Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

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Osteoinductive effect of the nanoparticulate form of *Cissus quadrangularis* ethanolic extract on implant surface in experimental animals

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Abstract

Background The purpose of this study was to evaluate the effect of nanoparticles (NPs) of a plant extract on implant osseointegration in vivo. NPs of the *Cissus quadrangularis* (CQ) extract were synthesized. Twenty Wistar rats were included in the study. The test group ($n=5$) had NPs-incorporated, the control group ($n=5$) had no incorporation of NPs, and the sham group ($n=5$) did not have implants placed. The toxicity profile of NPs was carried out by biochemical analysis before and after the implant placement. The rate of integration was assessed using Cone Beam Computed Tomography (CBCT), Digital X-ray, histology, and scanning electron microscopy (SEM) at intervals of 2, 4, and 6 weeks. Statistical significance between the groups was determined by Analysis of Variance (ANOVA) ($p<0.05$) and the differences were uncovered with post hoc Tukey's test.

Results Radiographs showed that the bone density around implants with NPs was considerably higher than those without NPs. The osteoid seam along the implant surface was found to be considerably higher in the test group. A noteworthy increase was observed in the bone formation around the implants in bone samples belonging to the test group. Comparing the values at different time intervals, the highest serum Alkaline phosphatase (ALP) activity was noted in the test group after six weeks and the lowest serum Acid phosphatase (ACP) activity was documented in the test group after 6 weeks.

Conclusion The test group samples exhibited an osseointegration rate of less than 2 weeks thus demonstrating CQ NPs to be a potent osteoinductive agent.

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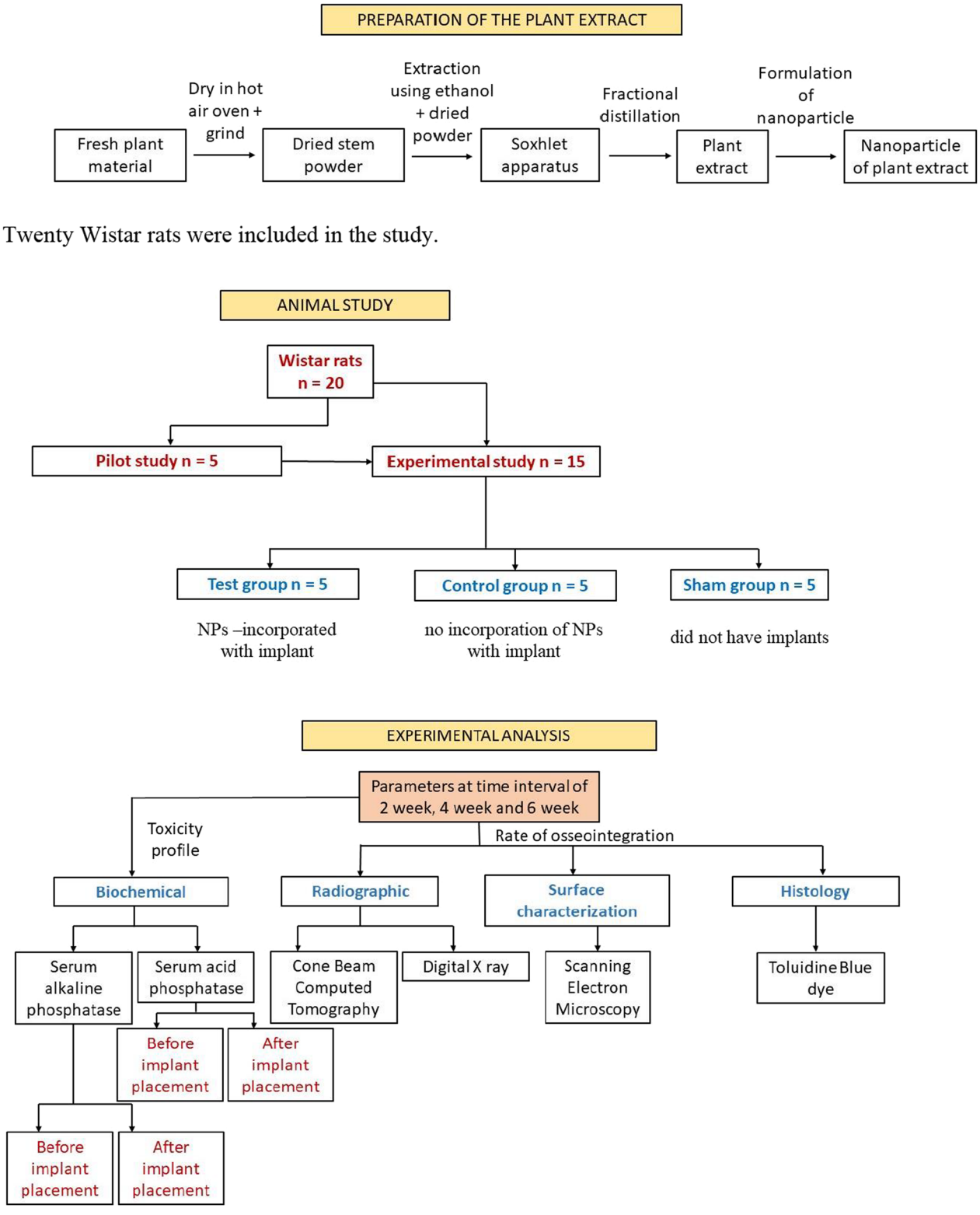
Keywords Osseointegration, Nanoparticles, Bone-implant interface, *Cissus quadrangularis*, Bone density

Graphical abstract



1 Background

Titanium has been long considered a benchmark for implant prosthesis. The sole purpose of osseointegration is that the functionality of the implant prosthesis is restored for an extended period [1]. It involves the role of mesenchymal stem cells in bone regeneration and propagation of cells at the bone-implant interface. Additionally, it includes spontaneous surface oxide formation and the implant host-response of implanted biomaterials [2]. Coating bioactive agents onto the titanium implant surface is to promote the revitalization of bone over the implant titanium surface [3]. However, abnormal biomaterial implantation could result in a foreign body reaction and the development of fibrous peri-implant tissue meshwork [4]. The chemical state of titanium on the surface is the determining factor for the tissue reclamation around an implant. Hence, an ideal surface biomaterial that has the potential for optimal bone formation with minimum adverse effects determines the success of implants.

Cissus quadrangularis (CQ) also known as veld grape is a native plant that has gained much attention for its bone-healing properties. It is routinely referred to as a "Bone Setter" in Ayurvedic Medicine due to its ability to accelerate bone healing, promoting osteoblastic proliferation, and collagen synthesis. CQ has also demonstrated anti-oxidant, anti-microbial, and anti-inflammatory actions [5].

This study was aimed at assessing the effect of plant extract CQ on the implant surface and to assess whether incorporation of nanoparticles of CQ extracts into the osteotomy site before implantation would enhance the rate of osseointegration on the implant surface in comparison to the ones excluded of NPs.

2 Methods

2.1 Study design

The animal experiment was approved by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, after approval of the Institutional Animal Ethics Committee (IAEC) [IAEC/KMC/115/2016 dated 16/12/2016]. Twenty Wistar rats, irrespective of gender, 90 days of age, weighing between 200 and 300 g were included in the study. Fifteen of them were arbitrarily allocated to three groups. The test group included rats in which implants were placed after the incorporation of plant extract-derived NPs into the osteotomy site. Bone sample numbers for the test group derived after different time intervals are as follows: Sample numbers 1 (2 weeks); 3 and 9 (4 weeks) and 5 and 7 (6 weeks). The control group comprised rats in which only implants were placed devoid of NPs. The samples are number 10 (2 weeks); 6 and 8 (4 weeks) and 2 and 4 (6 weeks). The sham group involved rats used for blood collection for biochemical analysis and no implant was placed in them. This group included sample numbers 11 (2 weeks), 12 and 13 (4 weeks), and 14 and 15 (6 weeks).

2.2 Preparation of plant extract

CQ plant material was collected locally as detailed below in "Preparation of plant extract". Ethanol (95%) was procured from Finar, Mumbai. Polyvinyl alcohol (PVA) and polycaprolactone (PCL) were purchased from Sigma-Aldrich, Bangalore. All other ingredients used were of analytical/ reagent grade.

