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Antibacterial activity of NBF jel against Streptococcus mutans isolated from orthodontic patients

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Abstract

Background: Streptococcus mutans is capable of demineralizing enamel by producing an acidic environment. Therefore, control of the bacterial biofilm on teeth is essential for the maintenance of oral health. The NBF jel with propolis ingredient that manufactured by nano-biofusion technology which has an antimicrobial activity as it is confirmed by many previous studies expected to induce more antimicrobial effect than traditional product and lastly overcome the bacterial biofilm and prevent the carries and others oral medical problems.

Aim of study : To evaluate the antibacterial action of NBF jel on Strep. mutans isolated from orthodontic appliance.

Material and method: Saliva samples were collected from 36 patients wearing appliance for different duration. Strep. mutans was isolated ,purified and diagnosis , then prepared two extracts from NBF jel (aqouse and alcoholic) in different concentration , chlorhexidine 2%(control) were added to isolated bacteria. After incubation for 48 hours; the inhibition zone of antibacterial activity of NBF was measured and colony count of bacteria was counted.

Results: The two types of extracts have had antimicrobial activity but the alcoholic extract was induced more action than aqouse one as well as their effect was less than the chlorhexidine 2%(control). The inhibition zone was increased with increasing the concentration of both extracts but the responsiveness was reduced with increased the period of using the appliance. The reduction in colony counts of strep.

Conclusion: NBF jel had antimicrobial activity against Strep. mutans isolated from appliance and this activity indirectly correlated with duration of wearing.

Key words: NBF jel, Streptococcus mutans; Propolis

Introduction

Nanotechnology is a novel scientific approach that involves materials and equipment's capable of manipulating physical as well as chemical properties of a substance at molecular levels. Biotechnology, uses the knowledge and techniques of biology to manipulate molecular, genetic and cellular processes to develop products and services and is used in diverse fields from medicine to agriculture.[1] Nanobiotechnology is considered to be the unique fusion of biotechnology and nanotechnology by

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which classical micro-technology can be merged to a molecular biological approach in real. The role of nanodentistry by means of the use of nanomaterials, biotechnology and nanorobotics will ensure better oral health. [2].

It has been proven that the basis of most pathological lesions due to the imbalance between the antioxidants and oxidants, caused by multiple heterogeneous causes. The reactive free radicals which are released are reactive oxygen radicals that are present in all biological systems. Oxidative stress occurs when the intracellular concentration of free radicals will increase beyond physiological values which lead to cell damage by lipid peroxidation, DNA and many other pro inflammatory cytokine factors [3].

The NBF gingival gel is the first product containing three biocompatible ingredients in nano-emulsion form which is manufactured with nano bio-fusion technology. These ingredients are vitamin C, E and propolis. The vitamin E acts synergistically with vitamin C to induce an antioxidant effect while propolis whose main ingredient is flavonoids having adhesive function. The antimicrobial activity of propolis is by far the most important biological property of propolis. In spite of the big compositional differences of the different propolis types, they all have antimicrobial activity. It seems that rather the sum of the propolis antimicrobial components than individual substances are responsible for the antimicrobial action [4].Propolis is applied in the different dental specialties: oral hygiene; periodontology and oral mucosa pathologies; oral surgery; orthodontics; restorative dentistry; endodontics and prosthetic dentistry. Propolis inhibits in the mouth different pathogenic microbes such as bacteria, fungi and viruses [5] and can be successfully applied against the different stomatological pathologic conditions: plaque formation, mouth wounds and ulcers, denture and aphthous stomatitis, paradontosis, periodontitis, gingivitis, dentinal hypersensitivity and caries [6,7].

S. mutans is a major matrix producer and can rapidly modulate the formation of cariogenic biofilms when dietary sucrose and starch are present [8]. Biofilms are highly structured microbial communities that are enmeshed in a self-produced extracellular matrix. Within the complex oral microbiome, Streptococcus mutans is a major producer of extracellular polymeric substances including exopolysaccharides (EPS), eDNA, and lipoteichoic acid (LTA). EPS produced by S. mutans-derived exoenzymes promote local accumulation of microbes on the teeth, while forming a spatially heterogeneous and diffusion-limiting matrix that protects embedded bacteria. The EPS-rich matrix provides mechanical stability/cohesiveness and facilitates the creation of highly acidic microenvironments, which are critical for the pathogenesis of dental caries [9]

Aims

1. To evaluate the Antibacterial effect of NBF jel on Streptococcus Mutans isolated from patients with orthodontic appliance**.**
2. To assess the activity against Strep. mutans if any with duration of using the appliance

Materials and methods

1. After approval from concerned authorities was obtained , un stimulated saliva samples (2-5 ml) were collected from (36) orthodontic patients wearing appliance for different period ($\leq$ 3, 3-6 and more

than 6 ≥ months) who were attending to outpatient clinics of Al-Mustansyria Dentistry College from 1st June-1st October-2016.

