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Evaluation of Effect of Curcumin Coated Titanium Discs on Osteoblasts Cells – An Invitro Study.

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ABSTRACT:

INTRODUCTION:

Implant treatment has become the first choice of treatment for replacement of missing teeth and oral rehabilitation over last few years. Osseointegration is the most important requirement for long term success of dental implant procedure.Fibrous encapsulation is detrimental to implant fixation, which is a strong contributor to later failure of the implants.Usually for most of the failed implants, a large amount of fibrous encapsulation can be found on implant– bone interfaces, which prevents effective osseointegration.So, it can be hypothesised that by coating implant surface with curcumin we could overcome fibrous encapsulation and enhance osseointegration.

AIM: To evaluate the osteoblastic activity on titanium discs coated with curcumin gel.

OBJECTIVES:

- To evaluate the osteoblastic activity of curcumin coated titanium discs.
- To evaluate the cytotoxicity.
- To evaluate the osteoblastic cell proliferation.

METHOD:

A total of 5 Titanium disc samples will be fabricated for the study of the above give dimension. In this study cell proliferation, cell toxicity and viability of osteoblasts will be assessed. curcumin gel was used to coat 5 discs to form a uniform coating of the material on Titanium discs. Curcumin is formulated and coated on titanium discs. MTT assay is done to evaluate cell viability.Evaluation of cytotoxicity of curcumin gel has been done using UMR 106 cells. ALP assays are done to evaluate cell proliferation. Tryphan blue staining is used to determine cell count in this sample which will help us to determine the osteoinductive property of these material on osteoblasts.

RESULTS: The study

CONCLUSION: This study will help us to find an alternative cheaper and naturally occurring material to enhance the Osseo inductive and osteoconductive property of titanium implants.

I. INTRODUCTION:

Implant treatment has become the first choice of treatment for replacement of missing teeth and oral rehabilitation over last few years. Osseointegration is the most important requirement for long term success of dental implant procedure.

Osseointegration is a biologic response leading to a direct structural connection between living bone and the surface of an implant under functional loading. Reliable osseointegration is dependent on six factors like implant biocompatibility, implant design, implant surface, state of host bed, surgical technique and loading conditions. The implant surface topography plays a key role in the early stages of bone to implant contact (BIC); peri-implant bone formation depends on the healing capacity of the bone.

Fibrous encapsulation is detrimental to implant fixation, which is a strong contributor to later failure of the implants. Usually for most of the failed implants, a large amount of fibrous encapsulation can be found on implant– bone interfaces, which prevents effective osseointegration (i.e., the direct contact between bone and the implant) and causes implant loosening. In addition, the fibrous tissue formed can also increase intra-articular pressure, resulting in the extension of the gap between the bone and the implant and subsequently resulting in loosening of the implant. [1]

However, prevention of fibrous encapsulation is difficult because of the lack of effective strategies which can selectively control the growth of fibroblasts and osteoblasts. Because

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curcumin, an extract from Curcuma longa, was recently found to reduce the formation of fibrous tissue, it is hypothesized that loading curcumin on implant surfaces would be efficacious in inhibiting fibrous encapsulation without adversely affecting the osteoblast functions.[1]

Curcuma longa, possesses anti-inflammatory, potent anti- oxidant and anti-carcinoma effects, and has been used as an alternative for drugs for a wide range of conditions such as colon cancer, arthritis and Alzheimer's disease." In particular, curcumin can prevent the formation of fibrotic tissues in lung, X-ray s kidney and liver fibrosis. At the tissue level, administration of curcumin results in a significant reduction of fibrous tissue. At a cellular level, it has been reported that curcumin can significantly inhibit the migration and proliferation of fibro blasts." In addition, curcumin has been proven to exhibit no deleterious side effects in the human body even with doses of 2-12 g per day. The characteristics of curcumin may be beneficial for orthopaedic applications to prevent fibrous encapsulation on the implant surfaces[1]

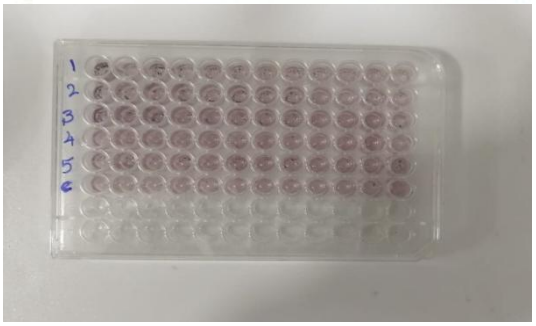
So it can be hypothesised that by coating implant surface with curcumin we could overcome fibrous encapsulation and enhance osseointegration.