Fresh CQ plant material was collected from a local nursery and authenticated by a senior pharmacist. The herbarium was prepared (PP 616, voucher no. 514) and preserved at the Herbarium and Botanic Museum of the Pharmacy school. Four kilograms of plant stem were dried in a hot air oven at 110 °C for 30 min and ground into a coarse powder. Extraction from the plant material was carried out using a Soxhlet Extractor by the application of heat. Approximately 300 g of the fine powder was used with 2L of 95% ethanol as solvent. The collected extract was separated from the solvent by fractional distillation. The ethanolic plant extract was collected in a distillation flask and transferred into a petri dish for drying in a hot water bath at ≈ 60 °C for about 7 h and then collected in a glass beaker [6]. The prepared ethanolic extract was characterized by Reverse Phase High Performance Liquid Chromatography (HPLC).

2.3 Formulation of NPs from the plant extract

Acetone (5 ml) was used as the solvent to dissolve 10 mg of the extract and 50 mg of PCL. Acetone was added dropwise to PVA solution (20 ml of 1% w/v in water) using a magnetic stirrer at 500 rpm and continued for 30 min. The dispersion was subjected to probe sonication (Pulse: 6 s; Amplitude of 40%; Time: 20 min) for size reduction. The nano-suspension was kept for stirring overnight on a magnetic stirrer at 500 rpm to remove acetone. It was centrifuged at 15,000 rpm at 4 °C for 30 min and the pellet was re-dispersed in 5 ml of 5% w/v mannitol solution in water. The resultant dispersion was then subjected to lyophilization for 48 h. The NPs were characterized based on size, zeta potential, and polydispersity index (PDI) using a ZetaSizer (NanoZS, Malvern Instruments, UK) before and after lyophilization. The surface morphology of the nanoparticles was assessed by Transmission electron microscopy (TEM; CM200, Philips, USA). A small quantity of nanoparticles was diluted with Milli-Q water and stained with 2% w/v phosphotungstic acid for 1 min. Then, the sample was placed on the copper grid for visualization of particles.

2.4 Estimation of the entrapment efficiency (EE) of the NPs

Entrapment efficiency was determined by the procedure explained below: The supernatant obtained after centrifugation was collected and free ethanolic extract present in the supernatant was measured for its absorbance using a UV spectrophotometer. The absorbance readings obtained with the extract alone and the nanoparticulate extract demonstrated the EE of the NPs. The EE was calculated to determine the percentage of the extract entrapped or absorbed by the nanoparticles using the equation, EE (%) = $[(T\text{-}F)/T] \times 100$, where T and F are total extract content and free or un-entrapped extract in the supernatant.

The lambda max of the extract was found to be 255.4 nm. The calibration curve was linear with an R^2 value of 0.9914 in the concentration range of 1–5 µg/ml. The calibration curve equation was found to be $y = 0.1997x – 0.0371$. The entrapment efficiency of the extract was found to be 94.5 ± 1.01%.

2.5 Pilot study

A pilot study was conducted in Two Wistar rats to check for the feasibility of molar tooth extraction under anesthesia. A 2 mm osteotomy site was prepared in the maxillary bone using a straight fissure bur. This procedure was carried out to ascertain whether the preparation of an implant receptor site was easier than the insertion of an implant into an extraction socket. As the thickness of the bone intra-orally was inadequate to place implants, we placed the implants in the femoral bone [7–9] following the Animal Research: Reporting of In Vivo Experiment (ARRIVE) guidelines.

2.6 Implant design and its configuration

In the present study, ten Straumann® Dental implants: SLA® Standard, Standard Plus, Tapered Effect, Bone Level, and Bone Level Tapered were used. (Test group: 5; Control group: 5; Dimensions: 3.3 mm in diameter and 8 mm in length).

2.7 Surgical implant placement procedure

Rats were anesthetized using an intraperitoneal injection of 500 mg of thiopental sodium in 20 ml of distilled water. The dosage was determined based on the body weight of the rodent and an approximate amount of 0.5–0.6 ml was used [10, 11].

About 2 ml of blood was collected from the retro-orbital site by the capillary method in plain vacutainer (without any anticoagulant) and sent to the laboratory for cryopreservation. The animals were immobilized supine, and the hind limbs were shaved and disinfected with 10% povidone-iodine. The knee joint was fully exposed. A cylindrical hole was prepared at the intercondylar notch, parallel to the femoral long axis, using implant drills (Straumann implant placement kit). The drills were used in an ascending diameter to prepare the osteotomy site such that the longitudinal axis of the drill hole was placed parallel to the longitudinal axis of the femoral diaphysis. The drilling sequence was as follows: initial punch with a round bur (1200 rpm), pilot drill (800 rpm), 2 mm diameter drill (600 rpm), and finally 3.3 mm diameter drill (500 rpm). A short drill for bone-level implants up to the mark of 8 mm (length) was used and continuous profuse irrigation with physiological saline via syringe was done to minimize frictional heat and thermal necrosis. One implant was placed in each of the osteotomy sites mechanically with the implant driver.

2.8 Experimental analysis

2mgs of NPs were incorporated into the osteotomy before implantation in the animals belonging to the test group. For the control group, the implants were directly placed after the osteotomy procedure without the incorporation of NPs. An average insertion torque of around 30N-cm was achieved. After the insertion of the implants, the operating field was cleaned, and the wound was closed with non-resorbable sutures (Polyamide 4.0; Ethicon Products). Animals were monitored daily for 1-week post-surgery to detect issues related to wound healing and mobility. Post-procedure, all rats had free access to the normal pellet of food and water.

The rats were euthanized after 2, 4, and 6 weeks, the femoral condyles were dissected and cleaned from adhering tissues. Specimens were then processed for the evaluation of peri-implant bone regeneration. The femoral bone harvested from all the rats was analyzed by Cone Beam Computed Tomography (CBCT) for the quantification of implant osseointegration. The histologic evaluation was done to assess the quality of bone-implant osseointegration. Biochemical analysis was performed before and after placing the implants to monitor the levels of Alkaline phosphatase (ALP) and Acid phosphatase (ACP). Finally, the surface topography of the implant post-osseointegration was analyzed with scanning electron microscopy (SEM).

2.9 Radiographic imaging of the implant site

The bone samples were scanned using a CBCT system (iCAT Vision Q) with a scan resolution of 0.25 Voxel (120–kVP, 20.2 mA, and an integration time of 14.7 s). The region of interest was marked as a circle of 0.5 mm diameter to evaluate the density of the trabecular bone around the implant. The samples were segmented and the

region of interest for Bone-Implant Contact (BIC) evaluation was defined 2 pixels away from the implant surface. CBCT machines have proved to cause beam attenuation in the presence of a metal [12, 13]. To avoid any discrepancy in the obtained results, digital radiographs were taken with an Intra-Oral X-ray System (Kodak Cs 2100, 60 kV, 7 mA, 0.125 secs).

2.10 Histological examination

The femoral bones that were retrieved were fixed in 10% buffered formalin. The implant site was excised using a carborundum disk. The samples were dehydrated with an increasing concentration of alcohol following which the tissue was submerged in self-cure acrylic resin (Dentsply, Rapid Repair Clear Acrylic), and the blocks were processed with the Leica SP1600 saw microtome (Leica Biosystems, Danaher Corporation). Thin ground sections were prepared parallel to the longitudinal axis of the femur. These sections were then decalcified, decreased to a thickness of 30 µm, and stained with Toluidine Blue dye. Digital light microscopic images were produced with an Olympus dotSlide 2.0 Virtual Microscopy system (dotSlide—Virtual Slide System, Olympus; 200 × magnification) [14, 15].

2.11 Scanning electron microscopy (SEM)

The calcified bone-implant samples mounted in polymethylmethacrylate were uniformly sputtered with silver using a sputtering system. Four different sides of the sputtered section were compared—an image presenting the entire length of the implant (longitudinal section) with the host bone, an image focused on the left side of the implant, an image focused on the right side of the implant and an image focused on the apex of the implant. These samples were then examined by SEM (EVO MA 18 with Oxford EDS (X-act) to characterize the surface morphology of the osseointegrated implants [16, 17]. The samples were visualized under different magnifications at 2, 4, and 6-week intervals and the results of the radiographic investigation were confirmed.

