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THE EFFECT OF SOME SELECTED CHEMICALS ON *STREPTOCOCCUS MUTANS* FROM DENTAL CARIES ONTO DENTURES AND AMALGAMS.

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ABSTRACT

In the present study 13 isolates of *Streptococcus mutans* were collected from 25 volunteers within the ages of 10 - 30 years. Diagnosis was obtained according to the colony of morphology, Gram stain, biochemical test and Vitek system. They found to be 100 % sensitive to azithromycin by disk diffusion method (Kirby Bauer test). It showed sensitivities against some selected chemicals such as zinc oxide nanoparticals, phenytoin B.P, potassium L-proline dithiocarbamate, potassium oxalic acid dihydrazide *bis*dithiocarbamate, potassium succinic acid dihydrazide *bis*dithiocarbamate, and potassium adipic acid dihydrazide *bis*dithiocarbamate, and adipic acid dihydrazide *bis*dithiocarbamate by well diffusion and broth dilution methods. Azithromycin showed very strong antibacterial effect, when mixed with the amalgam components at an amount of 0.8 to 25 mg per capsule, while oxalic acid dihydrazide bisdithiocarbamate showed antibacterial effect at an amount higher than 10 mg per capsule. The other selected chemicals showed 100 % sensitivities, while L-proline, oxalic acid dihydrazide, succinic acid dihydrazide, adipic acid dihydrazide showed no antibacterial activity. The measured MIC and MBC of the selected chemicals showed the following values: Zinc oxide NPs, 5, 10 mg/mL; phenytoin B.P, 1×10^{-3}, 2×10^{-3}; potassium L-proline dithiocarbamate, 2×10^{-3}, 2×10^{-3}; potassium oxalic acid dihydrazide bisdithiocarbamate, 0.5×10^{-3}, 1×10^{-3}; potassium succinic acid dihydrazide *bis*thiocarbomate, 1×10^{-3}, 2×10^{-3}, potassium adipic acid dihydrazide bisdithiocarbamate, $1 \times 10\text{-}^{3}$, $2 \times 10\text{-}^{3}$ respectively. The measured MIC and MBC of the selected chemicals loaded on PMMA resin showed the following values: zinc oxide NPs, 30,

40 mg/mL; phenytoin B.P, 2.5, 5 mg/mL and potassium oxalic acid dihydrazide *bis*dithiocarbamate, 1.25, 2.5 mg/mL, respectively.

KEYWORDS: *Streptococcus mutans*, azithromycin, dental caries, denture, amalgams, phenytoin B. P, dicarboxylic acid dihydrazide bisdithiocarbamate.

INTRODUCTION

Streptococcus mutans is the cariogenic bacteria from the oral cavity, it is one of the major causative bacterial groups in human dental decay by producing acids, aciduric potential, formation, utilization of storage polysaccharides, and formation of insoluble extracellular polysaccharide. [1] Dental caries forms through the complex interaction over time between acid producing bacteria, and fermentable carbohydrate, and many host factor including teeth and saliva. [2] Denture bases fabricated from heat-polymerized acrylic resins (PMMA) may act as a reservoir for *Streptococcus mutans* and other microorganisms and contribute to re-infection in denture wearers [3] and occurs in areas around orthodontic brackets bonding agents. [4] Thus there is a need to use a broad-spectrum antimicrobial resin [5] to prevent *Streptococcus mutans*, and other microorganisms from accumulation. Zinc oxide nanoparticals (50 nm), have been reported to the disruption of cell membrane activity. [6] The induction of intercellular reactive oxygen species, including hydrogen peroxide (H_2O_2), other strong oxidizing agent harmful to bacterial cells can be used. [7] It has also been reported that Zn can be activated by UV and visible light to generate highly reactive oxygen species such as OH^-, H_2O_2, and O_2^{2-}. [8] The negatively charged hydroxyl radicals and superoxide cannot penetrate into the cell membrane and are likely to remain on the cell surface, whereas H_2O_2 can penetrate into bacterial cells therefore it is has been used in PMMA resin component. Also It has been found that fifty to sixty percent rate of dental caries occurs after restoration and treatment with amalgam. [9-10] To prevent tooth decay after restoration there is a need to select a chemical with antibacterial activity within the amalgam components.

