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EFFECT OF ORAL APPLICATION OF KILLED *STREPTOCOCCUS MUTANS* CELLS ON IMMUNOGLOBULIN- A LEVEL IN ALBINO RATS

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ABSTRACT

This study was done to evaluate the effect of killed *Streptococcus mutans* to use as a dental caries vaccine in albino rats. To isolate *S. mutans* saliva was collected from persons suffering from dental caries. All specimens were inoculated on mitis salivarius bacitracin agar, which is a selective medium for isolation of *S. mutans*. All biochemical tests required to identify *S. mutans* were done.To obtain the killed *S. mutans* cells, the organism was cultured on mitis salivarius agar at 37^0c for 72hours. Some colonies were then transferred to a test tube containing 5ml of BHI broth and incubated at 37^0c for 16 hours. Twenty five microliter transferred to another test tube containing 10 ml of BHI broth with 2% sucrose. These tubes were incubated at 37^0c for 22hours and then the bacterial cells were collected by centrifugation. The bacterial cells were washed by normal saline and killed with 10% formalin. Thirty albino rats were bred in a suitable place, specially prepared for this target. The age range of these rats was between 50-60 days and of almost similar weights. The rats were then divided into 5 groups; each group containing 6 rats. Four groups of these were vaccinated using previously prepared vaccine (killed *S. mutans* cells). Immunization has been achieved via oral route. Meanwhile the other group was left not immunized as a control group. Blood and saliva specimens were collected from all groups before immunization and also one week, 2 weeks, and three weeks after immunization. The blood samples were used for a complete blood count by using autohematolyzer (sysmex), whereby saliva specimens were used for *S.mutans* cells count and the measurement immunoglobulin-A level. This study has shown a significant increase in white blood cells' count, which composes the front defense line in the immune system, accompanied by an increase in the immunoglobulin- A level. This increase has led to a substantial decrease in *Streptococcus mutans* cell count. This decrease appeared obviously in three weeks; the thing that proves that the use of killed *Streptococcus mutans* have a great role in protecting teeth against caries, which is mainly caused by *Streptococcus mutans*. It is noticeable, through this study, that there has been a stability in the several components of blood (as for instance the hemoglobin concentration, red blood cells count and the platelets count), which indicates that there are no adverse side effects from the employment of this vaccine. So, it is possible to state that the use of this vaccine can provide sufficient protection against dental cavity.

KEYWORDS: ***Streptococcus mutans***, immunoglobulin-A, dental caries, vaccine.

INTRODUCTION

Dental caries is described as tooth decay. It is an infectious disease which damages the structures of teeth.[1] The disease can lead to pain, tooth loss, infection, and in severe cases death.[2] Dental caries remains one of the most common and wide spread infectious diseases of mankind.[3] Cariogenic microorganisms enter the dental biofilm early in life and can subsequently emerge, under favorable environmental conditions, to cause disease.[4]

Oral diseases, such as dental caries should be considered as consequences of ecologically driven imbalances of oral microbial biofilms. Dental caries is cause by microorganisms belonging to the resident oral micro-flora rather than by classic microbial pathogens. Thus, most individual harbor the microorganisms involved in this disease.[5] The mouth contains a wide variety of bacteria, but only a few specific species of bacteria are believed to cause dental caries: *S. mutans* and lactobacilli are among them.[6] Particularly for root caries, the most closely associated bacteria frequently identified are

lactobacillus acidophilus, *Actinomyces viscosus*, Nocardia spp., and *S. mutans*.[7]

MATERIALS AND METHODS

Biological materials

- Albino Rats (12 rats)
- Rat whole saliva (to measure Immunoglobulin-A and count the bacteria)
- Sheep blood or/and horse blood (and serum) (for enrichment of media) they were fresh and free of antimicrobial agents.
- Glucosyltransferase enzyme (prepared from *Streptococcus mutans*).