An MTT Assay is a colorimetric assay based on assessing the cell metabolic activity. The biochemical mechanism behind the MTT assay involves NADPH -dependent cellular oxidoreductase enzyme that converts the Yellow tetrazolium MTT into Insoluble foramazan. Formazan can be dissolved with dimethyl Sulfoxide to give purple colour with characteristic absorption of 540 nm. Intensity of purple colour is directly proportional to the Cell number and thus indicating cell viability (6). We will be determining the osteoblastic osteoclastic activity of Curcumin gel coated on Titanium implants. This study will help us to find an alternative cheaper and naturally occurring material to enhance the Osseo inductive and osteoconductive property of titanium implants.

II. MATERIALS AND METHODS:

- Titanium discs
- Curcumin gel
- 3-(4,5-Dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide(MTT)
- Commercially pure Grade 4 titanium discs measuring 5mm diameter and 2 mm width are selected for the study.

A total of 5Titanium disc samples were fabricated for the study of the above give dimension. In this study cell proliferation, cell toxicity and viability of osteoblasts were assessed. curcumin gel was used to coat 5 discs to form a uniform coating of the material on Titanium discs. Curcumin is formulated and coated on titanium discs. MTT assay is done to evaluate cell viability .Evaluation of cytotoxicity of curcumin gel has been done using UMR 106 cells. ALP assays are done to evaluate cell proliferation. Tryphan blue staining is used to determine cell count in this sample which will help us to determine the osteoinductive property of these material on osteoblasts.



MTT assay was performed to Evaluate the Compatibility

Osteoblasts **cells culture.**

Osteoblasts cells were cultured in Eagle minimum essential medium with 15% (vol/vol) heat-inactivated FBS, 2 mM L-glutamine, 50 IU/mL penicillin, and 50 mg/mL streptomycin.cell grown in T-25 cm2 culture flasks under standard incubation conditions (37C, 95% air/5% CO2) until they reached confluence (*70%-80%). After 1 weeks, the cells were dissociated using trypsin solution and then replated in 6-well plates at a cell density of 2.5 x 10^5 cells per well. Two milliliters of complete DMEM medium were added to each well, and after 24 hours of cell attachment.

MTT assay.