This study aimed to explore the effect of some selected chemicals such as, azithromycin, zinc oxide NPS, phenytoin B.P [11], L-proline, oxalic acid dihydrazide, succinic acid dihydrazide, adipic acid dihydrazide, potassium L-proline dithiocarbamate, potassium oxalic acid dihydrazide *bis*dithiocarbamate, potassium succinic acid dihydrazide *bis*dithiocarbomate, and potassium adipic acid dihydrazide *bis*dithiocarbamate on the growth and a viable count of *Streptococcus mutans* in amalgam and denture formulations. [12]

MATERIALS

a. Equipment's and Apparatus

The following equipment's and apparatus were used during the present study: VITEK 2 system from bioMerieux, USA; dental vibrator from Bego, Germany; amalgamator (Triturator), Germany; Hydraulic press, Germany; Bench top lathe polishing machine from Sirio, Italy.

b. Chemicals and Selected Chemicals

Azithromycin powder from Jamjoom, Saudi Arabia used as it is; bacitracin powder from Appli-Chem, Germany; bromocresol purple from Qualkmis, India; sucrose from CDH, India; zinc oxide nanoparticals 50 nm from Nanoshel, USA; dental stone Type III thixotropic from Zhermack, Italy; Heat cure acrylic resin for denture (powder and liquid) from Ivoclar Vivadent AG, Italy; absolute ethanol 99.9 % from BDH, England; L-proline, oxalic acid dihydrazide, succinic acid dihydrazide, and adipic acid dihydrazide from Sigma-Aldrich, USA; phenytoin B.P was prepared according to Al-Nuzal [11], potassium L-proline dithiocarbamate, potassium oxalic acid dihydrazide *bis*dithiocarbamate, potassium succinic acid dihydrazide *bis*thiocarbomate, and potassium adipic acid dihydrazide *bis*dithiocarbamate prepared according to literature Al-Nuzal. [12]

c. Solutions of Selected Chemicals

1. Zinc oxide NPS suspension: each of the following amounts of zinc oxide 0.5, 10, 20, 30, 40, 50, 60, and 70 mg were suspended in 1.0 ml of sterilized distilled water.
2. Phenytoin solution: an amount of phenytoin of 100 mg was dissolved in 99 % alcohol; the solution needed some heat to enhance the complete solution.
3. Oxalic acid dihydrazide solution: an amount of oxalic acid dihydrazide of 40 mg was dissolved in 10 ml of sterilized distilled water.
4. Suucinic acid dihydrazide solution: an amount of succinic acid dihydrazide of 60 mg was dissolved in 10 ml of sterilized distilled water.
5. Adipic acid dihydrazide solution: an amount of adipic acid dihydrazide of 80 mg was dissolved in 10 ml of sterilized distilled water.
6. L-proline solution: an amount of L-proline dithiocarbamate of 100 mg was dissolved in 10 ml of sterilized distilled water.

7. Potassium oxalic acid dihydrazide *bis*dithiocarbamate solution: an amount of potassium oxalic acid *bis*dithiocarbamate of 130 mg was dissolved in 10 ml of sterilized distilled water.
8. Potassium succinic acid dihydrazide *bis*dithiocarbamate solution: an amount of potassium succinic acid *bis*dithiocarbamate of 150 mg was dissolved in 10 ml of sterilized distilled water.
9. Potassium adipic acid dihydrazide *bis*dithiocarbamate solution: an amount of potassium adipic acid dihydrazide *bis*dithiocarbamate of 160 mg was dissolved in 10 ml of sterilized distilled water.

d. Isolation of *Streptococcus mutans*

Dental swab was taken from human healthy volunteers, aged (10 - 30 years) having dental plaque or cavity after removing any remain food and push dental swab in the cavity or plaque and then culture. Selective media of Mitis Salivarius Bacitracin Agar incubated anaerobically using a candle for 48 hour at 37^0C then incubated aerobically for 24 hour at room temperature. [13] The colonies of *Streptococcus mutans* were identified on the basis of the following characteristics: morphology of the colonies, [14]; Gram's stain [15]; biochemical Test viz. Catalase production Test [16], and carbohydrate fermentation test. [17] VITEK 2 systems product information: The GP identification card is based on established biochemical methods and newly developed substrates. [18]

e. Azithromycin and Selected Chemicals Susceptibility of *Streptococcus Mutans*

Disk diffusion test of Kirby-Bauer method was performed to obtain the susceptibility *of Streptococcus mutans* towards azithromycin. Sensitivity of the selected chemicals was performed following well diffusion method [19] and broth dilution method. [20] The broth dilution method procedure was used to measure the sensitivity of the selected chemical solutions with the volume of the brain-heart infusion broth (8.9 ml) in the tubes set was made, and 1 ml of the selected chemical was added.