2.12 Biochemical analysis

Determination of serum alkaline phosphatase (ALP) was carried out by para-nitrophenyl phosphate (pNPP) Kinetic method (Diagnostic reagent kit, Tulip Diagnostics Ltd.). Estimation of Serum acid phosphatase (ACP) was done by α—Naphthylphosphate Kinetic method (Tulip Diagnostics Ltd.). The absorbance for ALP and ACP was measured at a wavelength of 405 nm. Animal experiments have evaluated ALP and ACP (Tartrate-resistant acid phosphatase; TRAP) levels to assess osteoblastic and osteoclastic activity respectively around dental implants [18, 19].

2.13 Statistical analysis

All statistical evaluations and data presentations were performed with SPSS (version 24.0, IBM Corporation). The data were subjected to a one-way analysis of variance test (ANOVA). Statistical significance was determined at a 95 percent confidence level. The normality of data was analyzed by the Shapiro–Wilk test. As the data followed normal distribution, the parametric tests were used to analyze the data. The one-way ANOVA test was used to check the mean dissimilarities among the groups. Post hoc analysis was done using Tukey's (honestly significant difference) HSD test.

3 Results

3.1 Characterization of extract and NPs

Out of a total of 300 g of the plant material used for extraction using 95% ethanol, 1.112 g of semi-solid extract was obtained (practical yield: 0.37%). The size of prepared nanoparticles before lyophilization was 625.1 nm, which after lyophilization was 705.2 nm. Likewise, PDI values were found to be 0.230 and 0.259 before and after lyophilization respectively. Zeta potential values were—4.10 mV before and − 3.26 mV after lyophilization. Minor variations in the zeta potential and PDI values before and after lyophilization may be due to the use of cryoprotectant (mannitol) during the preparation of nanoparticles. As shown in the TEM image (Fig. 1), most of the particles showed more than 500 nm in size. The particles were found to be discrete and spherical.

3.2 The outcome of the pilot study

Out of the total 20 Wistar rats, two were included in the pilot study. It was concluded that the available bone thickness intra-orally was insufficient for implant placement and thus we decided to place the implants in the femoral bone.

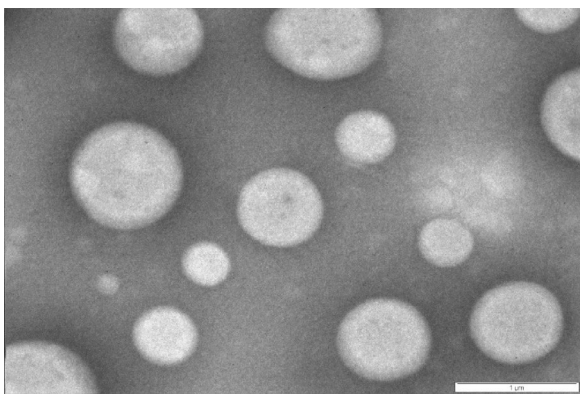


Fig. 1 TEM micrograph of Nanoparticles

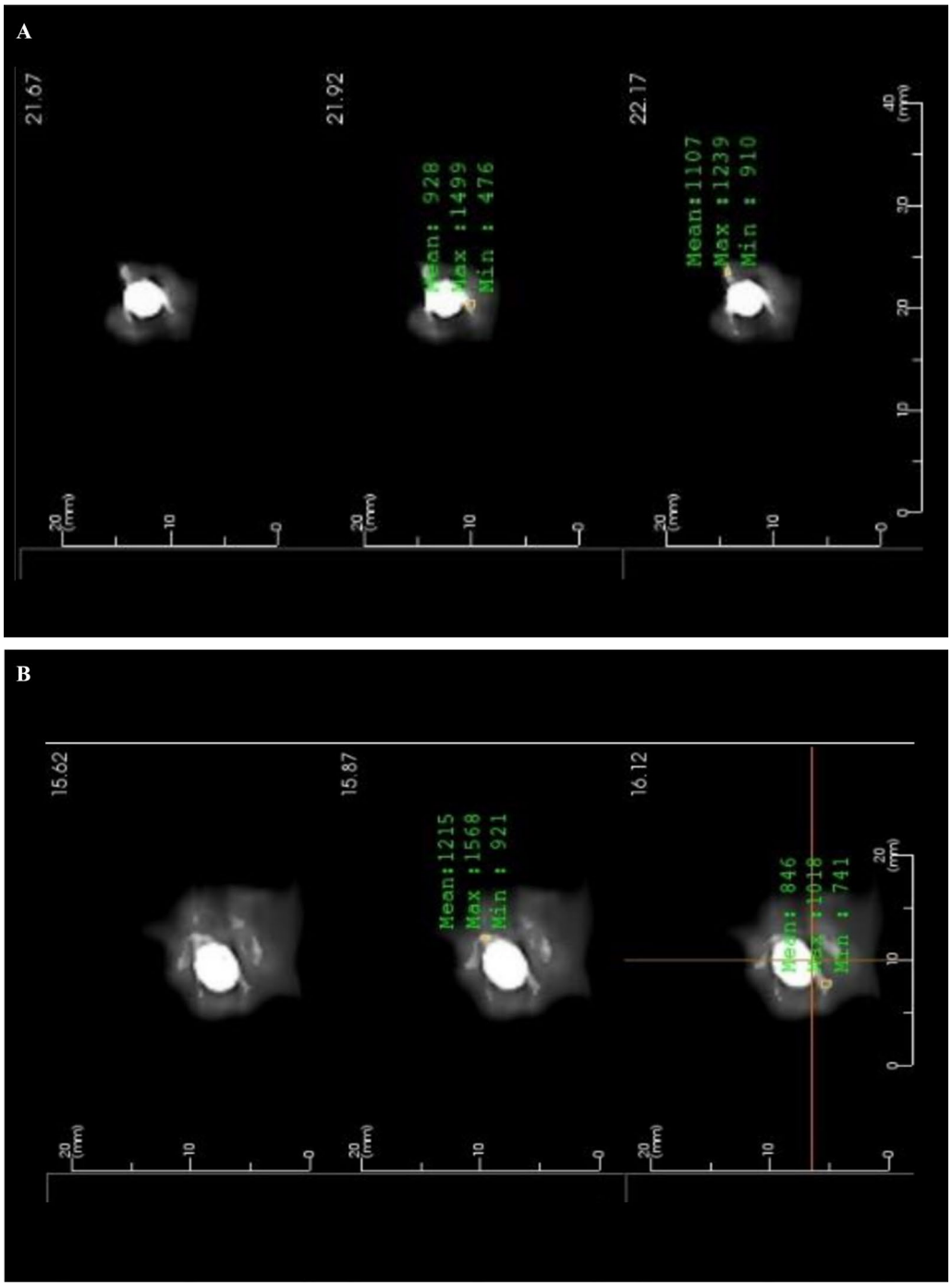


Fig. 2 **A** CBCT analysis of test group sample after 2 weeks. **B** CBCT analysis of test group sample after 4 weeks. **C** CBCT analysis of test group sample after 6 weeks