2. Isolation and purification of mutans Streptococci was done by dispersed saliva sample using vortex mixer for 1 minutes .Ten fold dilutions were performed by transferring 0.1 ml of sterile phosphate buffer saline (PH 7) ,from dilution 10^{-1} and 10^{-3} of salivary samples , 0.1 ml was taken and spread in duplicate on the selective media MSA (Mitis Salivarius Agar) for isolation Streptococci and on MSBA(Mitis Salivarius Bacitracin Agar) for Mutans streptococci .The plates were incubated an aerobically using gas pack for 48hour at 37 C^0, then aerobically for 24 hour at 37 C^0 [10] . Mutans streptococci were diagnosed according to their morphological characteristics on MSA plates [11] by using cultural, morphological, Gram stain and bio-chemical characteristics. The maintenance of bacterial isolates and checked for purity by re-inoculation on the MSB agar plates, incubated an aerobically for 48 hour at 37 C^0, followed by incubation aerobically for 24 hour at 37 C^0 .Broth were stored in fridge until use , this procedure were repeated monthly .while the activation of bacterial isolates was done by inoculated 0.9 ml of mutans streptococci isolate in 10 ml of TPB (Tryptose Phosphate Broth),then incubated aerobically at 37 C^0 for 18 hours for activation before the conduction of each in vitro experiment in the study .

3.Aqouse and alcoholic extracts in different concentrations (10,20,30,40 and 50%) were prepared by dissolving the material under study in distal water or alcohol by serial dilution method [4,6].

4.The sensitivities of the Mutans Streptococci to different concentrations of NBF was measured by using agar well technique by preparation of MHA (Mueller Hinton Agar) and then 0.1 ml of activated Mutans Streptococci inoculums by spread the MHA , left at room temperature for 20 minutes (the culture inoculum was aseptically transferred into sterile petri dishes and 15 ml of agar was poured into the same plate). Equal wells were prepared by using a sterile cork borer of 5 mm in diameter was used to punch holes in the agar creating a well/ditch. To each well was added 0.1 ml of different concentrations of extracts. All plates were labeled and allowed for 2 hours for proper diffusion of the extract before incubation at 37°C for 24 hours. The mean zones of inhibition were measured and recorded to the nearest mm [12] .

5.The total colony count of streptococcus mutans was counted to study the effect of different concentration of NBF on viable count according to procedure of [13] .by prepared BHI (Brain Heart Infusion) broth and distributed in test tubes (8.9ml to each tube) and I ml of tested agents was added to each tube except control which was broth and bacteria only , then 0.1 ml of activated bacterial inoculums ,incubated then from each broth 0.1 ml was transferred to 0.9 ml of sterile phosphate buffer saline (PH 7) and tenfold dilution were performed . from dilution 10^{-1} , 0.1 ml was taken and spread on duplicates of MSB agar and incubated the viable count of all plates were estimated .(viable cell count =mean number of the colony ×reciprocal of the dilution 10^{-1}×10).

Inclusion and exclusion criteria: - all patients wearing appliance were candidate for this study, patients currently on antibiotic were excluded.

Statistical analysis: -SPSS was used for data entry and analysis, suitable tests were used accordingly (ANOVA, student T test and dunnetti test, p≤0.05 considered significant).

Results

The results showed that there was a significant difference in mean value of inhibition zone with all concertation except conc.10% that was induced by different type of extracts and control and the mean value of inhibition zone was increased with increasing the concertation of extracts as showed in table.1 and fig.1, on multiple comparison with control by Dunnett test; same significant difference was reported as showed in table.2.

The result of current study demonstrated that the inhibition zone induced by two types of extracts in different concentration was decreased with increasing the duration of using of appliance. A significant reduction in the number of colonies in the samples as the result of the effect of the extract on bacterial growth was reduced with increasing the duration of using the appliance as seen in fig.3(A, B, C and D).