f. Preparation Amalgam-Azithromycin Mixture

A mixture of amalgam-azithromycin was prepared according to the following steps: A commercial capsule of dental amalgam was opened according to the manufacturer instruction. The total weight of the amalgam is 600 mg and different amount of the selected antibiotic (0.8, 1.6, 3.12, 6.25, 12.5, and 25 mg of azithromycin) was added to the silver alloy compartment and closed before releasing the mercury. The above amount of the antibiotic

will not change the physical nature of the amalgam. The mixture capsules were place in the amalgamator (triturator) apparatus, and mixed for 3000 rpm for 10 second. The amalgam was left to set for 5 minutes, and it is ready for studying its biological properties.

g. Preparation Amalgam-Oxalic Acid Dihydrazide *Bis*dithiocarbamate Mixture

Similar procedure was used to prepare amalgam-potassium oxalic acid dihydrazide *bis*dithiocarbamate mixture with the following amounts; 2.5, 5, 10, and 20 mg in amalgam capsule batch.

h. Sensitivity of Amalgam Mixtures

Sensitivity of the azithromycin-amalgam and potassium oxalic acid dihydrazide *bis*dithiocarbamate-amalgam mixtures were performed following well diffusion method [19], and broth dilution method [20]. The procedures were used to measure the sensitivity with slight change. The volume of the brain-heart infusion broth (9.9 ml) in the tubes set was made.

i. PMMA Loaded with Zinc Oxide Nps Disc Preparation

A polymethyl methacrylate resin discs mold were made by using stainless steel mold with diameter 5.0 mm and depth of 0.6 - 0.7 mm to the study the sensitivity of *Streptococcus mutans* by broth dilution method. For the preparation of PMMA resin specimens, a metallic round molds were made from stainless steel block into the desirable shape and thickness using turning machine. To obtain a PMMA impression discs, a similar procedure followed in the conventional flasking technique for complete dentures construction was used, and a regular discs of a 5.0 mm diameter and 0.6 - 0.7 mm thickness was obtained. The heat cure denture base PMMA resin was mixed according to manufacturer instruction with powder/liquid ratio (P/L ratio) *e. i* 2.25 g of powder was mixed with l ml liquid and varying amount of zinc oxide NPs powder (0.5, 10, 20, 30, 40, 50, 60, and 70 mg suspended in 200 µL distilled water and 800 µL of liquid monomer). Specimens devoid of zinc oxide NPs powder were used as controls. In a clean dry glass jar, the powder and liquid was thoroughly mixed well with a clean spatula, then the jar was covered until the mixture reached the dough stage. As the acrylic resin reached the dough stage, the resin was removed from the jar, rolled on the metallic mold and then packed into the holes previously coated with separating medium. A polyethylene sheet was used as separating medium between the upper and lower flask during the initial flask closure in a hydraulic press under 1 bar pressure. [21] The flask was removed from the press, opened carefully, then the polyethylene sheet was removed, the

PMMA resin excess was trimmed with a sharp wax knife, at this stage. After completing the curing cycle, the flasks were allowed to cool down slowly in the water bath for 30 min then removed from the water bath and allowed to be cool at the bench before deflasking. Then all specimens were carefully deflasked and cleaned, flashes of acrylic were removed with an acrylic bur to get a smooth surface. The specimens were grounded with silicon carbide papers with continuous dipping in water for cooling. Polishing was accomplished by using bristle brush and rag wheel with pumice in lathe polishing machine. A gloss surface was obtained by using chamois buff and polishing soap on dental lathe using low speed (1500 rpm) with continuous dipping in water to avoid overheating which may lead to distortion of the specimens. They were measured with electronic digital caliber to obtain the standard dimensions of all zinc oxide NPs-loaded resin disks (5.0 mm diameters, 0.6 - 0.7 mm thickness). These zinc oxide NPs-loaded resin disks were used in the antibacterial activity.