Collection of Rats whole- mouth saliva

Saliva was collected from 2 groups of rats before immunization and also one week, two weeks and three weeks after immunization. To collect saliva; concentration of acetyl-choline (0.75mg/ml) were infused at the rate of 0.1ml/minute for 15minutes[8] through the submandibular.[9] Whole saliva was collected with sterile 3ml plastic containers. After collected immediately EDTA was added to a final concentration of 5mM to prevent formation of heterotypic calcium ion-dependent immunoglobulin mucin complexes and to inhibit Ig-A1 protease activity in the sample.[10] The collected saliva was used for bacterial count and measured level of Ig-A.

- Collection of Rats whole blood

Whole blood was collected from all groups of rats before immunization and after its immunization by one week, two weeks and three weeks. Whole blood was collected by capillary tube from the eye of rat in plastic container containing (EDTA). Collected blood was used for complete blood cells count (CBC).

Bacterial strain

Streptococcus mutans obtained from collected human saliva by cultured it on selective media, mitis salivarius bacitracin agar with 10% sucrose. Also further biochemical tests were done to confer the identification of *S.mutans* (*vide* table No: 1).

Primary isolation

Whole –mouth saliva of dental caries human patient was collected and also Whole- mouth saliva was collected from each rat under study. Saliva was streaked directly on Mitis salivarius bacitracin agar (a selective medium for *Streptococci mutans*), incubated aerobically and aerobically with enriched carbon dioxide (Co_2) condition (approximately 5- 10%), at 37 ċ for 24 hours.

Incubation of cultures

All inoculated solid media were incubated aerobically and aerobically with enriched Co_2 at 37 ċ for 24-48 hours. Carbohydrate media were incubated aerobically at 37 ċ for up to 7 days, V.P test media was incubated aerobically at 37 ċ for 48 hours.

Biochemical examinations

These were done by using of biochemical tests, which were performed according to Barrow and Feltham (1993)[11], Ochei and Kolhatkar (2000)[12], and Cheesbrough (2000).[13] (*Vide* table No: 1).

Table (1): Biochemical Reaction of *Streptococcus mutans*:

Character	*S. mutans*
Shape	S
Haemolysis	-/α/β
Required CO_2	+
Growth at 45 ċ	-
Growth in 6.5% Na CL Broth	-
Growth on 40% Bile agar	D
Bile- aesculin test	D
Phosphatase	-
Bacitracin 0.04IU	-
Optochin	-
Hippurate	-
V.P	+
Pyruvate	-
Ribose	-
Mannitol	+
Sorbitol	+
Sucrose	+
Lactose	+
Trehalose	+
Raffinose	+
Inulin	+
Starch	-
Motility	-
Yellow pigment	-

Key: S: Spherical, +: Positive, -: Negative, D: Different strains give different results. V.P: Voges Proskauer test.

Preparation of killed *S. mutans*

Cell free GTF prepared from cultured supernatants of *S.mutans*.[14]

S.mutans was grown aerobically on mitis salivarius agar for 72 hours. The culture was used to inoculate tubes of Brain heart infusion broth (5ml). Twenty five micro litter of a 16 hours broth culture was then inoculated in to 10ml of BHI broth containing 2% sucrose. After 22 hours of incubation, the cells were collected by centrifugation at 8000 rpm for 30 minutes and washed twice with 0.85% saline solution.[15] The cells were killed in 10% formalin. The killed bacterial cells were washed in (PBS) and suspended in PBS to prepare the bacterial cells suspension (10^{10}cells/ml).[16]

Animal immunization: Rats were divided into 2 groups each of which involved 6 rats.

One group of rats was immunized by oral route with already prepared vaccine (killed *S.mutans* cells). In the other side one group of rats was used as a control group without vaccination.

Enzyme – Linked Immunosorbent Assay (ELISA)
Collected Saliva samples from all groups of rats were subjected to ELISA for measurement of immunoglobulin- A. Salivary IgA antibody levels were determined by Enzyme-Linked Immunosorbent Assay (ELISA). Polystyrene microtiter plates were coated with 25 µg/ml GTF (that already prepared from *S. mutans*). Then add rat's saliva (that already collected) to the wells. Then add anti Ig conjugated to an enzyme. The reaction of a substrate with an enzyme to produce a colored product, thus indicating a positive reaction.