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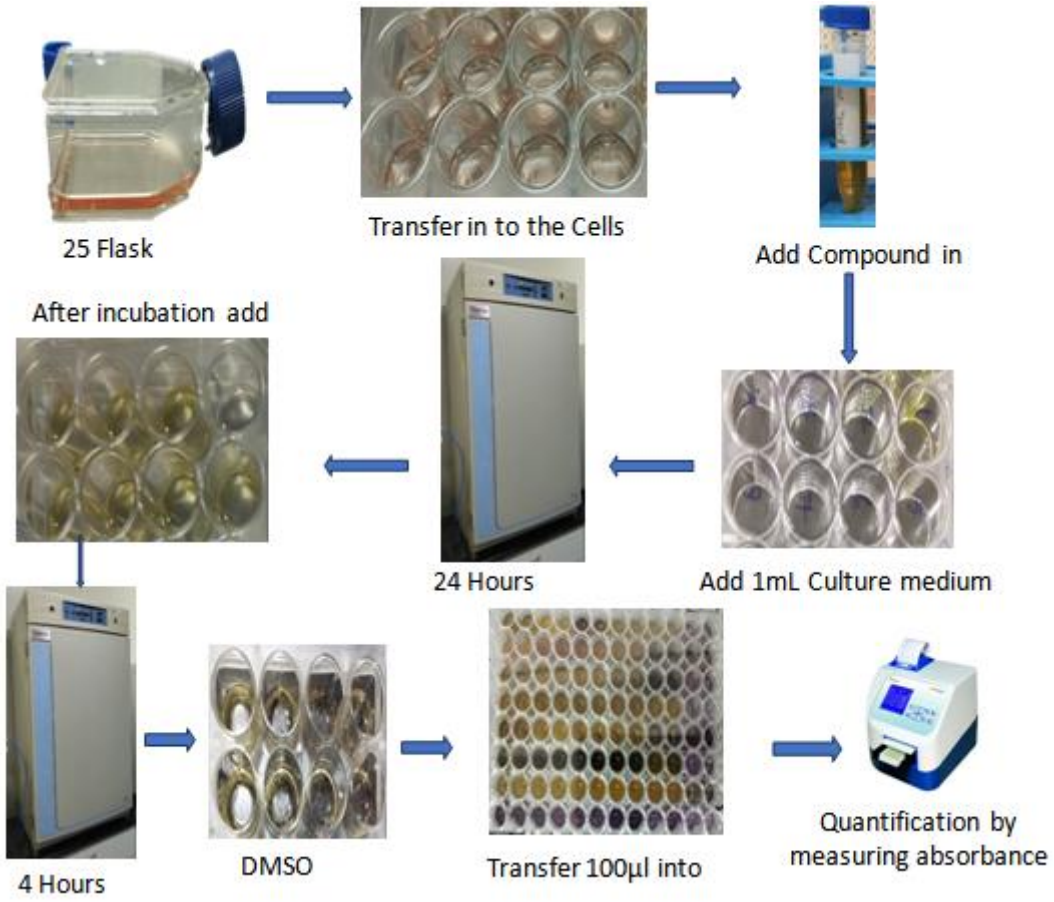
In the 6-well plate with 1 mL of complete culture medium per well. Next, 0.5 mg/mL MTT was added to the bottom well. The plate was then incubated at 37C for 4 hours. After incubation, the culture medium was aspirated from the insert and well, and the resulting formazan crystals were solubilized by adding 100µlof DMSO solution per well. The cell types were gently shaken for 2 minutes to mix the blue reaction product uniformly with the solvent. Finally, 100µl of the colored DMSO was transferred from each insert and each well to a new 96-well plate for the quantification of cell viability. Absorbance at 450 nm was measured using a microplate reader.



III. RESULTS:

Average	Group Average	Standard Deviation	% Cell Viability	
0.035667	0.180938	0.001775		
0.073167	0.20169	0.109237		
0.240083	0.223111	0.017212	100	CONTROL
0.219	0.219717	0.040016	91.21833	S1
0.230083	0.219896	0.046816	95.83478	S2
0.217333	0.2165	0.029296	90.52412	S3
0.208417	0.216083	0.036052	86.81014	S4
0.22375	7.898846	0.041757	93.19681	S5