j. PMMA Loaded with Selected Chemicals Disc Preparation

The procedure mentioned in above section (**i**) were followed for varying amounts of potassium oxalic acid dihydrazide *bis*dithiocarbamate (in 200 µL distilled water) and phenytoin (dissolved directly in the monomer), 0.5 - 40 mg/mL .

k. Effect of Zinc Oxide NPs Loaded Resins on Viable Count of *Streptococcus mutans*

The following method was used to compare the antibacterial properties of ZnO NPs -loaded resin discs in a direct contact test. *Streptococcus mutans* bacterial suspension in brain-heart infusion (BHI) broth with concentration of 0.5 McFarland were prepared (1 mL of this solution contains approximately 1.5×10^8 bacteria). Based on a pilot study, since a visual method was used to count bacteria, in order to decrease the number of bacterial colonies and make it easy to count them, 0.5 McFarland suspension was diluted 1,000 times to achieve a concentration of 1.5×10^5 bacteria in 1 mL. A sampler was used to place 0.01 mL of the bacterial suspension on the surface of the disc samples. Then the samples containing bacterial suspension were incubated in an incubator for 1 hour at 37℃ to vaporize the water. The samples were placed in test tubes containing sterile 0.5 mL BHI broth and incubated in an incubator for 24 hour at 37℃. After incubation, a sterile sampler was used to retrieve 0.01 mL from each liquid culture media to uniformly spread on MSB agar plates, incubated an aerobically at 37ºC for 48 hour and then aerobically for 24 hour at room temperature. The colony-forming unit per milliter (CFU/ml) was counted. This value from control tubes was considered as the initial count of bacteria. [22]

l. Effect of Oxalic Acid Dihydrazide *Bis*dithiocarbamate and Phenytoin Loaded Resins on Viable Count of *Streptococcus Mutans*

The same procedure mentioned in section (**k**) were followed for varying amounts of potassium oxalic acid dihydrazide *bis*dithiocarbamate and phenytoin.

RESULT AND DISCUSSION

This microorganism grows selectively on MSB agar plates with addition of 0.2 units/ml bacitracin and 20 % sucrose. [23] This medium supported the growth of *Streptococcus mutans* with good suppression of other organisms. Only just 13 isolates obtained for *Streptococcus mutans*, which were identified according to their colony morphology under microscopic, Gram stain, biochemical test, and VITEK 2 system.

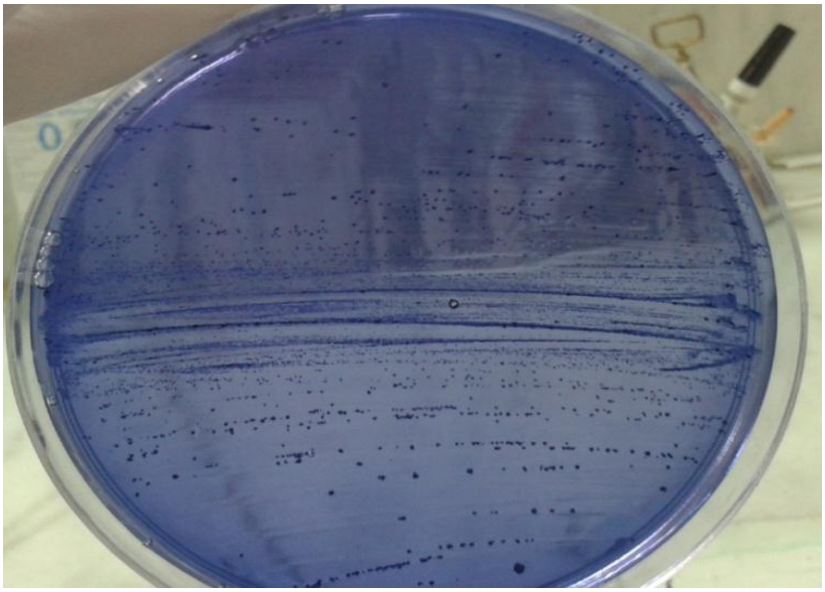

Figure 1: *Streptococcus mutans* on MSB agar.

