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Influence of Two Different Chitosan Modified Intracanal Medicaments on Fracture Resistance of Root Dentin: An in-vitro comparative evaluation.

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Abstract

Aims & Background: This study aimed to compare and evaluate the fracture resistance of root dentin treated with Triphala and Modified Triple Antibiotic Paste (MTAP) mixed with Chitosan.
Materials and Methods: Forty (40) single rooted human mandibular premolar teeth with single canal were chosen for this study. The teeth were sectioned to obtain a root length of 10mm. The canals were prepared till F3 Protaper Gold and irrigated with normal saline. The samples were then divided in four groups with each group having 10 teeth(n=10). Group A (Positive Control) was left untreated. Group B (Negative Control) was treated with Calcium Hydroxide. Then the root canals of two test groups were filled with the respective intracanal medicaments i.e. Triphala+Chitosan (Group C), MTAP+Chitosan (Group D). All the groups were sealed with temporary restorative material. The samples were stored in 100% humidity at 37°C for 15 days. After which they were mounted in self-cure acrylic resin till 5mm of root was exposed and then subjected to fracture resistance test in a universal testing machine and the data was tabulated and analyzed with SPSS software v.25 using one-way ANOVA and post-hoc Tukey's HSD test.
Results: Untreated i.e. Positive Control Group (1206.80 ± 63.33 N) shows highest fracture resistance followed by Triphala+Chitosan Group (1147.70 ± 54.58 N) and MTAP+Chitosan Group (1062.20 ± 59.66 N). Calcium Hydroxide i.e. Negative Control Group (895.90 ± 65.11 N) has the lowest fracture resistance.
Conclusion: Both Triphala and MTAP combined with Chitosan enhances the fracture resistance of root dentin than Calcium Hydroxide and thus can be considered as an adjunct in endodontic therapy. Further research needs to be conducted in in-vivo setup to gather more evidence.
Clinical Significance: The modified intracanal medicaments with chitosan may improve fracture resistance, leading to better clinical outcomes for endodontically treated teeth, especially in high-stress bearing areas.

Keywords: Intracanal Medicaments, Triphala, Modified Triple Antibiotic paste, Fracture Resistance, Chitosan, Calcium Hydroxide.

Introduction:

In order to avoid infection and inflammation, endodontic therapy aims to eliminate any pathologic microorganisms from the root canal and periapex. Therefore, the root canal system must be adequately disinfected and here comes the importance of intracanal medicaments.[1] In contrast to healthy tooth, an endodontically treated tooth has reduced fracture resistance because of dehydration and loss of dentin. In addition, some of the critical components that sustain the natural tooth such as the cusps, ridges, and pulp chamber roof are also removed, further compromising its fracture strength.[2] However, the chemo-mechanical preparation, irrespective of manual or rotary instrumentation, irrigation protocols and application of intracanal medicaments affect the chemistry and structural integrity of root dentin. Literature shows the

microhardness of radicular dentin is severely compromised leading to root fractures after completion of endodontic therapy.[3,4]

Root fracture is linked to a number of established conditions that decrease tooth structure, such dental caries, canal preparation, and potentially, intracanal medications. These intracanal medications may increase root fragility because of their demineralization action, which over time may negatively impact the radicular structure.[5] Although calcium hydroxide is considered as gold standard intra-canal dressing, research shows it has detrimental effect on root canal microhardness values upon its application. Compared to calcium hydroxide, triple antibiotic pastes and its modifications shows higher microhardness values of radicular dentin.[6]

For almost 2,000 years, Triphala has been used in Ayurvedic (Indian) treatment. It is obtained from dehydrated form of three different fruits: Haritaki (*Terminalia chebula*), Bibhitaki (*Terminalia bellirica*), and Amala (*Emblica officinalis*). Preliminary research indicates that triphala has antibacterial properties against a wide range of microorganisms. The advantages of triphala include its germicidal properties, convenience of use, affordability, substantivity, and superior biocompatibility.[7]

The complex, multi-microbial nature of root canal infections indicates that using just one antibiotic is unlikely to effectively eliminate all the microorganisms involved. Metronidazole, minocycline, and ciprofloxacin are combined to form triple antibiotic paste (TAP). The combination of these three medications boosts antibacterial activity and overcomes bacterial resistance. However, the tooth-discoloring medication minocycline can be substituted with another antibiotic, like amoxicillin, which has superior antibacterial efficacy, to get around the restrictions.[8]

Hence, this study aimed to compare and evaluate the effect of Triphala mixed with Chitosan (TRIPHALA+CHITOSAN) and Triple Antibiotic Paste modified with Amoxicillin mixed with Chitosan (MTAP+CHITOSAN) on the fracture resistance of root dentin.