The results demonstrated there was significant difference in mean count of streptococcus mutans after using different type of extracts and control (p≤0.05 for all) and as concentration of extract increased the colony count decreased as seen in table.3 and fig.2. On multiple comparison of each extract to control the results also reported significant difference in mean value of bacterial growth with all concentration as seen in table.4

Discussion

The protective role of NBF jel was well assessed by many studies but the antibacterial activity not well evaluated there for we are focusing on the antibacterial effect of the NBF jel and trying to determine the specific ingredient, which induce such action if any. Our finding revealed there was significant antibacterial effect induced by the NBF jel demonstrated in reduction in colony count of test organism and this effect was increased by increasing the concentration of used extracts as well as we found that the alcoholic extract induces more effect than aqouse one but the effect of both extracts against strep. Mutans was lower than that of chlorhexidine which was used as control agent. We believed that the antibacterial effect which was seen with using the NBF jel belongs to propolis compound which is one of the main constituent of the NBF jel. The antimicrobial effect of propolis, where confirmed by many studies, where the researchers Bankova [14,15] satiated that the antibacterial property of propolis was reported with all of it is types. A study conducted by Banskota and his co workers [16] stated that the Poplar propolis gathered by Apis mellifera caucasica had a higher antibacterial activity than one gathered by Apis mellifera anatolica and Apis mellifera carnica. A study conducted by [17] reported that the propolis is more active against Gram-positive pathogens but many Gram-negative bacteria are also inhibited. A study done by Victoria and his research [19] proved the antibacterial activity for propolis against Micrococcus luteus, Salmonella typhimurium [18], Klebsiella pneumonia. Although in previous studies [20] claimed that Listeria monocytogenes is not sensitive to propolis, recent works [21] revealed significant antibacterial activity of

propolis against Listeria. A study conducted by [22] shown that propolis has a strong antibacterial activity against 13 different pathogens. With the increasing of antibiotic resistance in the last years, there is a considerable interest of hospitals in propolis as an antibacterial agent. It has been shown that propolis has synergistic effects with antibiotic action against bacteria [23,24]. Pepeljnjak, S [25] reported that the antibacterial effect of propolis is bactericidal not bacteriostatic. Many studies show that propolis effectively limits the quantity of E. faecalis in root canals, but its effectiveness might be lower than that of chlorhexidine [26]. Popovska M et al [27] link the antimicrobial activity of propolis to flavonoids where they stated; Propolis provides a very good adhesion, and the nanoemulsion has a much lower surface tension due to the small size of the molecules of the vitamin that cause rapid absorption through the mucosa in the target cells. The main ingredient of propolis; flavonoids, which owed antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory properties. Banskota AH et al not linked the antimicrobial activity of propolis to flavonoids only but to derivatives of aromatic acids and esters such as fatty acids, terpenoids, and waxy acids. Results of several studies have demonstrated an in vitro inhibition of “mutans group streptococci” (MGS) growth by propolis [28].

The ability of strep. mutans to produce extracellular polysaccharides, mainly glucans, has been described as a critical factor in the pathogenesis of dental caries and plaque formation and accumulation. In this case, mutans streptococci should be a prime target for any therapeutic agent aimed at preventing dental caries. Therefore, the antimicrobial activity of propolis against these bacteria could play an important role in preventing the formation of dental plaque and caries [29].

The poor response of Strep. mutans which isolated from patients with prolonged duration of using the appliance to both types of extracts not well understood and the most explanation for this phenomenon is the increasing in the incidence of microbial resistance due to frequent using of antibiotic with increased frequency of infection in appliance using individuals.

Conclusion

It may be concluded that the NBF jel possesses antimicrobial activity against Strep. mutans and this effect mostly belong to propolis ingredient.

References

1- Fakruddin M D, Zakir H and Hafsa A, Prospects and applications of Nanobiotechnology: a medical perspective, Journal of Nanobiotechnology.2012,10:31

2- West JL, Halas NJ: Applications of nanotechnology to biotechnology commentary. Curr Opin Biotechnol. 2000, 11: 215-217

3- Momen-Beitollahi J, Mansourian A, Momen-Heravi F, Amanlou M, Obradov S, Sahebjamee M. Assessment of salivary and serum antioxidant status in patients with recurrent aphthous stomatitis. Med Oral Patol Oral Cir Bucal. 2010,15: e557–e561.

4- Kujumgiev, a; tsvetkova, i; serkedjieva, y; bankova, v s; christov, r; popov, s Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal of Ethnopharmacology.1999, 64 (3): 235-240.

5- Silici S; Koc A N; Mistik S; Comparison of in vitro activities of antifungal drugs and propolis against yeasts isolated from patients with superficial mycoses. Annals of Microbiology.2007 ,57 (2): 269-272.

6- Hayacibara M F; Koo, H; Rosalen P, et al; In vitro and in vivo effects of isolated fractions of Brazilian propolis on caries Development. Journal of

Ethnopharmacology.2005 ,101 (1-3): 110-115.