Bacterial count (according to Ochei, 2000)
The collected saliva from all groups of albino rats was subjected to bacterial cells count assay to counting *S. mutans* cells.

Complete blood cells count (CBC)
The collected Blood samples from all groups of rats were subjected to the hematological analysis to perform complete blood cells count by using automated hematology analyzer (Sysmex).

Statistical analysis
Collected data were subjected to statistical analysis using Completely Randomized Design (CRD) according to Steel and Torrie, (1960). Table (2) shows the diagram of Analysis of Variance (ANOVA) table of CRD according to Steel and Torrie, (1960). Treatments means were separated to determine the significant differences between them by using Least Significant Differences method (LSD). To check the homogeneity of applied experimental units, Coefficient of Variance (C.V %) was calculated according to Steel and Torrie, (1960),[17] by the formula:

$$C.V\ \% = \frac{\sqrt{E.M.S}}{G.T.mean} X100$$

E.M.S = Error mean square. G.T = Grand total $= \frac{Ex}{n}$

Table (2): Analysis of Variance (ANOVA) Table.

Source of variation	d.f	s.s	m.s	F.cal.	F. tab.	
					5%	1%
Treatments(t)	(t-1)	t.s.s	t.s.s / E.d.f	T.M.S / E.M.S		
Error (E)	(n-1) - (t- 1)	E.s.s	E.ss / E.d.f			

Key: d.f: degree of freedom. s.s: sum of square. M.s: mean square. F. cal: f. calculated. F. tab: F. tabulated.

RESULTS
In the present study, Statistical difference in mean score among the 5 groups of rats was assessed by ANOVA table. Multiple comparisons among groups indicated a significantly different mean score of GTF enzyme in comparison with not-immunized rats. These briefly exhibited as follow:

Effect of Oral Application of Bacterial Cells Vaccine Level of Salivary Immunoglobulin-A (IgA) (µg/ml)
Statistical analysis showed that oral application of bacterial cells vaccine was significantly ($P<0.01$) affected IgA of albino rats (Appendix: 1).

T3 (4.1 µg/ml) treatment recorded significantly higher mean of SIgA level as compared to T0 (1.3 µg/ml), T1 (1.8 µg/ml) and T2 (2.4 µg/ml) treatments (Table: 3). Also T2 had significantly higher mean of IgA as compared to T0 and T1 (Table: 3). Moreover T1 recorded significantly higher mean of this parameter as compared to T0 (Table: 3). The percentage increased of S.IgA under T3 treatment as compared to T0, T1 and T2 was (215.4%), (127.8%) and (70.8%) respectively (Table: 3). Also T2 had increased this character as compared to T0 and T1 by about (84.6%) and (33.3%) respectively (Table: 3). Whereas T1 increased this parameter as compared to T0 by about (38.5%) (Table: 3).

Bacterial Count ($x10^6$/ml)
When comparing the unimmunized normal albino rats with those having orally bacterial cells vaccine, there was statistically significant affected ($P<0.01$) on bacterial count in saliva of albino rats (Appendix: 1).

The third week after vaccination T3 ($0.6X10^6$/ml) recorded lower mean of bacterial count as compared to T0 ($1.31X10^6$/ml), T1 ($1.35X10^6$/ml) and T2 ($1.0X10^6$/ml) treatments (Table: 3). More over T2 showed lower mean of this parameter as compared to T0 and T1 (Table: 3). While T1 recorded lower mean of bacterial count as compared to T0 (Table: 3). T3 showed big differences of bacterial count when compared to other treatments, the percentage of decreased in bacterial number under T3 as compared to T0, T1 and T2 was (54.2%), (55.6%) and (40%) respectively (Table: 3). Also T2 was decreased this parameter as compared to T0 and T1 by about (23.7%) and (25.9%) respectively (Table: 3). Whilst T0 provided decreased on bacterial count when compared to T1 by about (3.0%) (Table: 3).

Table (3): Effect of oral application of Bacterial cells vaccine on some characteristics of albino rats according to duration of administration.