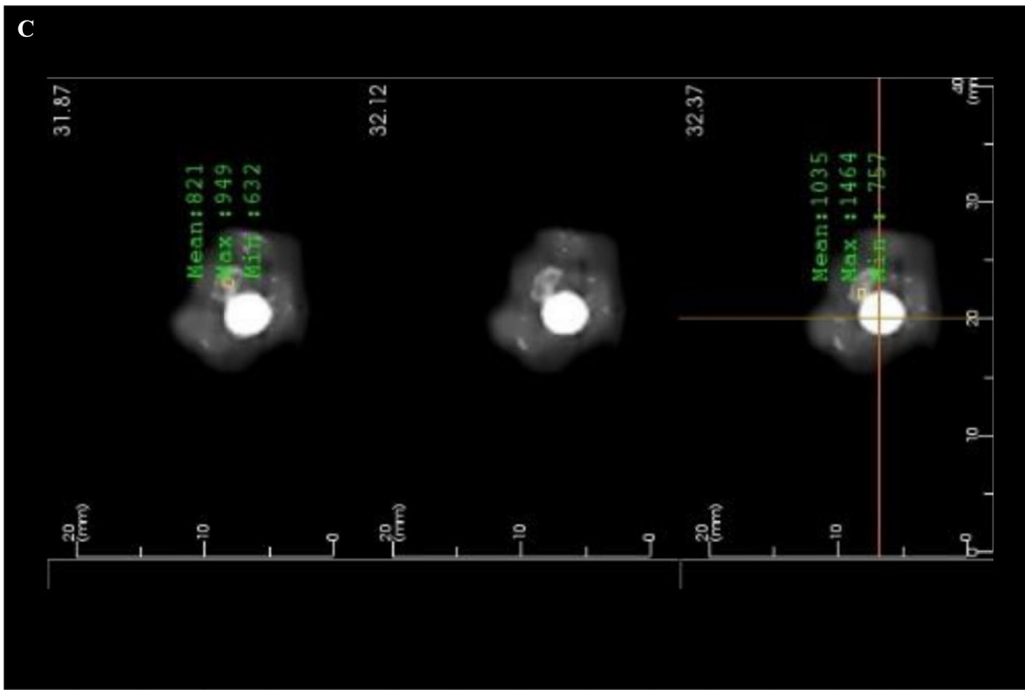


Fig. 2 continued

3.3 Post-operative investigations

Of the eighteen rats, three rats died during the surgical procedure before the placement of implants due to anesthetic complications. Two out of the total ten implants failed to integrate with necrosis of adjacent host bone. Both the rats (Sample No. 10 and 4) belonged to the control group and hence were not considered for any further investigation. The animals treated with the remaining eight implants survived and there was no clinical complication noted throughout implantation. Hence, these samples were considered for further analysis. All the investigators were blinded regarding the experimental and control group.

3.4 Radiographic interpretation

The bone density around the osseointegrated implant was measured in Hounsfield Units (HU). Radiographs showed that the bone density around implants with NPs was considerably higher than those without NPs (Figs. 2A–C and 3A–B). The highest bone density was observed with the rat bone sample No. 3 (test group) after 4 weeks of implant placement (approx.1030 HU); while the lowest bone density was recorded with the rat bone sample No. 6 (control group) after 4 weeks of implant placement (approx. 357 HU) (Table 1, Graph 1). Qualitative analysis using digital X-ray demonstrated slightly more opacity around the implant in bone Sample No. 3 incorporated with CQ NPs, followed by bone Sample No. 1, which was also treated with CQ NPs, while the samples belonging to rats of the control group exhibited lesser dense bone in comparison to the test group (Figs. 4A–C and 5A–B).

3.5 Histological analysis

Toluidine blue stained sections observed under an optical microscope at 200X magnification showed a clinically significant difference between the newly formed unmineralized bone tissues from the host bone. Dark blue staining depicted mineralized bone while the un-mineralized osteoid exhibited a pale blue or slightly pinkish hue (Fig. 8A–B). The osteoid seam along the implant surface was found to be considerably higher in the test group (Sample No. 3).

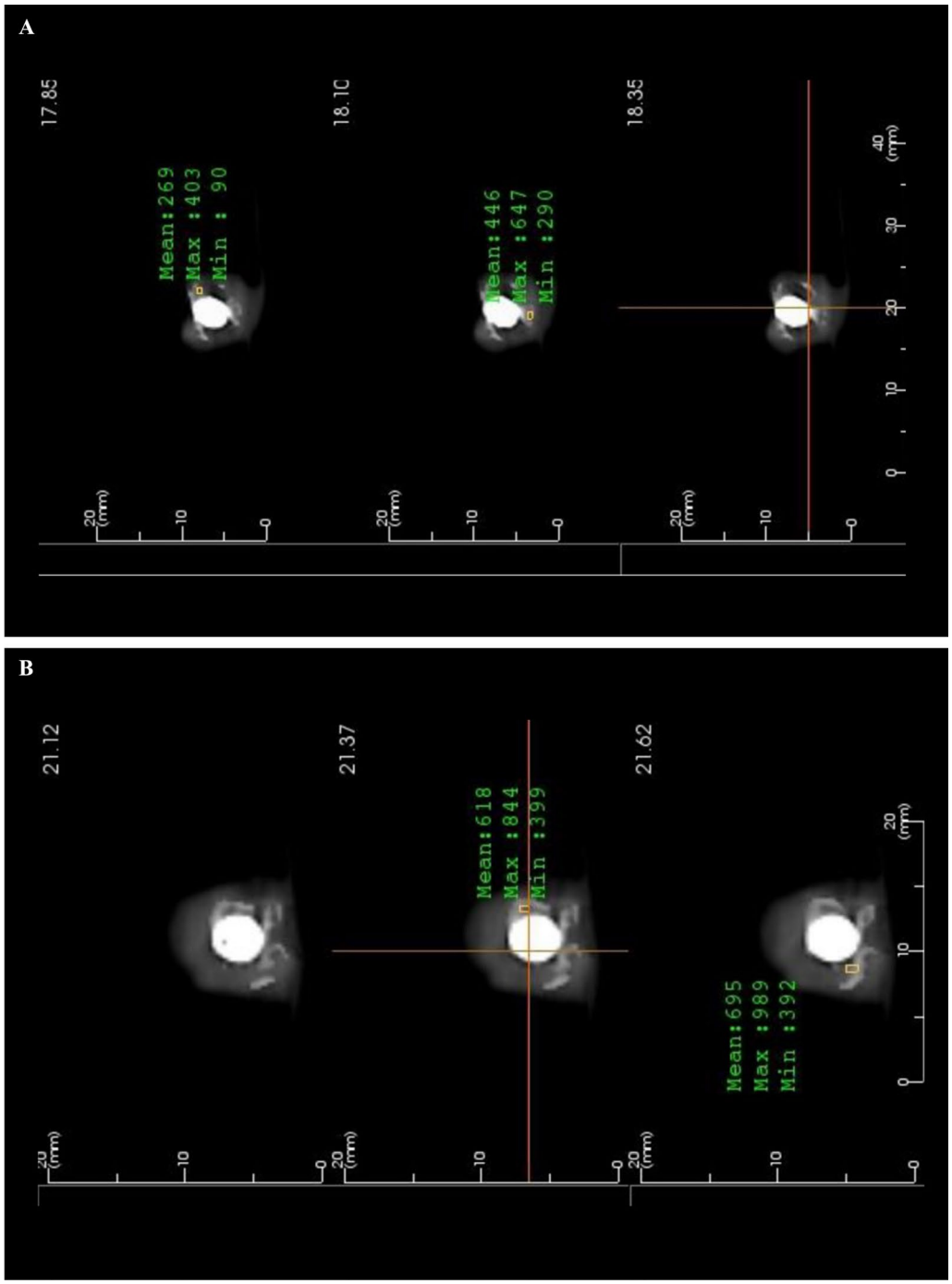
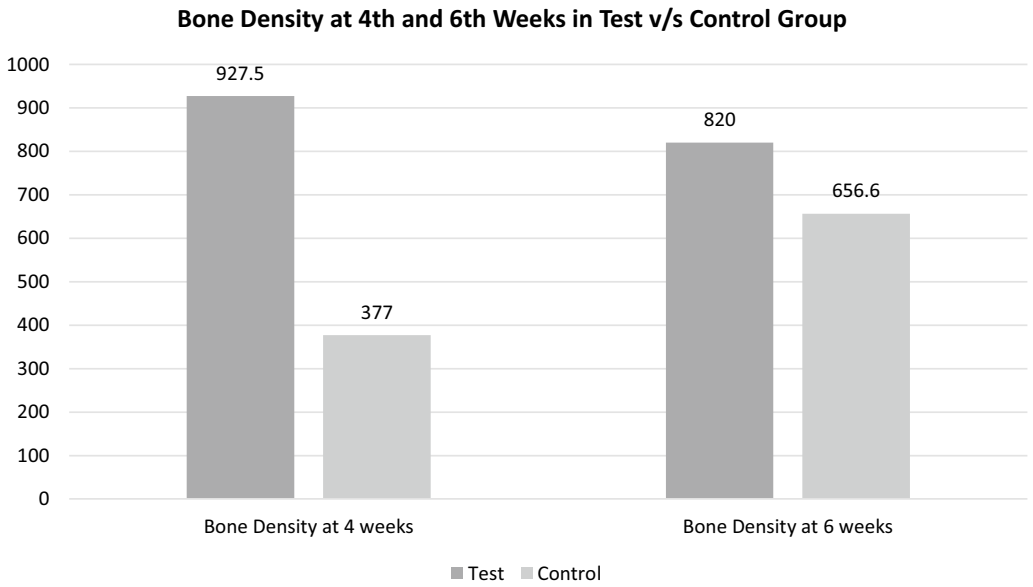