7- Liberio S A, Pereira A I, Araujo M et al; The potential use of Propolis as a cariostatic agent and its actions on mutans group streptococci, Journal of Ethnopharmacology.2009, 125 (1): 1-9.

8- Paes Leme, A. F., Koo, H., Bellato, C. M., Bedi, G., and Cury, J. A. The role of sucrose in cariogenic dental biofilm formation–new insight. J. Dent. Res.2006, 85, 878–887.

9- Klein M I et al, Streptococcus mutans-derived extracellular matrix in cariogenic oral biofilms, Frontiers in Cellular and Infection Microbiology, 2015 ,5(10); 1-8.

10- Hoolbrook W,Beighton D.Streptococcus mutans levels in saliva and distribution of serotypes among 9-year old Icelandic children .Scan .J.Dent.Res.(1986):95:37-42.

11- Hardie M.Oral Streptococci.In:Sneeath PHA ,Mair NS,Sharpe ME,and Holt JG (eds) : Bergey s Manual of Systemic Bacteriology . 2 nd William and Wilkins Co., Baltimor,1986.

12- Sandra S,Nadia A,Mitsuko T:Antimicrobial activity of four different dental gel formulas on cariogenic bacteria . J.Microbial .,2005,16:337-342 .

13- Baron E., Peteson ,L.Fingold,S.Methods for testing Antimicrobial Effectiveness .In Bailey and Scotts diagnostic Microbiology .9th ed.C.V.Mosby Co., St.,Louis ,USA,1994.

14- Bankova V; Recent trends and important developments in propolis research. Evidence-based complementary and alternative medicine,2005, 2 (1): 29-32.

15- Bankova V; Popova M et al;Plant origin of propolis: Latest developments and importance for research and medicinal use,2007 ; pp 40-46.

16- Banskota A H, Tezuka Y, Kadota S;Recent progress in pharmacological research of propolis.Phytotherapy Research,2001, 15 (7): 561-571.

17- Grance, J M; Davey R W; Antibacterial properties of propolis (bee glue), Journal of the Royal Society of Medicine.1990, 83: 159-160.

18- Uzeal A; S K; Oncag O et al; Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiological Research,2005 160 (2):189-195.

19- Victoria, F R; Franco et al; Pharmacological evaluation of Propolis solutions for endodontic use. Pharmaceutical Biology.2007. 45 (9): 721-727.

20- Grance, J M; Davey R W; Antibacterial properties of propolis (bee glue). Journal of the Royal Society of Medicine.1990. 83: 159-160.

21- Paulino N; Coutinho J; Coutinho L et al; Clinical evaluation of the anti-inflammatory effect of baccharis dracunculifolia propolis gel on cervicitis.2014 RCEAM 1

22- Basim E et al; Antibacterial activities of Turkish pollen and propolis extracts against plant bacterial pathogens.2006, Journal of Food Engineering 77 (4): 992-996

23- OnlenY et al; Antibacterial activity of propolis against MRSA and synergism with topical mupirocin. Journal of Alternative and Complementary Medicine .200713 (7): 713-718.

24- Orsi R D et al Synergistic effect of propolis and antibiotics on the Salmonella typhi. Brazilian Journal of Microbiology .2006,37 (2): 108-112.

25- Pepeljnjak, S and Kosalec I; Galangin expresses bactericidal activity against multiple- resistant bacteria: MRSA, Enterococcus spp. and Pseudomonas aeruginosa. FEMS Microbiology Letters.2004. 240 (1): 111-116.

26- Wieckiewicz, w et al. Does Propolis Help to Maintain Oral Health? Evidence-based complementary and alternative medicin.2013.

27- Popovska M, Fidovski J, Radojkova-Nikolovska V et al; The effects of NBF gingival gel in the treatment of recurrent oral ulcers: case report, Coresponding Author: Mirjana Popovska, University Cyril and Methodius, Faculty of dentistry, 1000, Skopje, Republic of Macedonia

28- Banskota AH, Tezuka Y, Kadota S. Recent progress in pharmacological research of propolis. Phytother Res 15: 561- 571, 2001

29- Arsalan S et al; Antimicrobial activity of poplar propolis on mutans streptococci and caries development in rats. Turk J Biol 36 (2012) 65-73

Results (Tables and Figurs)

Table.1- The mean value of inhibition zone (mm) by different extract for each concentration.