Treatment	B.C(x10^6/ml)	Ig-A	WEI	WBCs	Neu	Lym	Mon	Eas	Bas	RBCs	plts	Hb	Hcv	Mcv	Mch	Mchc
T0	13.1	1.3	117.8	7.3	29.2	65	2.0	3.0	0.8	7.1	762.5	12.9	41.6	58.9	18.2	31.0
T1	13.5	1.8	113.5	6.7	27.5	65.7	2.7	3.3	0.8	7.4	808.2	14.1	44.2	59.4	18.9	31.9
T2	10.0	2.4	132.3	6.5	29.8	66.3	1.3	2.0	0.5	7.5	739.5	14.0	43.5	56.4	18.1	32.0
T3	0.6	4.1	134.3	10.2	29.5	66.0	2.3	1.2	1.0	7.3	784.8	13.2	44.0	60.4	18.1	29.8
L.S.D	2.3	0.042	7.2	2.8			0.9	0.5				0.7				1.4

T0: One week before immunization. T1: One week after immunization. T2: Two weeks after immunization. T3: Three weeks after immunization. L.S.D: Least Significant Different.

Weight of Albino Rats

Appendix (1) showed that weight of albino rats had significantly affected ($P < 0.01$) by bacterial cells vaccine when used orally (Appendix 1).

T3 treatment (134.3 gram) was recorded significant higher mean of albino rats weight as compared to T0 (117.8 gram), T1 (113.5 gram) and T2 (132.3 gram) (Table: 3). Also T2 was provided higher mean of this parameter as compared to T0 and T1 (Table: 3). Whilst T0 had higher mean of this character when compared with T1 (Table: 3). The percentage increased of weight under T3 treatment as compared to T0, T1 and T2 treatments was (14%), (18.3%) and (1.5%) respectively (Table: 3). More over increased of this character under T2 treatment as compared to T0 and T1 treatments was (12.3%) and (16.6%) respectively (Table: 3). While T0 was increased this parameter when compare with T1 by about (3.8%) (Table: 3).

White Blood Cells Counts (WBCsX10^3/cumm)

Statistical analysis showed that oral application of bacterial cells vaccine was significantly affected ($P<0.05$) white blood cells count of albino rats (Appendix: 1).

Table 3 showed that T3 treatment (10.2X10^3/cumm) had significantly higher mean of leucocytes count as compared to T0 (7.3X10^3/cumm), T1 (6.7X10^3/cumm) and T2 (6.5X10^3/cumm) treatments (Table: 3). Whilst T0 had significantly higher mean of this parameter as compared to T1 and T2 (Table: 3). While T1 had higher mean of WBCs as compared to T2 (Table: 3). T3 treatment increased WBCs as compared to T0, T1 and T2 by about (39.7%), (52.2%) and (56.9%) respectively (Table: 3). More over the percentage increased of WBCs under T0 treatment as compared to T1 and T2 was (9.0%) and (12.3%) respectively (Table: 3). While T1 increased this parameter as compared to T2 only by about (3.1%) (Table: 3).

Neutrophiles (%)

As appear in appendix 1 oral application of bacterial cells vaccine was no significantly affected ($P>0.05$) Neutrophiles count of albino rats (Appendix: 1).

T2 treatment (29.8%) had higher mean of Neutrophiles count as compared to T0 (29.2%), T1 (27.5%) and T3 (29.5%) treatment (Table: 3). Also T3 recorded higher mean of this character as compared to T0 and T1 (Table: 3). Whilst T0 was recorded higher mean of this parameter as compared to T1 (Table: 3). The percentage increased of Neutrophiles count under T2 treatment as compared to T3 and T0 only was about (1%) and (2.1%) respectively, while when compared to T1 was (8.4%) (Table: 3). In addition increased of this parameter under T3 as compared to T0 only was about (1%), and as compared to T1 was (7.3%) (Table: 3). While T1 provided decreased in Neutrophiles number as compared to T0 by about (5.8%) (Table: 3).

Lymphocytes Count (%)

Statistical analysis showed that oral application of bacterial cells vaccine was no significantly affected ($P>0.05$) lymphocytes count of albino rats (Appendix: 1).