Fig. 3 **A** CBCT analysis of control group sample after 4 weeks. **B** CBCT analysis of control group sample after 6 weeks

Table 1 Comparison of bone density at 4 and 6 weeks between the test and control group

Timeline	Groups	*N*	Mean	S.D	S.E	M.D	95% CI	*Z* value	*P* value[#]
4 weeks	Test	2	927.50	145.66	103.00	550.50	99.45–1001.54	5.251	0.034[†]
	Control	2	377.00	27.57	19.50				
6 weeks	Test	2	820.00	148.49	105.00	–	–	–	
	Control	1	656.60	–	–	–	–	–	–

[#] *p* value derived from *t* test; [†]significant at $p < 0.05$



Graph 1 Comparison of bone density at 4 weeks and at 6 weeks between the test and control group

3.6 Evaluation of images obtained by SEM

SEM images presented a good judgment in the qualitative assessment of the newly formed bone. Out of the four different images, the image of the implant apex which was in contact with the host bone accurately demonstrated the newly formed bone along the implant surface. Un-mineralized osteoid matrix or osteoblast could be seen on the implant surface.

Overall, a noteworthy increase was observed in the bone formation around the implants in bone samples belonging to the test group (Figs. 6A–C and 7A–B).

3.7 Assessment of parameters for biochemical analysis

A remarkable increase in the ALP activity was recognized with increasing time in both the test and control group post-implant placement (Tables 2, 3, and 4). There was no significant difference in the ALP activity within the sham group at different time intervals (Table 5, Graph 2). Comparing the values at different time intervals, the highest ALP activity was noted in the test group after six weeks; low ALP activity in the control group after 4 weeks, and the least ALP activity was noted in the Sham group.

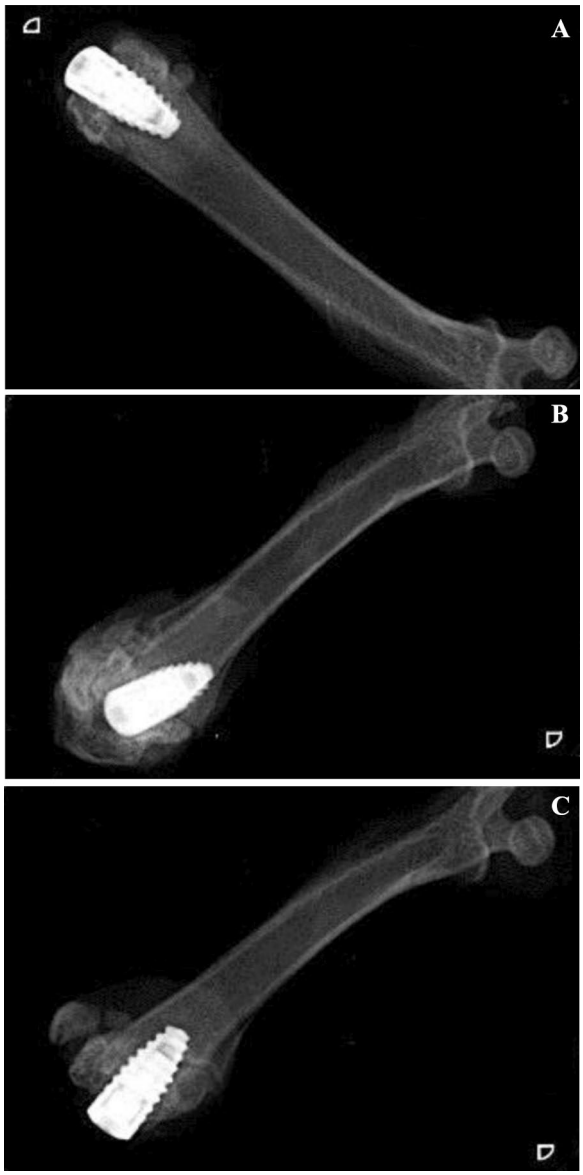


Fig. 4 **A** Digital radiograph of test group sample after 2 weeks. **B** Digital radiograph of test group sample after 4 weeks. **C** Digital radiograph of test group sample after 6 weeks

There was an increase in ACP activity in both the test and the control groups after 2 weeks (Table 2, Graph 3). This was attributable to the initial resorption process in bone remodeling in the early stages of the healing process. However, the test groups showed a decrease in ACP levels after 4 and 6-week intervals (Table 3, Graph 4). However, these readings increased in the control group at similar time intervals (Table 4, Graph 5). The lowest ACP activity was documented in the test group after 6 weeks.

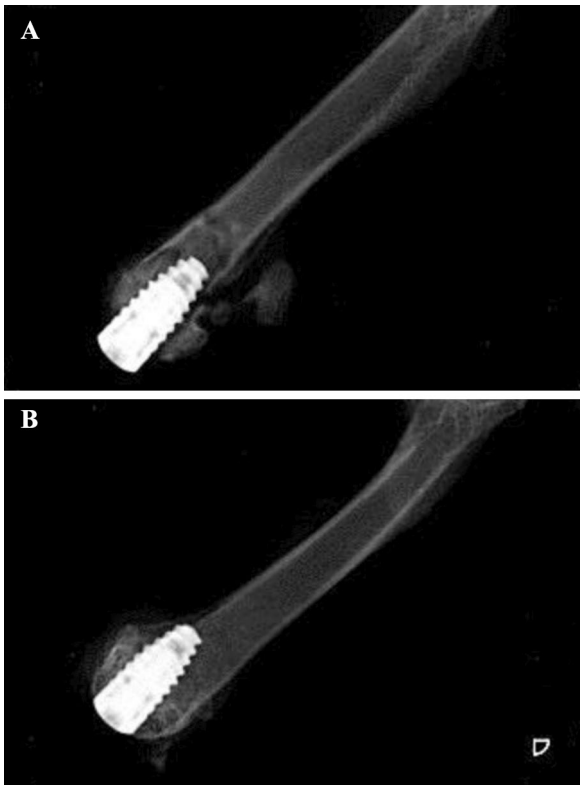


Fig. 5 **A** Digital radiograph of control group after 4 weeks. **B** Digital radiograph of control group after 6 weeks

4 Discussion

Based on the observations made in our study, it was evident that the use of CQ NPs demonstrates a significant improvement in bone regeneration and further promotes integration with the innate bone. CQ plant extract has been reported to have various properties such as specific effects on the healing of fractures and has been used as a bone regenerative material [20]. It promotes ALP activity and enhances collagen synthesis during the process of fracture healing [21]. The pharmacological and therapeutic activity of CQ involves chondroprotective effects and regenerative ability via the upregulation of surviving [22]. Active constituents of CQ encourage the growth and maturation of mesenchymal cells (MSCs) and foster novel bone generation through the WntLRP5-B-Creatnin signaling pathway of pre-osteoblast formation [23]. These properties could be retained when it is locally deposited, with demonstrable resistance against bone resorption, without diminishing the quality of innate bone. The plant material is easily available and has been contributing to enhancing fracture healing in humans.