Study parameters		Mean	Std. Deviation	95% Confidence Interval for Mean		p-value
				Lower Bound	Upper Bound	
Inhibition zone/10%	Aqueous	0.0	0.0	0.0	0.0	n/a
	Alcoholic	0.0	0.0	0.0	0.0	
Inhibition zone/20%	Aqueous	0.0	0.0	0.0	0.0	0.003
	Alcoholic	2.3	1.7	1.6	2.8	
Inhibition zone/30%	Aqueous	4.3	3.6	3.1	5.5	0.002
	Alcoholic	7.3	2.1	6.5	8.1	
Inhibition zone/40%	Aqueous	10.2	2.6	9.3	11.1	0.001
	Alcoholic	12.0	2.8	11.1	13.0	
Inhibition zone/50%	Aqueous	13.8	2.8	12.8	14.7	0.001
	Alcoholic	16.5	3.1	15.5	17.6	

N=36

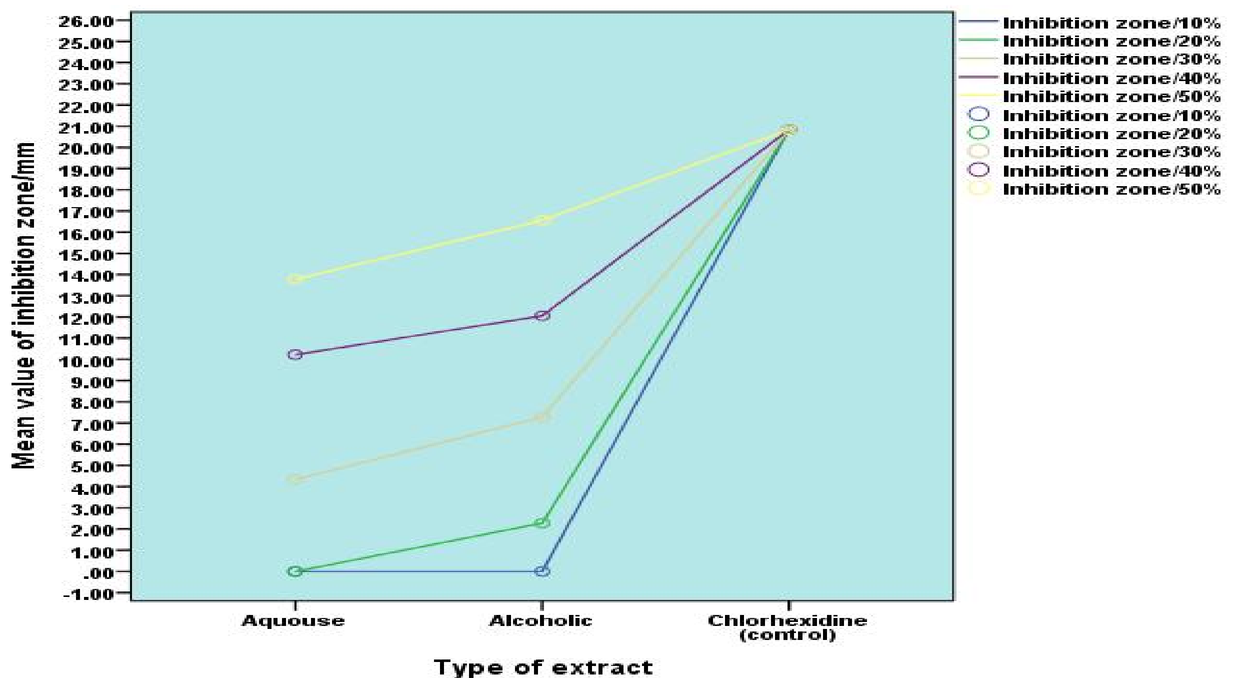

Fig.1-The mean value of inhibition zone by different extract for each concentration.

Table.2-Mean value of inhibition zone by different extract for each concentration.

Dependent Variable	(I) Type of extract	(J) Type of extract	Mean Difference (I-J)	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Inhibition zone/10%	Aqueous	Chlorhexidine(control)	20.8*	0.001	21.1	20.4
	Alcoholic	Chlorhexidine(control)	20.8*	0.001	21.1	20.4
Inhibition zone/20%	Aqueous	Chlorhexidine(control)	20.8	0.003	21.4	20.19
	Alcoholic	Chlorhexidine(control)	18.5	0.003	19.1	17.9
Inhibition zone/30%	Aqueous	Chlorhexidine(control)	16.5	0.002	17.8	15.1
	Alcoholic	Chlorhexidine(control)	13.5*	0.02	14.8	12.2
Inhibition zone/40%	Aqueous	Chlorhexidine(control)	10.6	0.001	11.8	9.3
	Alcoholic	Chlorhexidine(control)	8.7	0.001	10.0	7.5
Inhibition zone/50%	Aqueous	Chlorhexidine(control)	7.05	0.001	8.3	5.7
	Alcoholic	Chlorhexidine(control)	4.2	0.001	5.6	2.9

Table.3- The mean of colony count of Strep. Mutants ×10 by different extract for each concentration.