Streptococcus mutans colonies were further examined and diagnosed to their morphological characteristics on Mitis Salivarius Bacitracin agar plates. *Streptococcus mutans* colonies appeared as spherical or ovoid in shape with raised or convex surface, light blue in color, and about 1 - 2 mm in diameter, colonies adhered well to the agar surface as shown in Figure -1. Microscopic examination of our local isolates of *Streptococcus mutans* were found to be gram positive, spherical in shape, arranged in short or medium length forming chains similar to previously described microorganism. [15]

All the obtained local isolates of *Streptococcus mutans* were found to be catalase negative, and have the ability to ferment the carbohydrate sorbitol similar to the reported isolates. [16] A

positive reaction indicated by change in color from purple to yellow by formation of acid after incubation. [17] The VITEK 2 microbial identification system and GP card available was used for the automated identification of *Streptococcus mutans.* [24] Susceptibility of *Streptococcus mutans* to antibiotics were measured by using disk diffusion

Table-1: Susceptibility of *Streptococcus mutans* to antibiotics.

Antibiotics				
AMP 10 µg/mL	**TET 30 µg/mL**	**ERY 15 µg/mL**	**AZM 15 µg/mL**	**CFT 30 µg/mL**
S	S	S	S	S

* S: Sensitive bacteria.

method (Kirby Bauer test), and the results showed that bacterium isolates were sensitive to erythromycin (ERY), azithromycin (AZM), ceftriaxone (CFT), tetracycline (TET), and ampicillin (AMP) as shown in Table-1. [25]

The antimicrobial activity of azithromycin, zinc oxide NPs and some new *bis*dithiocarbamate viz, potassium L-proline *di*thiocarbamate, potassium oxalic acid *bis*dithiocarbamate, potassium succinic acid *bis*dithiocarbamate, potassium adipic acid *bis*dithiocarbamate [12], L-proline, oxalic acid dihydrazide, succinic acid dihydrazide, adipic acid dihydrazide, and phenytoin B.P [11], on the *Streptococcus mutans* were studied. The bisdithiocarbamate were prepared from the reaction of the corresponding dihydrazide with carbon disulfide in basic medium. The sensitivity of the compounds was measured by using two methods; the well diffusion [19], and the broth dilution methods. [20] In general azithromycin showed very strong bactericidal effect on the *Streptococcus mutans* in both methods as shown in Table-2. All acid dihydrazide

Table-2: Determination MIC, MBC and the killing concentration of the selected chemicals on *Streptococcus mutans*.

Selected chemical	Concentration (molar)					
	4×10^{-3}	3×10^{-3}	2×10^{-3}	1×10^{-3}	0.5×10^{-3}	1×10^{-4}
Azithromycin	K	K	K	K	K	K
Phenytoin	K	K	MBC	MIC	NK	NK
Oxalic dihydrazide	NK	NK	NK	NK	NK	NK
Succinic dihydrazide	NK	NK	NK	NK	NK	NK
Adipic acid dihydrazide	NK	NK	NK	NK	NK	NK
L-Proline	NK	NK	NK	NK	NK	NK
Potassium oxalic acid dihydrazide *bis*dithiocarbamate	K	K	K	MBC	MIC	NK
Potassium succinic acid	K	K	MBC	MIC	NK	NK

dihydrazide *bis*dithiocarbamate						
Potassium adipic acid dihydrazide *bis*dithiocarbamate	K	K	MBC	MIC	NK	NK
Potassium L-proline dithiocarbamate	K	MBC	MIC	NK	NK	NK

K: killing bacteria, MIC: minimum inhibition concentration, MBC: maximum bactericidal concentration and NK: not killing bacteria.