Materials and Method:

Sample Collection and Decoronation:
Forty (40) mandibular premolars having single root and single canal which were extracted for orthodontic and periodontal reasons were used in our study (Fig 1). They were cleaned of any debris, deposits with the help of an ultrasonic scaler device after which the samples were examined with a stereomicroscope at 10X magnification to exclude teeth with any defects. The teeth were then kept in distilled water till further use. After that, the teeth were sectioned with a diamond disc under copius water flow, leaving behind a root length of 10mm (Fig 2).

Canal Preparation:
Access was refined with #2 GG drill and a 15K file was used to record the working length under stereomicroscope at 10X magnification till the file was visible from the apex, the working length was kept 1mm short of that and verified radiographically. The canals were then prepared till F3 Protaper Gold Rotary file (Fig 3) under continuous irrigation with normal saline with every file change.

Grouping:
The samples were the grouped as follows depending upon the placement of different Intracanal Medicaments:

GROUP A (n=10): UNTREATED (POSITIVE CONTROL GROUP)
GROUP B (n=10): CALCIUM HYDROXIDE (NEGATIVE CONTROL GROUP)
GROUP C (n=10): TRIPHALA+CHITOSAN
GROUP D (n=10): MTAP+CHITOSAN

Chitosan Solution Preparation:
1.8 millilitres of glacial acetic acid was mixed with 100 millilitres of distilled water to make a 0.3 Normal (N) acetic acid solution. Next, 20 milligrams of Chitosan was weighed and then dissolved in the 0.3 N acetic acid solution to obtain 0.2% Chitosan solution.

Triphala+Chitosan Intracanal Medicament Preparation:
Dried Triphala Fruits were collected from an Ayurvedic Vendor and ground in a mortar pestle. The fruits were ground till a course mix was formed. The coarse mix was then shifted to a electric grinder to get a fine mix which was sieved and collected. The fine mix was then diluted with 0.2% chitosan solution in 1:4 volumetric ratio to make the TRIPHALA + CHITOSAN Intra-canal Medicament (Fig 4).

MTAP+Chitosan Intracanal Medicament preparation:
Amoxicillin (ALMOX 500, Alkem Laboratories Ltd.), Ciprofloxacin (CIPLOX 500, Cipla Ltd.) and Metronidazole (METROGYL 400, J B Chemicals and Pharmaceuticals Ltd) were crushed in a mortar pestle in a coarse mix and then mixed together in 1:1:1 volumetric ratio and mixed in an electric grinder to obtain a fine mix which was sieved. The fine mix was then diluted with 0.2% chitosan solution in 1:4 volumetric ratio to make the Modified Triple Antibiotic Paste (MTAP+CHITOSAN) Intra-canal Medicament (Fig 5).

Intracanal Medicament Placement:
Group A i.e positive control group was left untreated. Group B i.e negative control was treated with Calcium Hydroxide Intracanal Medicament (Neocal, Orikam, India). The two different intracanal medicaments were filled in a 2ml syringe and the root canals of two test groups were filled with the two respective intracanal medicaments i.e. Triphala+Chitosan (Group C) and MTAP+Chitosan (Group D) (Fig 6). All the groups were sealed with temporary restorative material of 3mm thickness. The samples were stored in 100% humidity at 37°C for 15 days.

Fracture Resistance Evaluation:
Each sample were embedded in self-cure acrylic resin using standardised cylindrical moulds of dimension of 3cm height and 1cm width, such that 5mm of the root length was exposed and then fracture resistance testing of the samples was conducted using a universal testing machine.
Each mounted sample was secured to the universal testing machine's jig, and load was delivered at a crosshead speed of 1 mm/minute to the orifice in vertical downward direction parallel the long axis of the root till it fractured (Fig 7).

Statistical analysis:
Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software V.25. One-way Analysis of Variance (One-way ANOVA) was employed for multiple group comparison, followed by the Post-Hoc Tukey test for pairwise intergroup comparisons to evaluate differences in mean fracture resistance values between the samples. A p-value of 0.05 was considered statistically significant.