Study parameters (colony count in each concentration)		Mean	Std. Deviation	95% Confidence Interval for Mean		p-value
				Lower Bound	Upper Bound	
Colony count-10%	Aqueous	84.5	10.7	80.9	88.1	0.001
	Alcoholic	77.3	11.6	73.3	81.2	
Colony count-20%	Aqueous	78.6	9.5	75.4	81.9	0.002
	Alcoholic	69.3	10.	65.9	72.8	
Colony count-30%	aqueous	70.5	9.2	67.4	73.6	0.002
	Alcoholic	59.9	10.8	56.2	63.6	
Colony count-40%	aqueous	61.0	8.5	58.1	63.9	0.001
	Alcoholic	51.9	9.6	48.6	55.2	
Colony count-50%	aqueous	53.3	10.1	49.9	56.7	0.01
	Alcoholic	41.7	10.9	38.0	45.4	

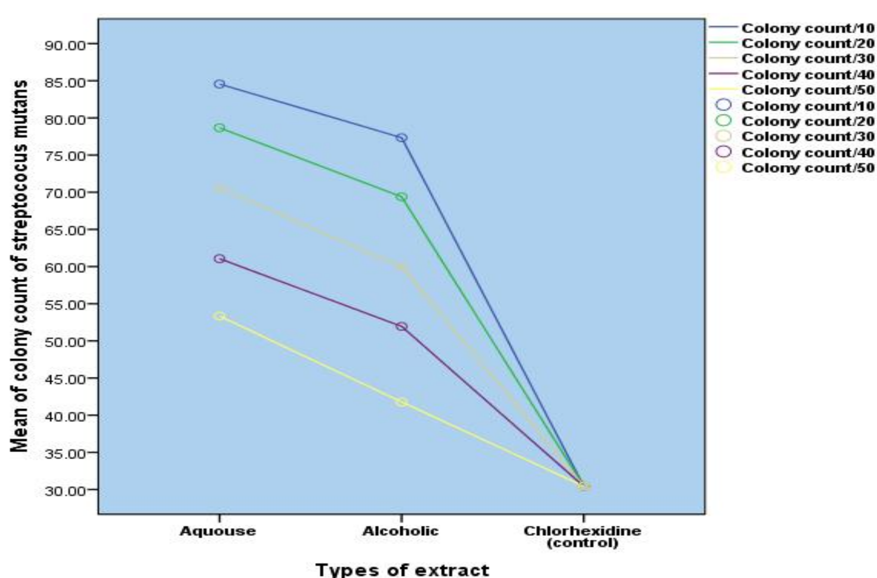

Fig.2-The mean of colony count of strep. mutants×10 by different extract for each concentration.

Table.4-The mean of colony count of streptococcus mutans ×10 by different extract for each concentration.

Dependent Variable	(I) Type of extract	(J) Type of extract	Mean Difference (I-J)	p-value.	95% Confidence Interval	
					Lower Bound	Upper Bound
Colony count-10%	Aqouse	Chlorhexidine(control)	54.1	0.001	48.6	59.6
	Alcoholic	Chlorhexidine(control)	46.8	0.001	41.4	52.3
Colony count-20%	Aqueous	Chlorhexidine(control)	48.2	0.002	43.0	53.5
	Alcoholic	Chlorhexidine(control)	38.9	0.002	34.2	43.7
Colony count-30%	Aqueous	Chlorhexidine(control)	40.1	0.002	35.5	45.8
	Alcoholic	Chlorhexidine(control)	29.5	0.002	24.3	34.7
Colony count-40%	Aqueous	Chlorhexidine(control)	30.6	0.001	25.0	35.3
	Alcoholic	Chlorhexidine(control)	21.5	0.001	16.8	26.2
Colony count-50%	Aqueous	Chlorhexidine(control)	22.8	0.001	17.4	28.3
	Alcoholic	Chlorhexidine(control)	11.3	0.001	6.19	16.8