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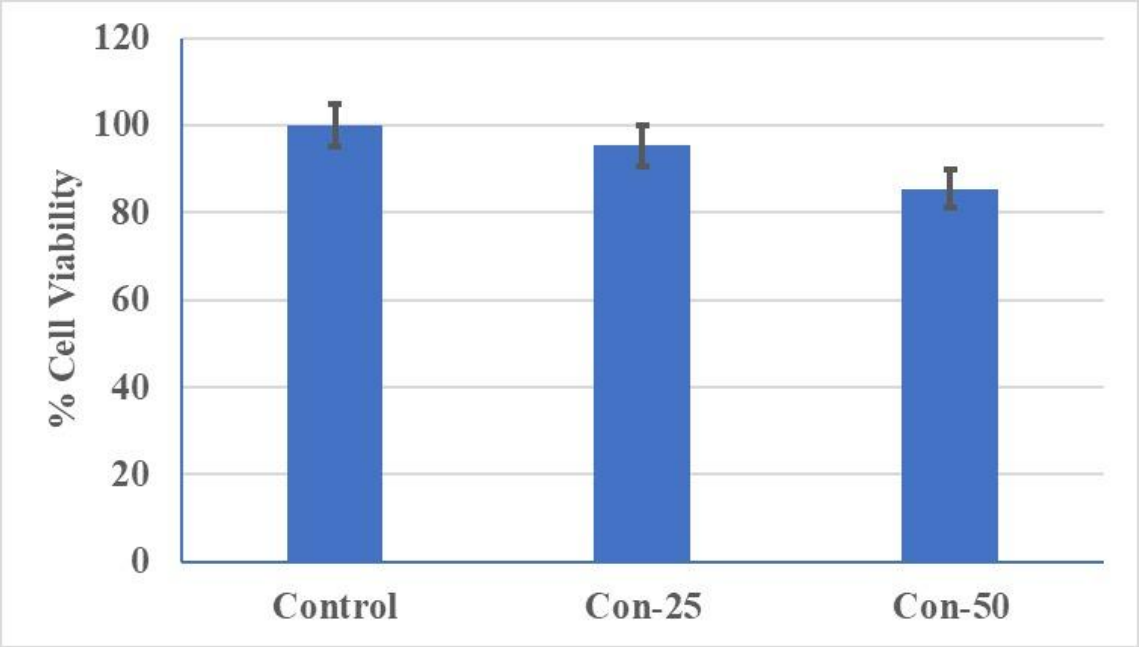

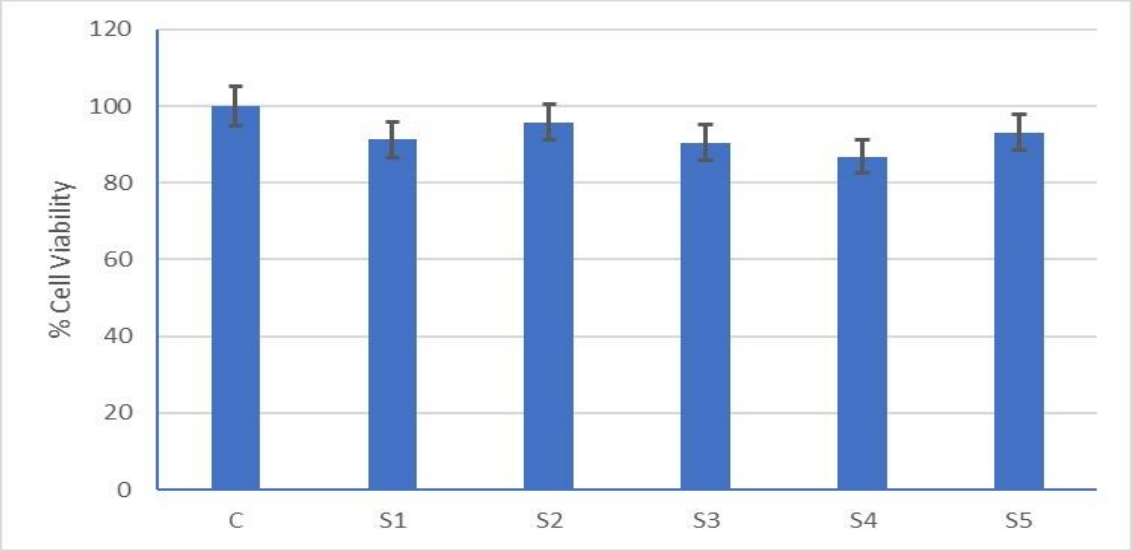

IV. STATISTICAL ANALYSIS:

Comparison of Mean MTT Assay values at 450 nm b/w 3 groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	p-value
Control	3	0.3862	0.0114	0.376	0.398	0.008*
25% Conc.	3	0.3830	0.0065	0.380	0.392	
50% Conc.	3	0.3272	0.0260	0.298	0.346	

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* - Statistically Significant

Multiple comparison of mean difference in mean MTT Assay values b/w groups using Tukey's post hoc Test					
(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Control	25% Conc.	0.0032	-0.0410	0.0434	1.00
	50% Conc.	0.0590	0.0169	0.1012	0.01*
25% Conc.	50% Conc.	0.0558	0.1000	0.0157	0.01*

* - Statistically Significant

The mean MTT Assay value for control group was 0.3862 ± 0.114, for 25% concentration of curcumin group was 0.3830 ± 0.0065 and for 50% concentration of curcumin group was 0.3272 ± 0.0260. This difference in the mean MTT assay values between 3 groups was statistically significant at p=0.008.