showed no antibacterial effects by both method viz, oxalic acid dihydrazide, succinic acid dihydrazide, adipic acid dihydrazide and L-proline. These compounds have not been reported to have antibacterial activity. The antimicrobial activity measured of these new bisdithiocarbamate by both methods showed a very clear concentration dependency, i.e. higher concentration showed higher effect (highest inhibition zone in mm) in both methods. Potassium oxalic acid dihydrazide *bis*dithiocarbamate showed the highest inhibition zone among its homologues, probably due to the high inorganic character of its molecule as shown in Figure-2a. potassium succinic acid dihydrazide *bis*dithiocarbamate and potassium adipic acid dihydrazide *bis*dithiocarbamate showed similar antibacterial activity, while potassium L-proline dithiocarbamate showed the lowest value of inhibition among their dithiocarbamate. Oxalic acid dihydrazide, succinic acid dihydrazide, adipic acid dihydrazide, and L-proline showed no killing at the used concentrations. Phenytoin B. P showed low antibacterial activity at MIC value at a concentration of 1×10^{-3} molar, due to its low water solubility. Many water soluble phenytoin B.P derivatives showed higher antibacterial activity. [26] The other three *bis*dithiocarbamate showed similar value of MIC, except potassium oxalic acid dihydrazide *bis*dithiocarbamate which showed a value at 0.5×10^{-3} molar. Potassium L-Proline dithiocarbamate showed higher value of MIC at 2×10^{-3} molar. Results showed that *Streptococcus mutans* sensitive 100 % to zinc oxide NPs at concentration (50 to 70) mg/mL. The diameter of inhibition zone increases with increase of the amount zinc oxide NPs, while it was resistant 100% to Zinc oxide NPs at concentrations (5 to 20) mg/ml as shown in Table 3 in Figure-2 (b).

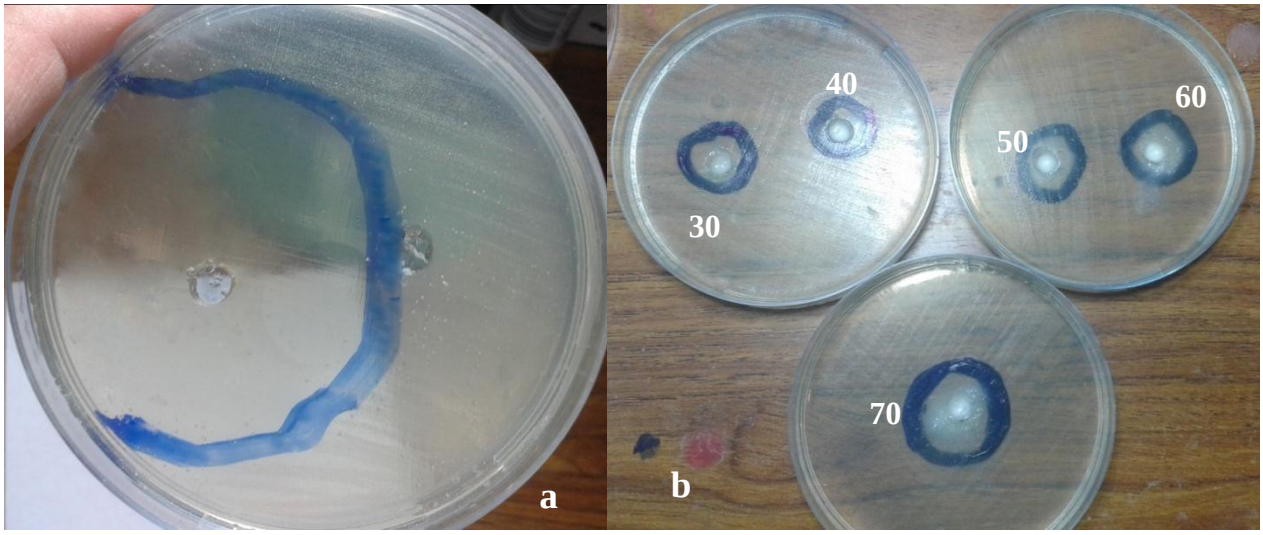

Figure -2: (a) The high inhibition zone of Potassium oxalic acid dihydrazide *bis*dithiocarbamate (left) compared to its precursor oxalic acid dihydrazide (right) which showed no apparent inhibition zone. (b) The inhibition zone of different amount of zinc oxide NPs suspended in water (30 - 70 mg/mL).

Zinc oxide NPs showed much more antibacterial activity in the broth dilution method than in the well method. This is not a surprising result because it is a bite difficult to the nano-particle to diffuse through the agar medium than the liquid culture media, as shown in Table-3.