Results:

Amongst the groups in the study, Group A (Untreated i.e. Positive Control) (1206.80 ± 63.33 N) exhibited the highest fracture resistance followed by Group C (Triphala+Chitosan ICM) (1147.70 ± 54.58 N) and Group D (MTAP+Chitosan Group) (1062.20 ± 59.66 N). Group B (Calcium Hydroxide i.e. Negative Control) (895.90 ± 65.11 N) has the lowest fracture resistance. One-way ANOVA (Table 1) shows that all the three groups show statistical significant differences (p=0.000).
Pairwise Comparison with post hoc Tukey's Test (Table 2) shows statistically significant difference between all groups except Group A and C(p=0.150).

Discussion:

The microbial biofilm plays a crucial role in root canal infections, making it essential to develop effective antimicrobial strategies to combat resistance and manage infections. However, even with treatment, it's challenging to eliminate all bacteria from infected canal systems, which can lead to persistent bacterial growth.[9] Unfortunately, excessive reliance on intracanal medicaments extended treatment through multiple appointments without achieving the desired results. Intracanal medicaments have unpredictable efficacy in the presence of debris, highlighting the importance of thorough root canal debridement as a non-negotiable step.[10]

Conventional cleaning and shaping protocols such as the use of manual or rotary files with different speed and torque, different root-canal irrigants and ICM results in microcracks within the tubular structure and altered collagen architecture leading to reduced dentinal microhardness.[11,12] Additionally, previous research indicates that prolonged exposure to calcium hydroxide may weaken the dentin, making the tooth more prone to fracture.[13] Keeping that in mind, this study asessed the effects of Calcium Hydroxide, Triphala+Chitosan and Modified Triple Antibiotic Paste (MTAP)+Chitosan on the fracture resistance of root canal dentin with the null hypothesis being both the experimental preparations had no effect on the fracture resistance values of root dentin.

In our study, Group A (Untreated i.e. Positive Control) (1206.80 ± 63.33 N) exhibited the highest fracture resistance followed by Group C (Triphala+Chitosan ICM) (1147.70 ± 54.58 N) and Group D (MTAP+Chitosan Group) (1062.20 ± 59.66 N). Group B (Calcium Hydroxide i.e. Negative Control) (895.90 ± 65.11 N) has the lowest fracture resistance. One-way ANOVA demonstrated statistically significant difference in the fracture resistance values in all groups (p=0.000). However, pairwise comparison showed no statistically significant difference in Positive Control and

Triphala+Chitosan Group. Thus, in the present study, Triphala along with Chitosan and MTAP combined with Chitosan shows higher fracture resistance values of root dentin as compared to Calcium Hydroxide, but were not able to produce the values of that of Untreated Group yet Triphala+Chitosan shows no statistical difference in fracture resistance values compared to Untreated Group. Thus, the null hypothesis was rejected.

'Triphala' is a well-known miracle drug of Ayurvedic Medicine. Triphala consists of equal parts of the Amla (*Emblica officinalis*), Haritaki (*Terminalia chebula*), and Bibhitaki (*Terminalia belerica*). It contains Flavonoids, Gallic Acid, Vitamin C, Quinones and Tannins. In dentistry it has been used as anti caries agent and mouthwash. It also shows antimicrobial action, anti-collagenase action and anti-oxidant effect.[7] According to Swati Chainani et al. Triphala was effective against *Lactobacillus* and *Candida albicans.* Selvi MM et al. documented that Triphala exhibited notable antimicrobial activity against *Enterococcus faecalis*, comparable to that of calcium hydroxide.[14,15]

Triple Antibiotic Pastes (TAP) were superior or equal to calcium hydroxide in their action on *E. faecalis* (Vatankhah M et al.) and showed the best antibacterial property followed by calcium hydroxide plus proton pump inhibitors against both the *E. faecalis* and *Candida albicans* strains. (Mehta S et al.)[16,17]

Mahsa et al. concluded that the use of Triphala as an irrigating solution had a minimal impact on the microhardness of root canal dentin, due to its anti-collagenase activity and antioxidant effects, which help maintain collagen integrity.[18] Vaishnavi Elika et al. stated Triphala has shown little to no changes in the microhardness values of root dentin when compared to 5% NaOCl and 17% EDTA.[19]