T2 treatment (66.3%) had higher mean of lymphocytes count as compared to T0 (65%), T1 (65.7%) and T3 (66%) (Table: 3). Also T3 recorded higher mean of this parameter as compared to T0 and T1 (Table: 3). More over T1 appeared higher mean of this character as compared to T0 (Table: 3). T2 treatment had percentage increased of lymphocytes count when compared to T0, T1 and T3 treatments by about (2%), (0.9%) and (0.5%) respectively (Table: 3). Also T3 had increased this parameter as compared to

T0 and T1 by about (1.5%) and (0.5%) respectively (Table: 3). Whilst T1 increased this parameter as compared to T0 by about (1.1%) (Table: 3).

Monocytes Count (%)

Appendix 1 showed that oral application of bacterial cells vaccine was significantly ($P<0.05$) affected monocytes count of albino rats (Appendix: 1).

T1 (2.7%) treatment recorded significantly higher mean of monocytes count as compared to T0 (2%), T2 (1.3%) and T3 (2.3%) (Table: 3). More over T3 showed significant higher mean of this character as compared to

T0 and T2 (Table: 3). While T2 showed lower mean of this parameter as compared to T0 (Table: 3). The percentage increased of monocytes count under T1 treatment as compared to T0, T2 and T3 was (35%), (107%) and (17.4%) respectively (Table: 3). But under T3 as compared to T0 and T2 the percentage increased was (15%) and (76.9%) respectively (Table: 3). Also T0 had increased this parameter as compared to T2 by about (53.8%) (Table: 3).

Eosinophils Count (%)
Oral application of bacterial cells vaccine was significantly (P<0.01) affected eosinophils count of albino rats (Appendix: 1).

Table 3 showed that T1 (3.3%) had significantly higher mean of eosinophils count as compared to T0 (3%), T2 (2%) and T3 (1.2%) (Table: 3). More over T0 had significantly higher mean of this character as compared to T2 and T3 (Table: 3). Also T2 had higher mean of this parameter as compared to T3 (Table: 3). Eosinophils count was increased under T1 treatment as compared to T0, T2 and T3 treatments by about (10%), (65%) and (175%) respectively (Table: 3). Also T0 was increased this parameter as compared to T2 and T3 by about (50%) and (150%) respectively (Table: 3). While T2 was increased this character as compared to T3 by about (66.7%) (Table: 3).

Basophiles Count (%)
Statistical analysis showed that oral application of bacterial cells vaccine was no significantly (P>0.05) affected basophiles count of albino rats (Appendix: 3).

Although there were slightly variance of basophiles count but T3 (1%) had higher mean of basophiles count as compared to T0 (.8%), T1 (.8%) and T2 (.5%) (Table: 3). In addition T0 and T2 which had equal number had higher mean of this parameter as compared to T2 (Table: 3). The percentage increased of basophiles number under T3 as compared to both T0 and T1 was (25%) and as compared to T2 was (100%) (Table: 3).whilst under T0 and T1 as compared to T2 was (60%) (Table: 3).

Red Blood Cells Counts ($X10^6$/cumm)
Red blood cells count before and after oral application of bacterial cells vaccine in albino rats showed there was no significantly (P>0.05) affected (Appendix: 3).

T2 ($7.5X10^6$/cumm) had higher mean of red blood cells count as compared to T0 (7.110^6/cumm), T1 (7.410^6/cumm) and T3 (7.310^6/cumm) (Table: 3). Also T1 had higher mean of this character as compared to T0 and T3 (Table: 3). While T3 had higher mean of this parameter as compared to T0 (Table: 3). The percentage increased of red blood cells count under T2 treatment as compared to T0, T1 and T3 was (5.6%), (1.4%) and (2.7%) respectively (Table: 3). Meanwhile T1 increased this parameter as compared to T0 and T3 by about (4.2%) and (1.4%) respectively (Table: 3). Whilst T3 was increased this character as compared to T0 by about (2.8%) (Table: 3).

Platelet Count ($X10^3$/cumm):
Statistical analysis showed that oral application of bacterial cells vaccine was no significantly (P>0.05) affected platelets count of albino rats (Appendix: 1).

