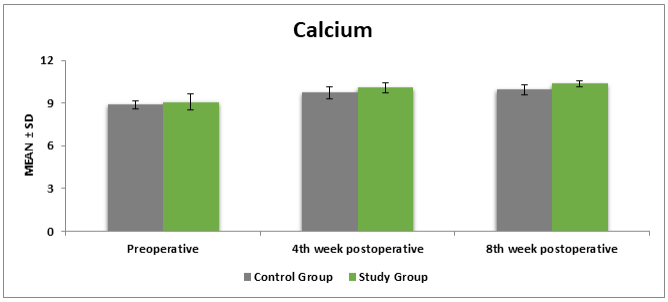
The test shows that there is a significant difference in calcium levels between the groups. 8th week has a higher value of calcium level as compared with preoperative and 4th week values.

Cell viability of all the groups after 24 hours and 72 hours is shown in graphs 1and 2 respectively. Mean serum alkaline phosphatase and calcium values of the control group and study group are shown in graphs 1 and 2 respectively.

Graph 1: Graphical representation of mean serum alkaline phosphatase values of control group and study group.




Graph 2: Graphical representation of mean serum calcium values of control group and study group.




**Discussion**

Herbs are employed by different cultures around the world for years for the treatment of different diseases. Cissus quadrangularis, a perennial climber widely used in traditional medicinal systems of India has been reported to possess bone fracture healing, analgesic properties and can increase osteoblastic activity. [5,11]

Serum alkaline phosphatase is an important serum biomarker for analysing bone layover and formation, so evaluating alkaline phosphatase levels during specific intervals can guide the evaluation of healing process and osseointegration. [6,12] Calcium utilization and absorption should be increased for healing purpose in regards to fracture healing, CQ works by high usage of minerals like calcium, sulphur by the osteoblasts in bone healing.[16] Cissus quadrangularis consists of vitamin A and C which can promote collagen formation.[17]

In-vitro part of the present study, Cissus quadrangularis improved the osteoblastic proliferation of MG-63 cells, a well-known osteoblastic cell line. The in-vitro part study was done to evaluate the osteoblastic proliferation and viability by using human osteoblast like MG-63 cells after coating cissus quadrangularis on titanium discs by MTT (methyl thiazolyl tetrazolium) assay and scanning electron microscope.

Human osteoblast-like MG 63 cells serves to analyze the effects of biomaterials and the corresponding cell response.[18] Osteoblastic cell growth majorly depends on surface microarchitecture. MG-63 cells exhibit various types of osteoblastic genes that are responsible for bone - forming cells.[19] Cell number reflects cell attachment, adhesion, spreading, and proliferation. [20]

Cell feasibility and cytotoxicity were checked utilizing viability/cytotoxicity packs. Afterwards, MG-63 cells were cultivated on the samples. After development, the samples were flushed with “PBS solution” for 30- 62 minutes. Multiplication test was done to survey cell growth, the discs are inspected with a time of 3 days following cell cultivation. Every unit territory (1 $cm^2$ of the embed) was cultivated with 5 × 104 cells/well and afterwards refined. At that point, the supernatant was taken and estimated at 490nm utilizing a micro plate reader. Analysis was done and the concentration necessary for a 50% inhibition (IC50) was verified graphically and percent of cell feasibility was determined.

The results showed that though the cells are binding on the surface of the disc, it does not show any toxicity on the tested cell line, MG-63. Finally, the reports of this study revealed that the significance level of the two groups by comparisons between the groups for MG-63 cells absorbance at 24 and 72 hours. The results obtained indicates significant variation of mean cell proliferation of MG -63 cell line after 24 hours in two groups. It was observed that there was the highest mean value (79.69) in titanium discs with cissus quadrangularis while the lowest mean value (49.83) was found in pure titanium discs. Levene's Test for Equality of Variances which

was used to assess the equality of variances, F value of 2.923 was obtained for 24 hours and 0.823 for 72 hours respectively and the value of P was lower than 0.05 which is relevant according to the statistics. Tests proved that CQ titanium discs showed better results than the other group and it was statistically significant. The study inferred high statistical significance towards titanium with cissus quadrangularis discs that showed increased cell proliferation in comparison with pure titanium discs.

In the in-vivo part, we utilised chemical biomarker serum alkaline phosphatase (ALKPO4) to analyse Osseo integration with subsequent placement of implant. Tablet form of Cissus quadrangularis was used to assess the influence of Cissus quadrangularis on the state of bone layover by measuring numerous serum biochemical markers. The serum alkaline phosphatase values were statistically analyzed by ANOVA, in which it was observed that there was the highest mean value (106.664) in 8th week postoperative of study group while the lowest mean value (97.091) was found in preoperative of control group.

Calcium supplement forms the major factor for bone healing. Lysine is an important amino acid aid in interest of calcium from the gut. Vitamin A and C is present in CQ which are effective in the formation of collagen.[21,22] By promoting the proliferating sequences within bone, CQ facilitates bone healing. Bone regeneration rate was increased and it improved blood circulation as well as nutrient supply to the bone. It preserves bone tissue anabolism and regeneration and promotes osteoblastic proliferation and differentiation. [23]

The study done by D. K. Deka et al. [24] proved that cissus causes dissolution of bone and periosteal reaction at the end of 11$^{th}$ day in study group. When evaluated radiographically Cissus group had showed significant deposition of bone and reaction in periosteum compared to control group after 21 days. They concluded that Cissus group showed shortened healing period by two weeks.

The research done by R. Sinha et al. [25] proved that serum alkaline phosphatase levels are high in bone healing and in patients who has malunion of bony fragments. Study concluded that by monitoring the alkaline phosphatase levels can give information about bone layover and bone resorption in a short period of time. Positive correlation was found between serum ALP levels and it was statistically relevant indicating a progress towards satisfactory bone healing.

Bhagath Kumar Potu et al [26] analysed the reactions of petroleum ether extract of CQ on the multiplication rate of bone marrow mesenchymal stem cells. By downregulating proinflammatory cytokines, Cissus quadrangularis decreases the amount of bone resorption. The material proved to have a better biological potential in the cell proliferation study than the titanium material. The physical properties are also superior to the comparative group. Cissus quadrangularis can be considered as a good alternative to improve bone formation after the insertion of implants. To reduce the osteointegration period Cissus quadrangularis can be given along with the regular antibiotic regimen.

**Limitations of the study**

In this study titanium discs were coated with cissus quadrangularis and then transferred to in-vitro culture analysis. Being a plant derived product, effective ways have to be established to coat cissus quadrangularis on implant surface to conduct more clinical studies. In this study only 32 in vitro samples and 22 in vivo samples are included but larger sample size can give better clinical outcome. Further studies are required to enhance the scope of this study and for its effective clinical application.

**Conclusion**

This study evaluated the osteoblastic proliferation and viability by using human osteoblast like MG-63 cells after coating cissus quadrangularis on titanium discs. Titanium discs with cissus quadrangularis showed increased confluence of osteogenic MG-63 cells compared to pure titanium discs. Among both of the groups, titanium with cissus quadrangularis showed increased adherence of MG-63 cells (p value ≤0.05).

Cissus quadrangularis elevated serum alkaline phosphatase and serum calcium level, thereby promoting bone healing.

With all these results, it is inferred that cissus quadrangularis is a potential material to promote osseointegration and to shorten the treatment time between tooth implant placement and prosthetic loading, as it possesses both local and systemic beneficiary effects.

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