Hence, the results of TRIPHALA+CHITOSAN group in our study could be associated with the abovementioned reasons. Conventional MTAP is acidic in nature. This characteristic may contribute to dentin demineralization, resulting in decreased microhardness values. However, conventional MTAP exhibits lesser decrease in microhardness values of root dentin as compared to Calcium hydroxide, thus lowering the risk of root fracture.[6]

We used Amoxicillin instead of Minocycline to counter the staining effect of Minocycline. In our study, because of the near neutral pH of Amoxicillin (Madhukumar M et al.)[20] and collagen crosslinking potential of Chitosan (Halkai R et al.), our MTAP+CHITOSAN group shows better fracture resistance values compared to Calcium Hydroxide group.[21]

A study by Thienngern P et al. shows Chitosan based ICM proved better than calcium hydroxide against *E. faecalis* and *Candida albicans.*[22] Research show chitosan has similar effect as EDTA on the microhardness values of root dentin.[23] A study by Raith DN et al. shows that when compared to 17% EDTA, 0.2% chitosan resulted in increased microhardness and reduced surface roughness of root dentin because of its collagen cross-linking potential.[24] Hence, 0.2% chitosan was chosen as a vehicle for the respective ICMs in our research model.[25]

The limitations of the present in-vitro study were such that, only a single tooth variant was studied. Stress pattern and stress concentration in oral cavity and the temperature and pH shifts of oral cavity varies in an in-vitro study. The findings of an this in-vitro study must be extensively researched and clinically assessed. Further studies may show more detailed explanations regarding the use of such novel intracanal medicament preparations in common clinical practise.

Conclusion:

The study reveals that both Triphala along with Chitosan and MTAP with Chitosan shows significantly higher fracture resistance values when compared with root canals treated with Calcium Hydroxide. This suggests that both the preparations could be used as intra-canal medicaments while maintaining the strength of the root dentin in endodontic therapy. Further research is required to get a more comprehensive idea regarding the use of such experimental preparations in clinical practise.

Clinical significance:

The improvement in the fracture resistance highlights that these chitosan modified medicaments improve fracture resistance, indicating their potential to enhance the clinical longevity of endodontically treated teeth. This is especially significant for regions of the mouth subjected to high functional and masticatory stresses. By reinforcing the structural integrity of root dentin, these medicaments may contribute to better long-term results and a decreased risk of fractures in patients who undergo root canal treatment.

List of abbreviations:

Modified Triple Antibiotic Paste (MTAP), Intracanal Medicament (ICM), Gates-Glidden Drill(GG Drill), Sodium Hypochlorite (NaOCl) and Ethylenediaminetetraacetic acid (EDTA)

References:

1. Kumar A, Tamanna S, Iftekhar H. Intracanal medicaments–Their use in modern endodontics: A narrative review. Journal of Oral Research and Review. 2019 Jul 1;11(2):94-9.
2. Belli S, Erdemir A, Yildirim C. Reinforcement effect of polyethylene fibre in root-filled teeth: comparison of two restoration techniques. Int Endod J. 2006 Feb;39(2):136-42.
3. Salem Milani A, Ganjpour S, Dehghani F, Rahimi S, Sabanik P. Comparison of the fracture resistance of the teeth prepared with ProTaper Universal, ProTaper Next, and ProTaper Gold rotary files. Clin Exp Dent Res. 2022 Dec;8(6):1421-1425.
4. Pascon FM, Kantovitz KR, Sacramento PA, Nobre-dos-Santos M, Puppin-Rontani RM. Effect of sodium hypochlorite on dentine mechanical properties. A review. J Dent. 2009 Dec;37(12):903-8.
5. Yassen GH, Platt JA. The effect of non-setting calcium hydroxide on root fracture and mechanical properties of radicular dentine: A systematic review. Int Endod J. 2013;46:112–8.
6. Palekar A, Mantri P, Awinashe M, Biradar B, Singh M. Comparative evaluation of effect of modified triple antibiotic paste and calcium hydroxide as intracanal medicament on microhardness of root dentin: An in vitro study. Endodontology. 2024 Apr 1;36(2):143-7.
7. Prakash S, Shelke AU. Role of Triphala in dentistry. J Indian Soc Periodontol. 2014 Mar;18(2):132-5.
8. Prakhyath A, Parvathaneni KP, Raju TB, Seshadri A, Kintada VS, Parepalli PS. Evaluation of the antibacterial efficacy of nanomodified triple antibiotic paste with nano anti-inflammatory drug and nano calcium hydroxide as intracanal medicament using confocal laser scanning microscope. Endodontology. 2024 Jan 1;36(1):25-9.
9. Mohammadi Z, Jafarzadeh H, Shalavi S, Palazzi F. Recent Advances in Root Canal Disinfection: A Review. Iran Endod J. 2017 Fall;12(4):402-406.
10. Ordinola-Zapata R, Noblett WC, Perez-Ron A, Ye Z, Vera J. Present status and future directions of intracanal medicaments. Int Endod J. 2022 May;55 Suppl 3(Suppl 3):613-636.
11. Dane A, Capar ID, Arslan H, Akçay M, Uysal B. Effect of Different Torque Settings on Crack Formation in Root Dentin. J Endod. 2016 Feb;42(2):304-6.
12. Dotto L, Sarkis Onofre R, Bacchi A, Rocha Pereira GK. Effect of Root Canal Irrigants on the Mechanical Properties of Endodontically Treated Teeth: A Scoping Review. J Endod. 2020 May;46(5):596-604.e3
13. Sethi S, Gupta A, Kurian AH, Abraham D, Chauhan P, Aneja K, Jala S, Singh A. Effect of calcium hydroxide on fracture resistance and microhardness of dentin in human teeth: A systematic review. Endodontology. 2022 Oct 1;34(4):223-8.
14. Chainani S, Siddana S, Reddy CV, Thippeswamy M, Maurya M, Rudraswamy S. Antimicrobial activity of Triphala on Lactobacilli and Candida albicans: An: in vitro: study. Journal of Orofacial Sciences. 2015 Jul 1;7(2):104-7.
15. Selvi MM, Selvabalaji A, Chonat A, Ananthan V, Mani E, Chinnaswamy AD. A Comparative Evaluation of Antimicrobial Efficacy of Triphala and Calcium Hydroxide as Intracanal Medicament: An In Vitro Study. J Pharm Bioallied Sci. 2022 Jul;14(Suppl 1):S774-S780.
16. Vatankhah M, Khosravi K, Zargar N, Shirvani A, Nekoofar MH, Dianat O. Antibacterial efficacy of antibiotic pastes versus calcium hydroxide intracanal dressing: A systematic review and meta-analysis of ex vivo studies. J Conserv Dent. 2022 Sep-Oct;25(5):463-480.
17. Mehta S, Verma P, Tikku AP, Chandra A, Bains R, Banerjee G. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste, calcium hydroxide, and a proton pump inhibitor against resistant root canal pathogens. Eur J Dent. 2017 Jan-Mar;11(1):53-57.
18. Eskandarinezhad M, Asghari V, Janani M, Reihani MF, Rahimi S, Lotfi M. Evaluation of the Effects of Triphala, 2% Chlorhexidine and 5.25% hypochlorite sodium on Dentin Micro-hardness as Irrigation Solutions. Journal of Ayurveda and Holistic Medicine (JAHM). 2016 Jan 12;3(6):58-67.
19. Elika V, Kunam D, Anumula L, Chinni SK, Govula K. Comparative evaluation of Chloroquick with Triphala, sodium hypochlorite, and ethylenediaminetetraacetic acid on the microhardness of root canal dentin: An in vitro study. J Clin Transl Res. 2021 Jan 21;7(1):72-76.
20. Madhukumar M, Geetha P, Nair KR, Unnikrishnan M. The Effects of Double Antibiotic Paste and Amoxicillin-clavulanate Paste Used in Endodontic Regeneration on Microhardness of Radicular Dentine: An In vitro Study. J Pharm Bioallied Sci. 2021 Jun;13(Suppl 1):S510-S515
21. Halkai R, Halkai KR, Mahveen SU. Effect of different intracanal medicaments combined with chitosan nanoparticles on microhardness and fracture resistance of root dentin–An in vitro study. Saudi Endodontic Journal. 2024 May 1;14(2):218-23.

22. Thienngern P, Panichuttra A, Ratisoontorn C, Aumnate C, Matangkasombut O. Efficacy of chitosan paste as intracanal medication against Enterococcus faecalis and Candida albicans biofilm compared with calcium hydroxide in an in vitro root canal infection model. BMC Oral Health. 2022 Aug 16;22(1):354
23. Pimenta JA, Zaparolli D, Pécora JD, Cruz-Filho AM. Chitosan: effect of a new chelating agent on the microhardness of root dentin. Braz Dent J. 2012;23(3):212-7.
24. Ratih DN, Enggardipta RA, Kartikaningtyas AT. The effect of chitosan nanoparticle as a final irrigation solution on the smear layer removal, micro-hardness and surface roughness of root canal dentin. The Open Dentistry Journal. 2020 Feb 14;14(1).
25. Sajjeev A, Shivkumar AT, Kalgeri SH. Evaluation of chitosan modified glass ionomer cement for microleakage under Spectrophotometer–An in-vitro study. J Evol Med Dent Sci. 2021 Aug 16;10:2769.