Multiple comparison of mean differences between 3 groups revealed that 50% Curcumin group showed significantly lesser MTT Assay values as compared to 25% Curcumin group and Control group and these mean differences were statistically significant at p=0.01. However, no significant difference in the MTT Assay value was noted between Control and 25% Curcumin group.

The Calculated Percentage of cell viability using MTT Assay values for Control group was 100.0%, for 25% Curcumin group was 95.32% and 50% Curcumin group was 85.48%.

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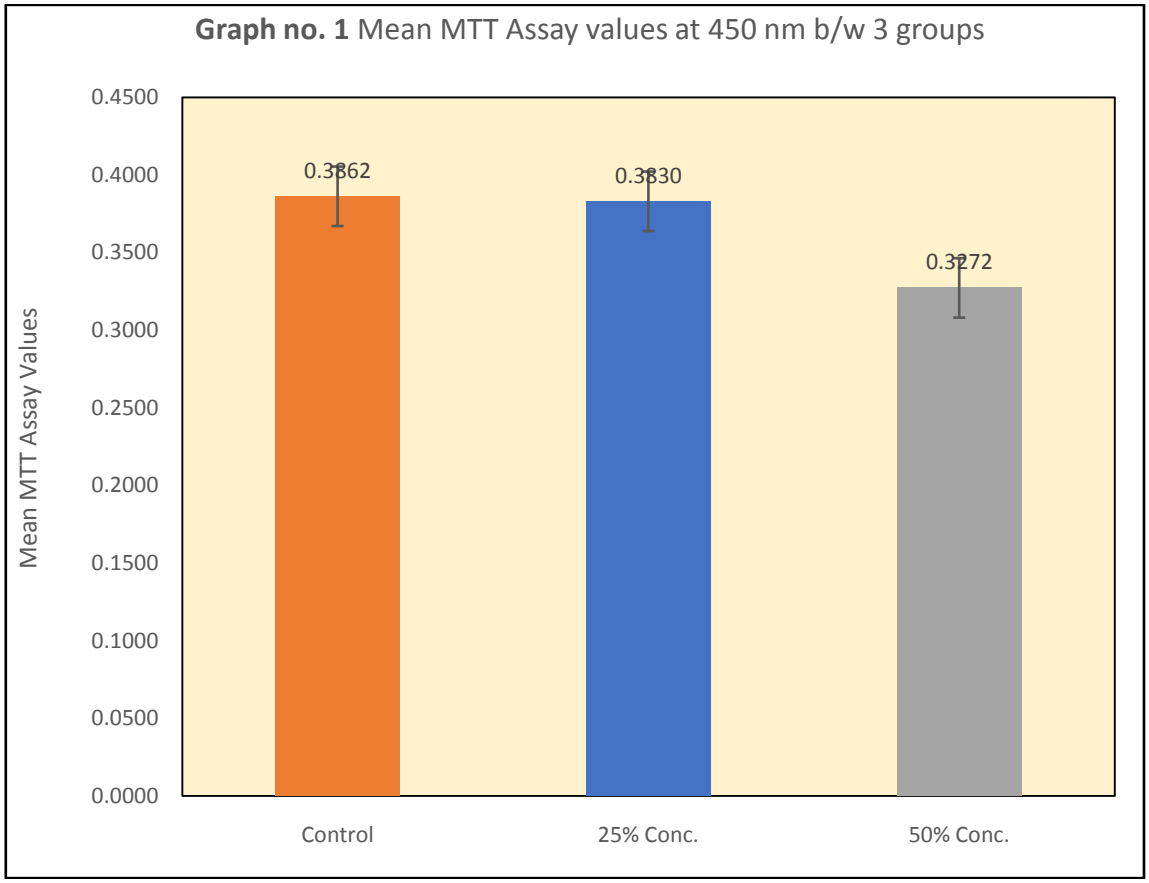