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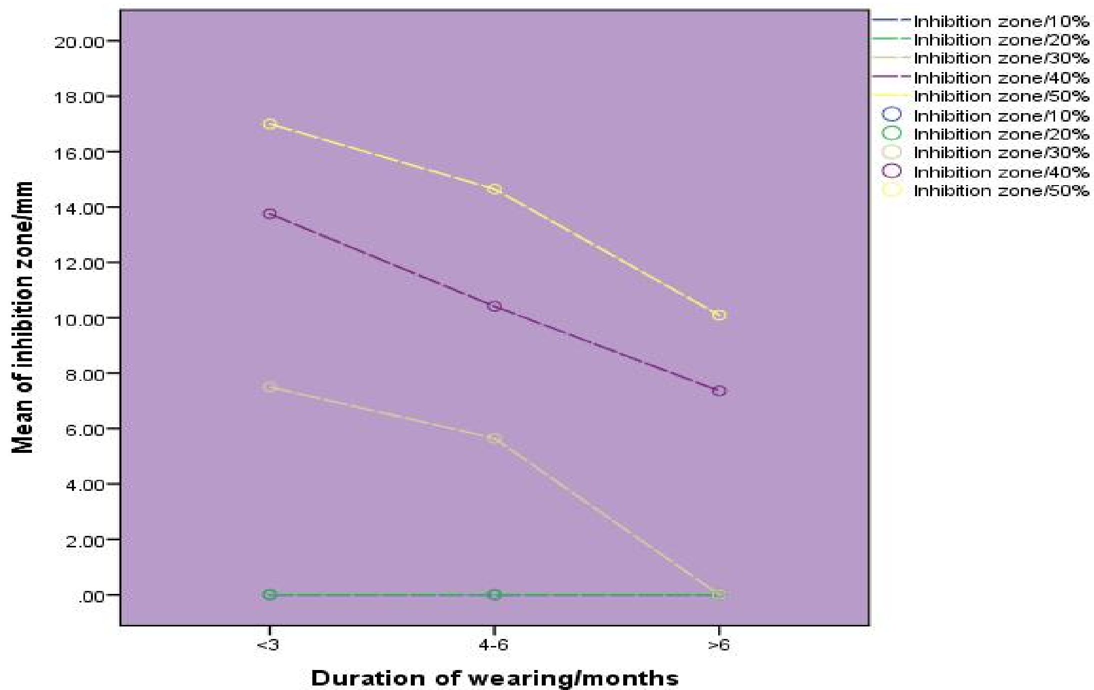

Fig.3-A-Relathionship of mean of inhibition zone by aqouse extract in different conc. and duration of wearing of appliance

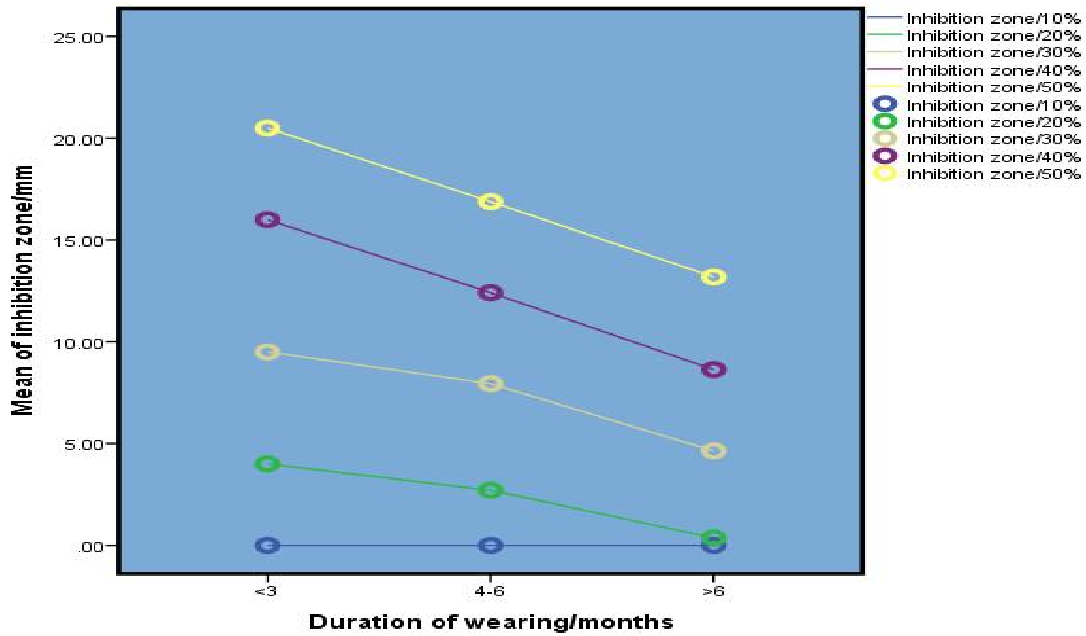

Fig.3-B-Relathionship of mean of inhibition zone by alcoholic extract in different conc. and duration of wearing of appliance