Azithromycin cannot be used in PMMA resin denature, for it has no thermal stability at 100º C for 45 minutes, included within the manufacturer steps of preparation. The suitability of the selected materials vis, phenytoin B.P and the *bis*thiocarbamates mentioned in Table-2, for use instead of azithromycin in the PMMA resin denature. Acid dihydrazide; Potassium oxalic acid dihydrazide *bis*dithiocarbamate, potassium succinic acid dihydrazide *bis*dithiocarbamate, potassium adipic acid dihydrazide *bis*dithiocarbamate, and potassium L-proline dithiocarbamate as well as L-proline have no antibacterial effect, so they were excluded. Zinc oxide NPs, phenytoin B.P, oxalic acid dihydrazide *bis*dithiocarbamate, succinic acid dihydrazide *bis*dithiocarbamate, adipic acid dihydrazide *bis*dithiocarbamate, and L-proline dithiocarbamate showed antibacterial activity and they all have thermal stability to be used in amalgam and denture. Among the dithiocarbamate is the potassium oxalic acid dihydrazide *bis*dithiocarbamate showed unusually higher antibacterial effect, and will be the focus of this study to be used in PMMA resin denture. Phenytoin B.P showed a good solubility in the liquid monomer component, this can be a reason to obtain a homogenous distribution within denature. An experiment was performed to show the effect of adding azithromycin to the

amalgam formula without changing its physical properties. Different amount of azithromycin (0.8, 1.56, 3.12, 6.25, 12.5, and 25 mg) was added to the silver alloy powder of the amalgam capsule, the capsule was well closed and then the

Table-3: The antibacterial effect of ZnO NPs on *Streptococcus mutans* in different method; well diffusion, zone diameter (mm), broth dilution, and loaded on PMMA resin by broth dilution method.

	Antibacterial effect of ZnO NPs	Amount of, mg/mL							
		5	10	20	30	40	50	60	70
1.	Well diffusion Method Zone Diameter, mm	7.5 (R)	10 (R)	12.25 (R)	12.5 (I)	15 (I)	17.5 (S)	20 (S)	22.5 (S)
2.	Broth dilution method	MIC	MBC	K	K	K	K	K	K
3.	Broth dilution method PMMA loaded with ZnO	NK	NK	NK	MIC	MBC	K	K	K

K: killing Bacteria, MIC: minimum inhibition concentration, MBC: maximum bactericidal concentration. R: Resist bacteria, S: sensitive bacteria, and I: intermediate. The results were interpreted according to the recommendation of (CLSI, 2011).

Table-4: Antibacterial effect of varying amounts of azithromycin in amalgam on *Streptococcus mutans*.

Antibacterial effect on *Streptococci mutans*	Amount of Azithromycin mg					
	0.8	1.56	3.12	6.25	12.5	25
Well diffusion method	S	S	S	S	S	S
Broth dilution method	K	K	K	K	K	K

K: killing Concentration, and S:sensitive bacteria.

mercury was released. The capsule was fitted in the amalgamator apparatus and mixing continues for 20 second to get homogenous amalgam, and the amalgam lump was left for 5 minutes to set into the right solid.

The sensitivity of the *Streptococcus mutans* was determined by well diffusion method. [19] From this experiment it was found that the control amalgam, showed 100 % resistance to amalgam. On increasing the amount of azithromycin made the amalgam much more effective to stop the growth of *Streptococcus mutans*, *i. e*. the bacteria was 100 % sensitive to the amalgam mixture. The diameter of inhibition zone was increased from 18 to 30 mm with increase of the amount of the added azithromycin. This result was interpreted according to the recommendation of. [25] It is difficult to go below the used minimum amount (0.8 mg), because the practical lower balance sensitivity was (± 0.2 mg), and it is not possible to use

dilute aqueous solution within the amalgam batch. On using broth dilution method [20], a lump of the amalgam control and amalgam with above mentioned varying amount of azithromycin were added to different tubes containing Brain-Heart infusion broth. The control amalgam, showed an increase in the turbidity, *i. e* 100 % resistance to amalgam, while all the culture liquid media with azithromycin showed no increase in the

Table-5: Antibacterial effect of varying amounts of Potassium oxalic acid dihydrazide *bis*dithiocarbamate in amalgam on *Streptococcus mutans*.