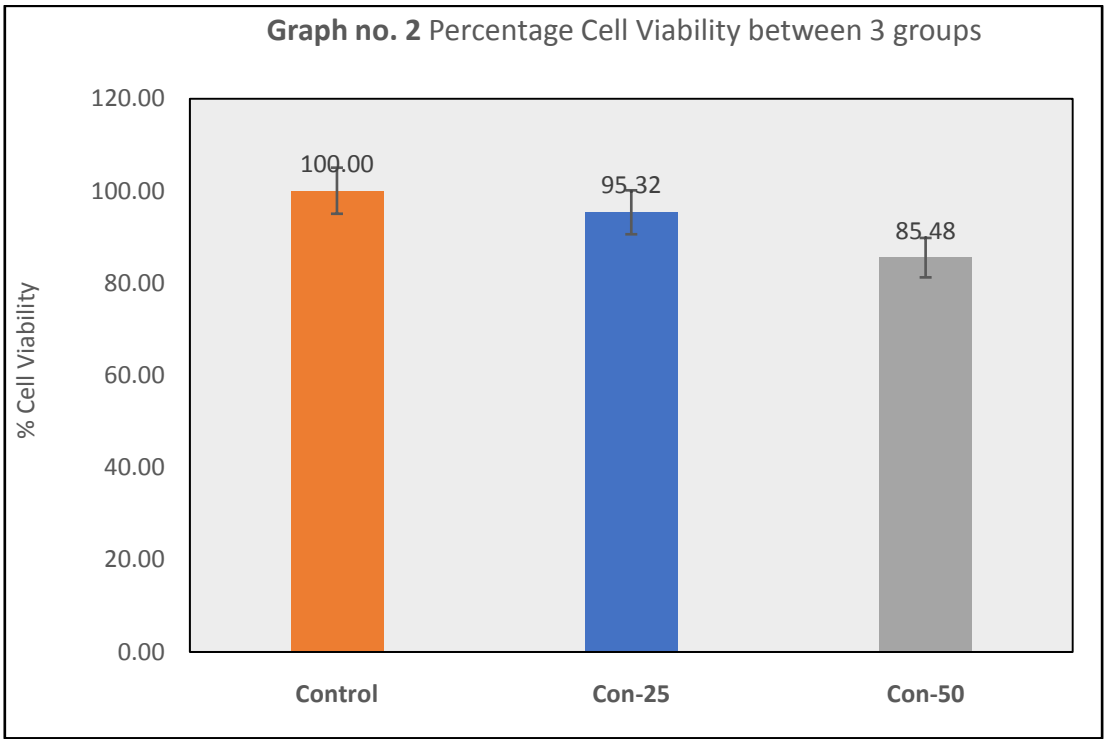

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Comparison of Mean MTT Assay values at 450 nm b/w Control & Curcumin Extract at diff. time intervals using Kruskal Wallis Test

Groups	N	Mean	SD	Min	Max	p-value
Control	12	0.2401	0.0172	0.222	0.284	0.03*
S1	12	0.2190	0.0400	0.182	0.328	
S2	12	0.2301	0.0468	0.162	0.304	
S3	12	0.2173	0.0293	0.183	0.275	
S4	12	0.2084	0.0361	0.152	0.289	
S5	12	0.2238	0.0418	0.153	0.338	

* - Statistically Significant

Multiple comparison of mean difference in mean MTT Assay values b/w Control & Curcumin Extract at diff. time intervals using Dunn's post hoc Test