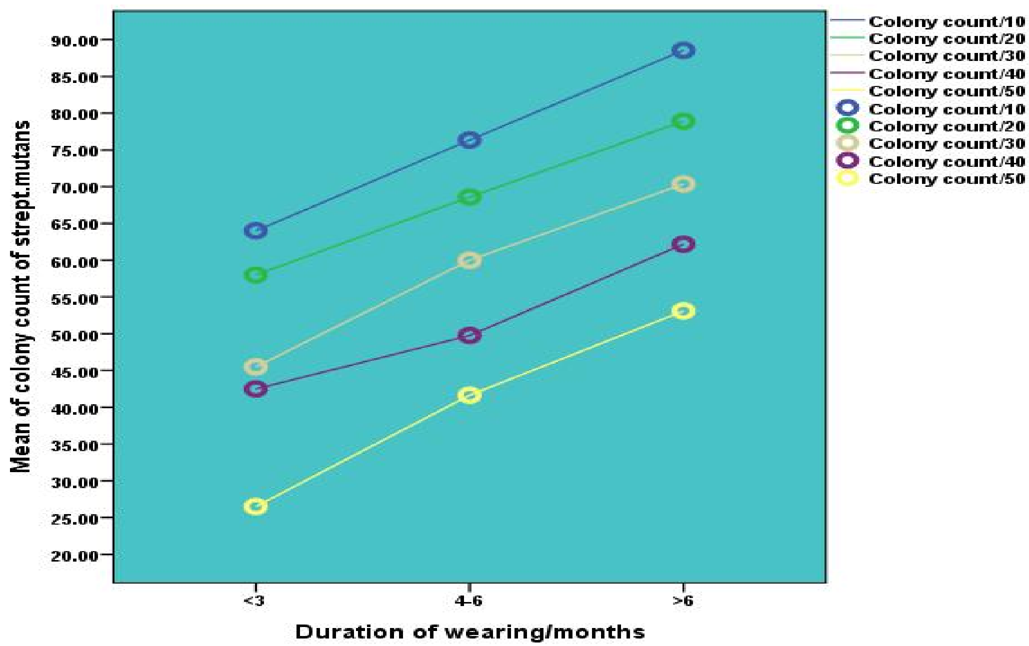

Fig.3-C-Relathionship of mean of colony count of strep. Mutans with using aqouse extract in different conc. and duration of wearing of appliance

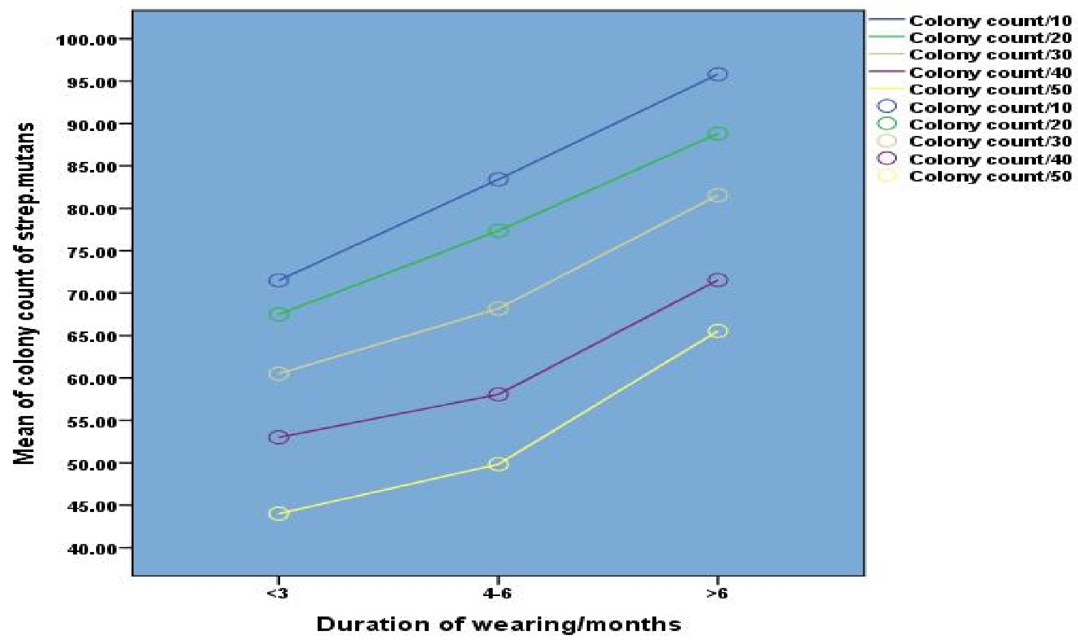

Fig.3-D-Relathionship of mean of colony count of strep. Mutans with using alcoholic extract in different conc. and duration of wearing of appliance