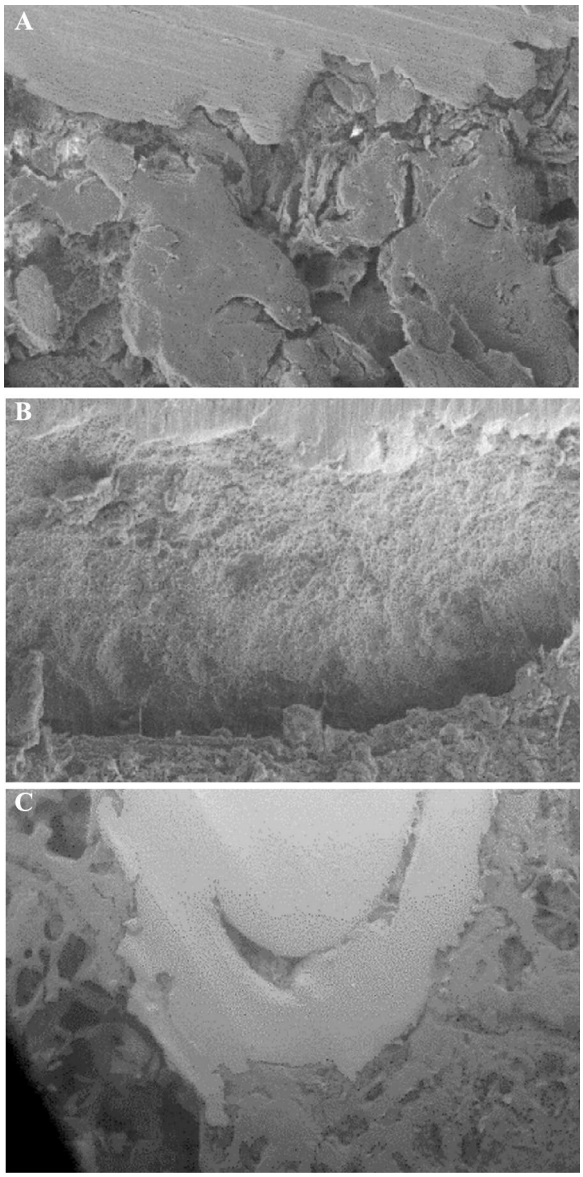


Fig. 6 **A** SEM analysis of test group sample after 2 weeks. **B** SEM analysis of test group sample after 4 weeks. **C** SEM analysis of test group sample after 6 weeks

PCL is a potential polymer of a biodegradable nature, with better chemical and thermal stability, good tissue compatibility, and solute permeability [24]. PCL-NPs containing ethanol extract of CQ were formulated to regulate slow—yet more effective drug delivery [25].

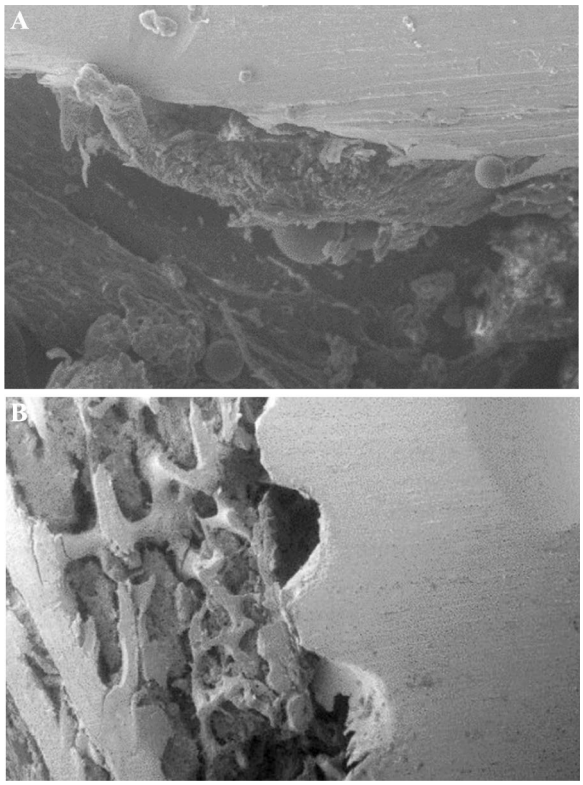


Fig. 7 **A** SEM analysis of control group sample after 4 weeks. **B** SEM analysis of control group sample after 6 weeks

Radiographic interpretation, which is a subjective elucidation, provides both qualitative and quantitative measures for the clinical outcome of implant therapy. Bone growth or loss can be calibrated either by setting fixed points where it is measured in both the compared groups and it can be objectively assessed by evaluating the bone density at the said point around the implant [12, 13]. In this study, the bone density of the test group sample in the early stages was indistinguishable from those in later stages, and also, it was significantly higher than the control group.

Studies have been performed to examine bone behavior using SEM along with different types of implant surfaces [16, 26]. SEM analysis in this study presented a good impression of the bone growth around the implants (Graph 1A–E). Histological investigation revealed a significant increase in osteoid accumulation along the implant surface as seen in the experimental group (Fig. 7A).

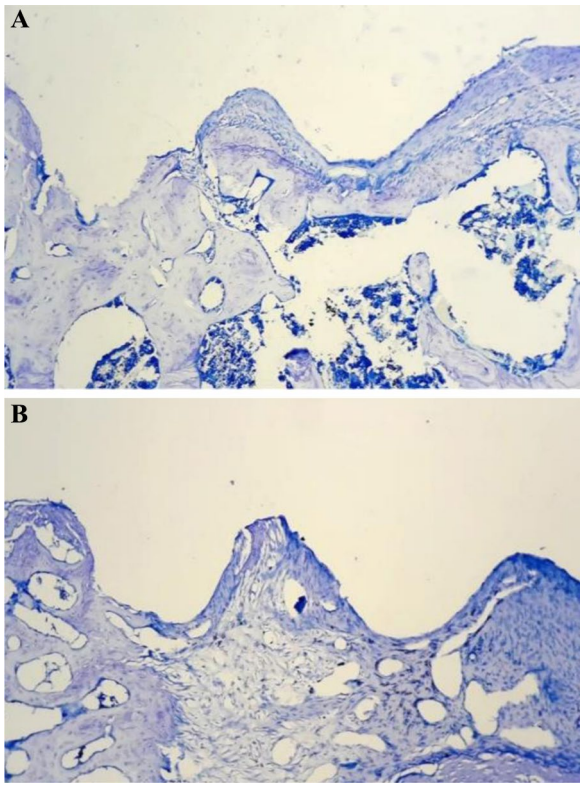


Fig. 8 **A** Toluidine blue staining of test group sample. **B** Toluidine blue staining of control group sample

Table 2 Alkaline phosphatase assay (ALP assay) and Acid phosphatase assay (ACP assay) after 2 weeks

Groups	N	ALP assay (Mean)		ACP assay (Mean)	
		Before implant placement (U/L)	After implant placement (U/L)	Before implant placement (U/L)	After implant placement (U/L)
Test	1	16.5	50.90	1.2	1.95
Control	1	8.26	37.9	3	5.75
Sham	1	15.5	16.5	4	4.25

No comparisons were done as there is only 1 sample in each group

A remarkable increase in ALP activity was noted post-implant placement in test groups. The ACP values were found to gradually decrease at further time intervals. The difference within the Sham group was not noteworthy for both ALP and ACP at any time interval.

CQ has been used for diabetes, obesity, high cholesterol, bone fractures, allergies, cancer, stomach upset, painful menstrual periods, asthma, malaria, wound healing, peptic ulcer disease, weak bones, (osteoporosis), and as body-building supplements as an alternative to anabolic steroid for a long period time [5]. In the field of dentistry, the activity of CQ has been tested as a medication by oral administration to improve healing post-implant surgery. It has demonstrated better bone healing when placed in periodontal osseous defects [18, 27, 28].

The null hypothesis was rejected because the NPs showed an improvement in the rate of osseointegration in the bone samples belonging to the test group when compared to the control group. The results of the present study support the use of CQ NPs in implant dentistry as an osteoinductive agent. It is the earliest original study to demonstrate the osteoinductive activity of CQ NPs against the implant surface when incorporated into the osteotomy site. However, further studies are warranted in higher animals to gain better insight into the therapeutic properties of CQ extract for enhancing osseous growth (Additional file 1).