Antibacterial effect on *Streptococci mutans*	Concentration, mg/capsule							
	0.5	1	2.5	5	10	20	30	40
Well diffusion method	R	R	R	I	S	S	S	S
Broth dilution method	NK	NK	NK	MIC	MBC	K	K	K

K: killing bacteria, MIC: minimum inhibition concentration, MBC: maximum bactericidal concentration. R: Resist bacteria, and S: sensitive bacteria.

turbidity, i.e all of them is at killing amount as shown in Table -4. Similar procedure was used to prepare amalgam with varying amount of Potassium oxalic acid dihydrazide *bis*dithiocarbamate; 0.5, 1.0, 2.5, 5, 10, 20, 30, and 40 mg in the amalgam capsule. It was found effective in the range of 10 - 40 mg/capsule, as shown in Table-5, much higher amount than that present in the solution of MBC value of 1×10^{-3} M, in Table-2. This is not extraordinary, simply for the reason that not all the added amount of Potassium oxalic acid dihydrazide *bis*dithiocarbamate was exposed in the solution.

It is not possible to follow the disc diffusion method to estimate the effect of PMMA resin loaded with varying amount of ZnO NPs, because the effect of nanoparticals is direct surface contact killing. Varying amounts of ZnO NPs were loaded on PMMA resin discs, and inoculated with *Streptococcus mutans* bacterial suspension and the submerged in brain-heart infusion (BHI) broth. From Table-3, no bacterial killing was observed at lower amount 40 mg/mL, compared to the broth dilution method, in same table. This result can be explained on the basis of weaker release of the ZnO NPs, and it seems that its function mainly through direct surface contact. This is in agreement with some other studies that confirmed "contact kill" hypothesis like for the PMMA-based polymeric silver sulfadiazine against *E. coli* and *S. aureus* [27] (Cao *et al*., 2009). Introducing variety of medical tools and elementary form to create surfaces resistance to bacterial adhesion and colonization like burns and traumatic wound dressing, dental work, scaffold, hip prosthetics, wound sutures, artifcial tendons ,surgical masks antimicrobial glass to fight hospital- acquired infection. [28]

Table-6: Determination MIC, MBC and the killing concentration of suspended PMMA resin loaded with varying concentration of phenytoin B.P and Oxalic acid dihydrazide *bis*dithiocarbamate on *Streptococcus mutans* by Broth dilution method.

	Concentration, mg/mL							
	0.5	**1**	**2.5**	**5**	**10**	**20**	**30**	**40**
Phenytoin B. P	NK	NK	MIC	MBC	K	K	K	K
Oxalic acid dihydrazide *bis*dithiocarbamate	NK	MIC	MBC	K	K	K	K	K

K: killing Bacteria, MIC: minimum inhibition concentration, MBC: maximum bactericidal concentration. R: Resist bacteria, and S: sensitive bacteria.

The MIC, MBC and the killing concentration of PMMA resin loaded with varying concentrations of phenytoin B.P, and oxalic acid dihydrazide *bis*dithiocarbamate on *Streptococcus mutans* by Broth dilution method were obtained. The values of MBC were 5 and 2.5 mg/mL for phenytoin and oxalic acid dihydrazide *bis*dithiocarbamate, respectively. These results were very promising for their use as antibacterial materials for loading PMMA resin for denature manufacturing as shown in Table-6.

CONCLUSION

Azithromycin showed very strong antibacterial effect, when mixed with the amalgam components at an amount of 0.8 to 25 mg per capsule, while Potassium oxalic acid dihydrazide bisdithiocarbamate showed antibacterial effect at an amount higher than 10 mg. The other selected chemicals showed 100 % sensitivities, with MIC and MBC of the selected chemicals showed the following values: zinc oxide NPs, 5, 10 mg/mL; phenytoin B.P, 1×10^{-3}, 2×10^{-3} M; potassium L-proline dithiocarbamate, 2×10^{-3}, 2×10^{-3} M; potassium oxalic acid dihydrazide bisdithiocarbamate, 0.5×10^{-3}, 1×10^{-3} M; potassium succinic acid dihydrazide *bis*thiocarbomate,1×10^{-3}, 2×10^{-3} M, potassium adipic acid dihydrazide bisdithiocarbamate, 2×10^{-3}, 2×10^{-3} M respectively. The measured MIC and MBC of the selected chemicals loaded on PMMA resin showed the following values: zinc oxide NPs, 30, 40 mg/mL; phenytoin B.P, 2.5, 5 mg/mL and Potassium oxalic acid dihydrazide bisdithiocarbamate, 1.25, 2.5 mg/mL, respectively.

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