Figures and Figure Legends:

Fig 1: Sample Size consisting of 30 human mandibular premolars.

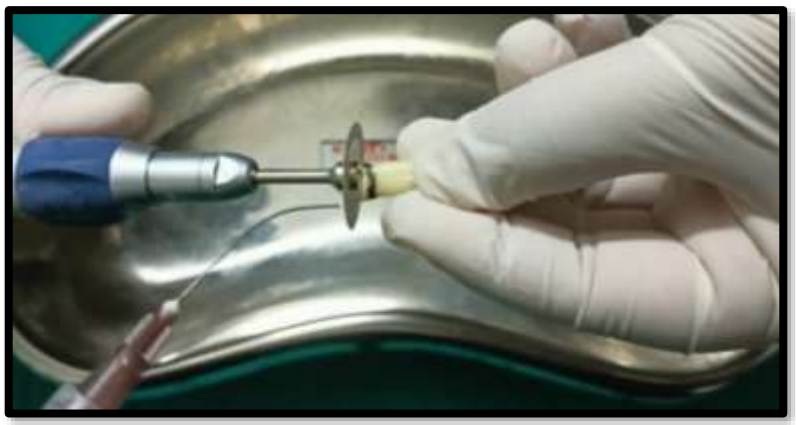

Fig 2: Decoronation of samples leaving behind a root length of 10mm.

Fig 3: Canal Preparation with Protaper Gold Files.

Fig 4: Triphala+Chitosan Intracanal Medicament.

Fig 5: MTAP+Chitosan Intracanal Medicament.

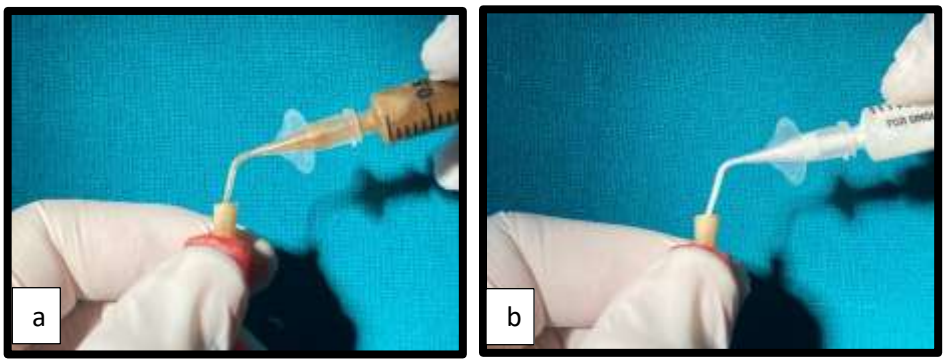

Fig 6: A: Triphala+Chitosan ICM Placement. B: MTAP+Chitosan ICM Placement.

Table 1: One-Way ANOVA analysis for fracture resistance of all groups.				
Groups	**Sample Size (N)**	**Mean (Newton)**	**Standard Deviation**	**p-Value**
Group A (Untreated i.e. Positive Control Group)	10	1206.80	63.33	0.000
Group B (Calcium Hydroxide i.e. Negative Control Group)	10	895.90	65.11	
Group C (Triphala+Chitosan Group)	10	1147.70	54.58	
Group D (MTAP+Chitosan Group)	10	1062.20	59.66	
p-Value was significant at 0.05 level				

Table 2: Pairwise Comparision of all groups for fracture resistance values using Tukey's HSD Test.				
Groups	**Comparison Groups**	**Mean Difference**	**Standard Error**	**p-Value**
GROUP A	GROUP B	310.90	27.19	0.000
	GROUP C	59.10	27.19	0.150
	GROUP D	144.60	27.19	0.000
GROUP B	GROUP C	251.80	27.19	0.000
	GROUP D	166.30	27.19	0.000
GROUP C	GROUP D	85.50	27.19	0.017
p-Value was significant at 0.05 level				