5 Conclusion

Within the constraints of the present in vivo study, it could be inferred that CQ plant extract is a very potent osteoinductive agent. The effect of the CQ NP incorporated into the osteotomy site before implantation had a favorable effect in promoting osseointegration over the implant surface, in laboratory animals. Preclinical studies in higher animals and clinical studies are required to establish long-term benefits of CQ NPs over implant surfaces for clinical use.

Table 3 Comparison of alkaline phosphatase assay (ALP assay) and Acid phosphatase assay (ACP assay) before and after placement at 4 weeks and at 6 weeks of the test group

Timeline	Assay	Placement	*N*	Mean	S.D	S.E	M.D	95% CI	*Z* value	*P* value[#]
4 weeks	ALP	Before	2	3.44	0.97	0.68	− 32.02	− 44.88–19.16	− 31.648	0.020[†]
		After	2	35.47	2.40	1.70				
	ACP	Before	2	2.37	1.59	1.13	0.39	− 4.11–4.90	1.113	0.466
		After	2	1.97	1.09	0.77				
6 weeks	ALP	Before	2	10.32	0.97	0.69	− 25.79	− 73.65–22.05	− 6.849	0.042[†]
		After	2	36.12	4.35	3.07				
	ACP	Before[a]	2	3.25	0.35	0.25	–	–	–	–
		After[a]	2	1.50	0.35	0.25				

[#] *p* value derived from paired *t* test; [a] the correlation and t cannot be computed because the standard error of the difference is 0. [†]significant at $p < 0.05$

Table 4 Comparison of alkaline phosphatase assay (ALP assay) and Acid phosphatase assay (ACP assay) before and after placement at 4 weeks and at 6 weeks of control group

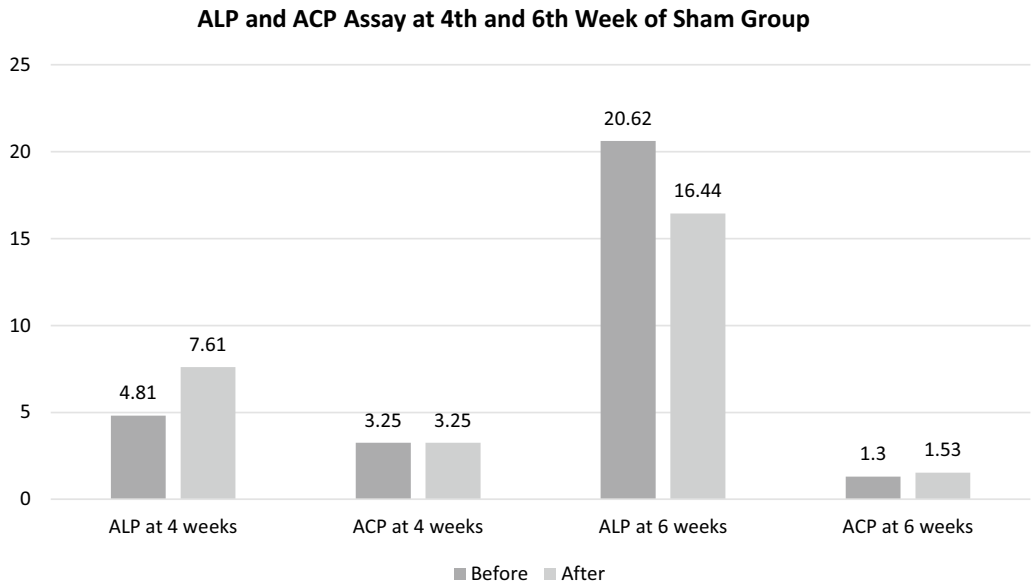
Timeline	Assay	Placement	*N*	Mean	S.D	S.E	M.D	95% CI	*Z* value	*P* value[#]
4 weeks	ALP	Before	2	4.81	0.97	0.68	− 20.43	− 23.23–17.62	− 92.448	0.007[†]
		After	2	25.25	1.28	0.91				
	ACP	Before	2	6.00	2.12	1.50	− 0.50	− 6.85–5.85	− 1.000	0.500
		After	2	6.50	2.82	2.00				
6 weeks	ALP	Before	2	8.44	4.15	2.93	− 17.46	− 40.68–5.75	− 9.558	0.046[†]
		After	2	25.90	1.56	1.10				
	ACP	Before	2	1.32	0.60	0.42	− 1.80	− 15.14–11.54	− 1.714	0.336
		After	2	3.12	0.88	0.62				

[#] *p* value derived from paired *t* test; [†]significant at $p < 0.05$

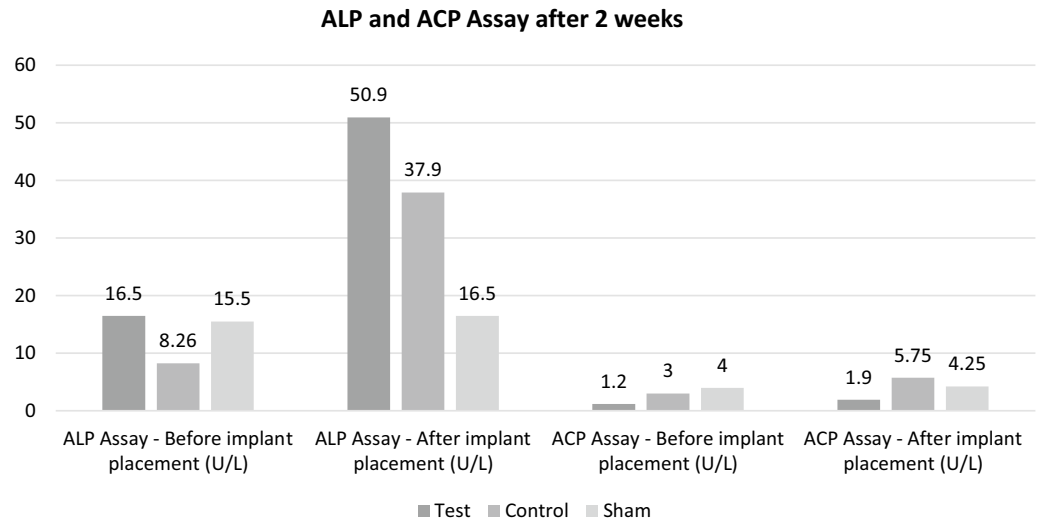
Table 5 Comparison of alkaline phosphatase assay (ALP assay) and Acid phosphatase assay (ACP assay) before and after placement at 4 weeks and at 6 weeks of sham group

Timeline	Assay	Placement	*N*	Mean	S.D	S.E	M.D	95% CI	*Z* value	*P* value[#]
4 weeks	ALP	Before	2	4.81	0.96	0.68	− 2.80	− 11.74–6.14	− 3.983	0.157
		After	2	7.61	0.02	0.01				
	ACP	Before[a]	2	3.25	2.47	1.75	–	–	–	–
		After[a]	2	3.25	2.47	1.75				
6 weeks	ALP	Before	2	20.62	7.74	5.48	4.18	− 36.09–44.45	1.319	0.413
		After	2	16.44	3.26	2.31				
	ACP	Before	2	1.30	0.63	0.45	− 0.23	− 1.06–0.59	− 3.615	0.172
		After	2	1.53	0.72	0.51				