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		p-value
			Lower	Upper	
Control	S1	0.0211	-0.0227	0.0648	0.007*
	S2	0.0100	-0.0337	0.0537	0.14
	S3	0.0228	-0.0210	0.0665	0.01*
	S4	0.0317	-0.0121	0.0754	0.002*
	S5	0.0163	-0.0274	0.0601	0.04*
S1	S2	-0.0111	-0.0548	0.0327	0.24
	S3	0.0017	-0.0421	0.0454	0.92
	S4	0.0106	-0.0332	0.0543	0.71
	S5	-0.0048	-0.0485	0.0390	0.44
S2	S3	0.0128	-0.0310	0.0565	0.28
	S4	0.0217	-0.0221	0.0654	0.12
	S5	0.0063	-0.0374	0.0501	0.68
S3	S4	0.0089	-0.0348	0.0527	0.63
	S5	-0.0064	-0.0502	0.0373	0.50
S4	S5	-0.0153	-0.0591	0.0284	0.25

* - Statistically Significant

The mean MTT Assay value for control group was 0.2401 ± 0.0172, for Curcumin group at S1 time interval was 0.2190 ± 0.0400, S2 time interval was 0.2301 ± 0.0468, S3 time interval was 0.2173 ± 0.0293, for S4 time interval was 0.2084 ± 0.0361 and for S5 time interval was 0.2238 ± 0.0418. This difference in the mean MTT assay values between b/w Control & Curcumin Extract at diff. time intervals was statistically significant at p=0.03.

Multiple comparison of mean differences revealed that Control group showed significantly highest MTT Assay values as compared to Curcumin extract group at S1, S3, S4 & S5 time intervals and these mean differences were statistically significant at p=0.007, p=0.01, p=0.002 & p=0.04 respectively. However, no significant difference in the MTT Assay value was noted between Control and Curcumin group at S2 time interval [p=0.14]. And further no significant differences were observed in Curcumin group between samples measured at different time intervals.

The Calculated Percentage of cell viability using MTT Assay values for Control group was 100.0%, for Curcumin group at S1 time interval was 91.21, S2 time interval was 95.83%, S2 time interval was 90.52%, S3 time interval was 90.52%, S4 time interval was 86.81% and S5 time interval was 93.20%.

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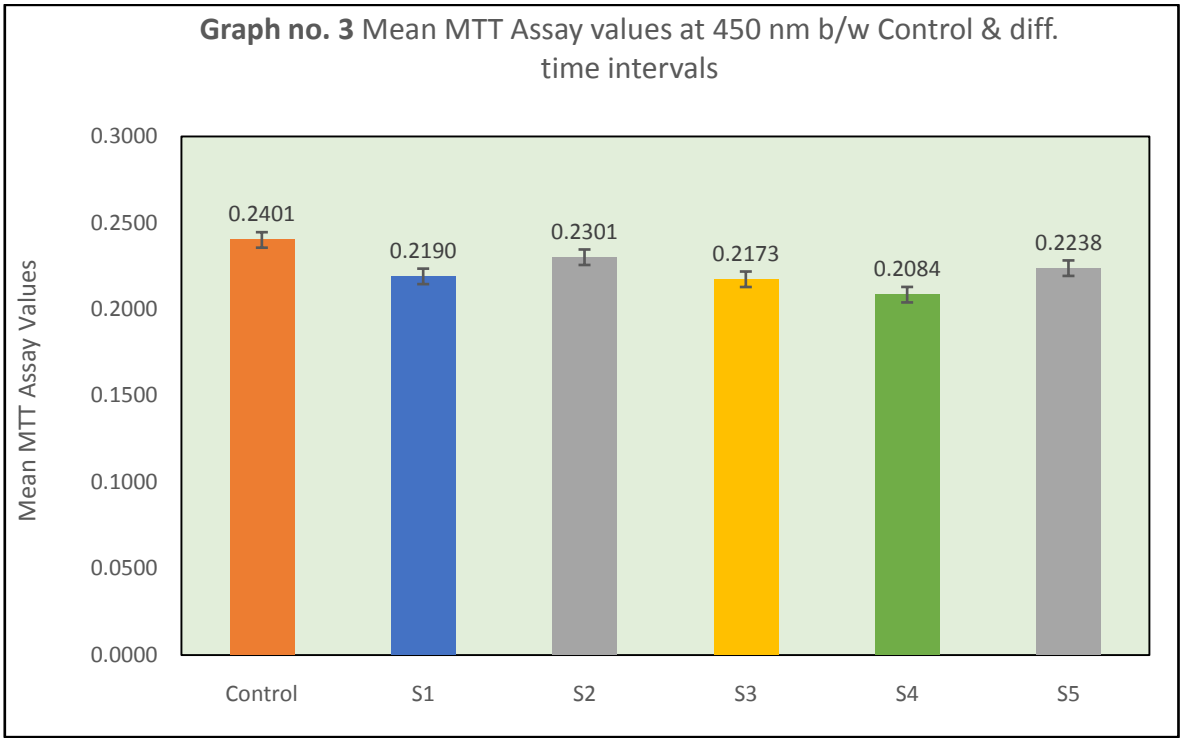

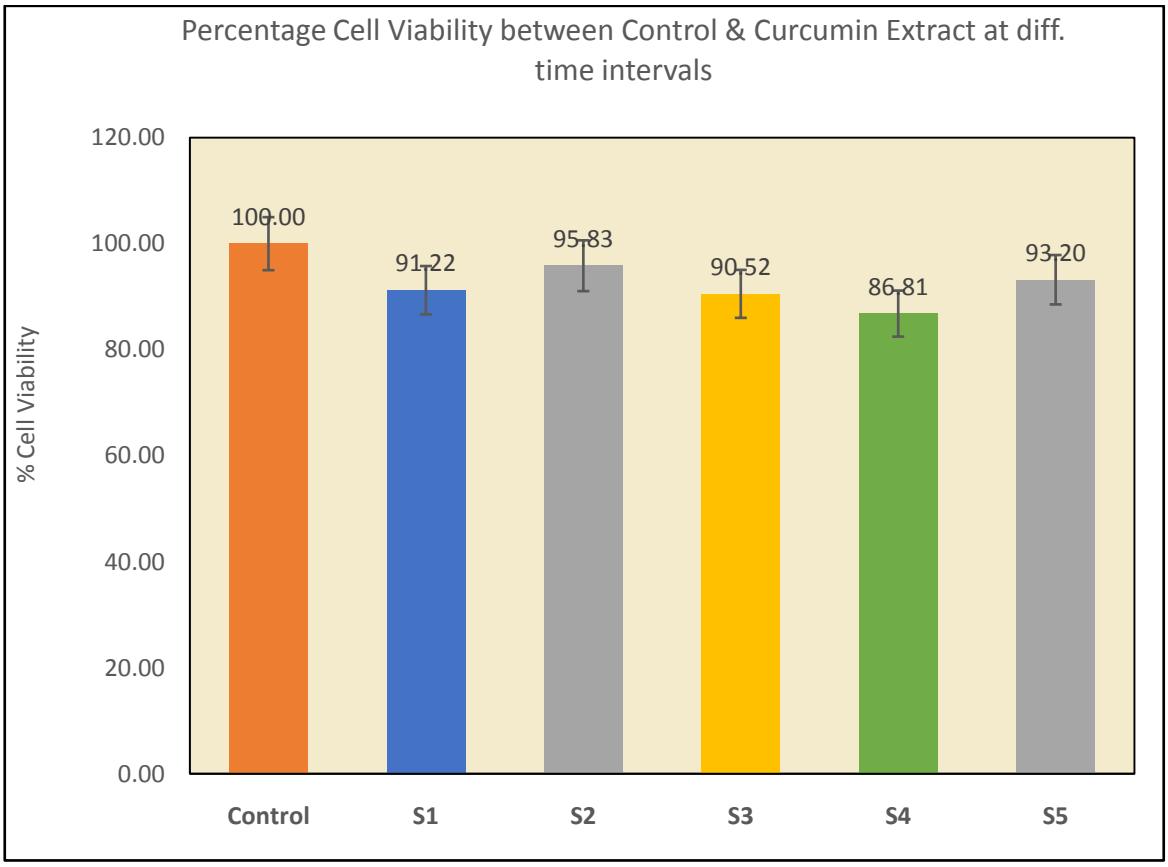

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V. CONCLUSION:

- **The curcumin coated discs showed increase in cell viability with increase in time and concentration.**

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