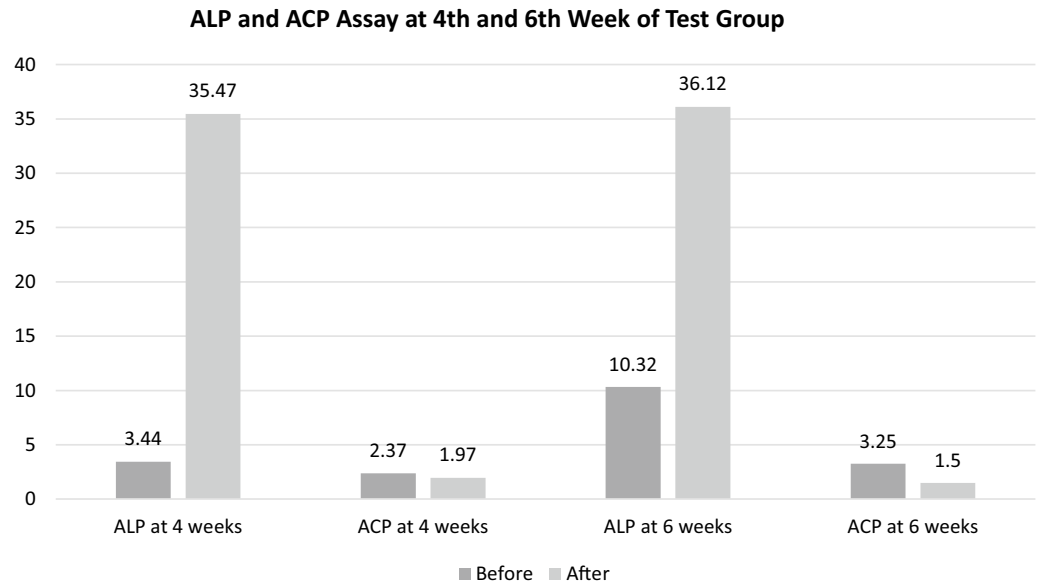
[#] *p* value derived from paired *t* test; [a] the correlation and *t* cannot be computed because the standard error of the difference is 0



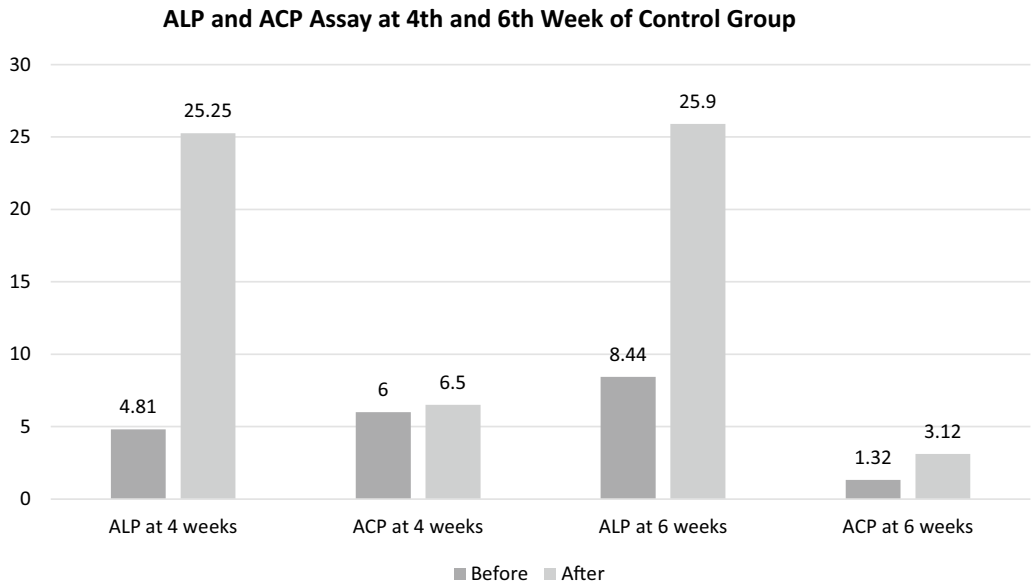
Graph 2 Comparison of alkaline phosphatase assay (ALP assay) and Acid phosphatase assay (ACP assay) before and after placement at 4 weeks and at 6 weeks of sham group



Graph 3 Alkaline phosphatase assay (ALP assay) and Acid phosphatase assay (ACP assay) after 2 weeks—before and after implant placement



Graph 4 Comparison of alkaline phosphatase assay (ALP assay) and Acid phosphatase assay (ACP assay) before and after placement at 4 weeks and at 6 weeks of test group



Graph 5 Comparison of alkaline phosphatase assay (ALP assay) and Acid phosphatase assay (ACP assay) before and after placement at 4 weeks and at 6 weeks of control group

Abbreviations

ACP	Acid phosphatase
ALP	Alkaline phosphatase
ANOVA	Analysis of variance test
ARRIVE guidelines	Animal Research: Reporting of In Vivo Experiments guidelines.
BIC	Bone-implant contact
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CQ	Cissus quadrangularis
EE	Entrapment efficiency
HU	Hounsfield units
IAEC	Institutional Animal Ethics Committee
MSC	Mesenchymal cells
NP	Nanoparticles
PCL	Polycaprolactone
PDI	Poly dispersity index
PVA	Polyvinyl Alcohol
SEM	Scanning electron microscopy
SLA Implants	Sandblasted, large grit, acid-etched treated Implants
SPSS	Statistical package for the social sciences
TRAP	Tartrate-resistant acid phosphatase
Tukey's HSD test	Tukey's honestly significant difference test
UV spectrophotometer	Ultraviolet spectrophotometer

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-024-00463-3.

Additional file 1. ARRIVE guidelines and source of funding (implant procurement).

Acknowledgements

We would like to acknowledge Mr. Ramesh, Straumann Dental Implants, India, for providing SLA modified surface titanium implants to carry out the study. We would like to acknowledge Dr Ahmed Mujib, Department of Oral Pathology, Bapuji Dental College, Karnataka, India, for his help in providing hard tissue microtome for histological sections. We are thankful to Dr Deepika Ratna (Dept of Oral Pathology, MCODS, Manipal, for staining and microscopic evaluation) and Dr Komal Smriti (Dept of Oral Medicine and Radiology, MCODS, Manipal, for the evaluation of radiographs). We appreciate Manipal Institute of Technology for performing SEM analysis. We would like to acknowledge Mr Shridhar (Animal officer, School of Life Sciences, MAHE, Manipal), Dr Bose Babu and Dr Manas Chakravarthy (Dept of Prosthodontics and Crown & Bridge, MCODS, Manipal) for assisting during implant placement surgery in rats and Dr Budhaditya Paul (Department of Public Health Dentistry) for the statistical analysis. Authors are thankful to Manipal Academy of Higher Education, Manipal, for providing all the necessary facilities and support.

Source of plant extract

Fresh CQ plant material was collected from Upavan Gardens (Life member of Indian Nurserymen's Association & Mysore horticultural Society), Udupi, Karnataka, India, and authenticated by Dr Manjunath Setty, Pharmacognosist, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India. Herbarium sample was prepared (PP 616, voucher no 514) and kept in the crude drug and herbarium museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal.

Source of animals

Central Animal Research Facility, Life Sciences, MAHE, Manipal.

Author contributions

Author 1 (SP) designed the study, analyzed the data, and wrote the manuscript. Author 2 (AN) participated in study design, procurement of implants, provided guidance during the procedure of implant placement and

discussion of results. Author 3 (SM) participated in study design, discussion, analysis of results and provided guidance during preparation of nanoparticles. Author 4 (S Khan) assisted in ethanolic extract preparation from Cissus Quadrangularis plant. Author 5 (SK) provided analysis of results obtained through biochemical parameters. Author 6 (RR) provided guidance for histology of bone samples. Author 7 (DB) helped in procurement of implants and provided guidance during the procedure of implant placement. Author 8 (ABS) prepared nanoparticles from the derived plant extract and performed UV spectrophotometry of the same. Author 9 (TD) performed biochemical analysis of different parameters. All authors have read and approved the manuscript.

Funding
SLA modified surface titanium implants (*n = 10) were provided by Straumann Dental Implants, India. Number of implants placed: 10 (Test group—5 and Control group—5). Implant used—Straumann® Dental implants: SLA® Standard, Standard Plus, Tapered Effect, Bone Level and Bone Level Tapered. Diameter—3.3 mm, length—8 mm. No other funding to declare. All authors have read and approved the manuscript.*

Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials. Any other data apart from the one uploaded in the article will be available from the corresponding author and first author upon request.

Declarations

Ethics approval and consent to participate
All animal experiments were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, after approval of the Institutional Animal Ethics Committee (IAEC).

Consent for publication
All authors agree with the content of the manuscript and have provided consent for publication.

Competing interests
The authors declare no conflict of interest.

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Received: 2 August 2023 Accepted: 4 January 2024
Published online: 09 April 2024

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