T1 treatment (808.2 $X10^3$/cumm) recorded higher mean of platelets count as compared to T0 (762.5% $X10^3$/cumm), T2 (739.5% $X10^3$/cumm) and T3 (784.8 $X10^3$/cumm) (Table: 3). Also T3 had higher mean of this parameter as compared to T0 and T2 (Table: 3). In contrast T2 had lower mean of this character as compared to T0 (Table: 3). The percentage increased of platelets count under T1 as compared to T0, T2 and T3 treatments was (6%), (9.3%) and (3%) respectively (Table: 3). In addition the percentage increased of this character under T3 as compared toT0 and T2 was (2.9%) and (6.1%) respectively (Table: 3). Whilst T2 was decreased this parameter as compared to T0 by about (3%) (Table: 3).

Measurement of Hemoglobin Concentration (g/dl)
Appendix 1 showed that oral application of bacterial cells vaccine was significantly (P<0.01) affected hemoglobin concentration of albino rats (Appendix: 1).

Table 3 showed that T1 (14.1g/dl) had higher mean of hemoglobin concentration as compared to T0 (12.9g/dl), T2 (14g/dl) and T3 (13.2g/dl) (Table: 3). Moreover T2 had higher mean of this character as compared toT0 and T3 (Table: 3). Whilst T3 had higher mean of this parameter as compared to T0 (Table: 3). T1 treatment provided percentage increased of hemoglobin concentration as compared to T0, T2 and T3 by about (9.3%), (.7%) and (6.8%) respectively (Table: 3). Also the percentage increased of this parameter under T2 as compared to T0 and T3 was (8.5%) and (6.1%) respectively (Table: 3). While T3 had increased this parameter as compared to T0 by about (2.3%) (Table: 3).

Packed Cell Volume (Hematocrite) (PCV %)
Statistical analysis showed that oral application of bacterial cells vaccine was no significantly (P>0.05) affected PCV of albino rats (Appendix: 1).

T1 treatment (44.2%) had higher mean of PCV as compared to T0 (41.6%), T2 (43.5%) and T3 (44%) (Table: 3). Also T3 recorded higher mean of this character as compared to T0 and T2 (Table: 3). Moreover T2 had higher mean of this parameter as compared to T0 (Table: 3). There was slightly percentage increased of PCV under T1 as compared to T3 (.5%) and also when compared to T0 and T2 was (6.3%) and (1.6%) respectively (Table: 3). T3 had percentage increased of PCV as compared to T0 and T2 by about (5.8%) and (1.1%) respectively (Table: 3). Also T2 increased this character as compared to T0 by about (4.6%) (Table: 3).

Mean Cell Volume (MCV/pg)
Oral application of bacterial cells vaccine was no significant (P>0.05) affected mean cell volume of albino rats (Appendix: 1).

Table 3 showed that T3 (60.4pg) had higher mean of MCV as compared to T0 (58.9pg), T1 (59.4%) and T2 (56.4%) (Table: 3). Moreover T1 recorded higher mean of this parameter as compared to T0 and T2 (Table: 3).in contrast T2 had lower mean of this character as compared to T0 (Table: 3). T3 gave percentage increased of MCV as compared to T0, T1and T2 by about (2.5%), (1.7%) and (7.1%) respectively (Table: 3). Whilst the percentage increased of this character under T1 as compared to T0 and T2 was (.8%) and (5.3%) respectively (Table: 3). In opposite of all above T2 had decreased this character as compared to T0 by about (4.2%) (Table: 3).

Mean Cell Hemoglobin (MCH ft)
Statistical analysis showed that oral application of bacterial cells vaccine was no significantly (P>0.05) affected MCH of albino rats (Appendix: 1).

Table 3 showed that there is slightly differ in MCH under all treatments. T1 (18.9ft) had higher mean of MCH as compared to T0 (18.8ft), T2 (18.1ft) and T3 (18.1ft) (Table: 3). Whilst T2 and T3 which provided equal results had lower mean of this character as compared to T0 (Table: 3).

The percentage increased of MCH under T1 as compared to T0, T2 and T3 was (3.8%), (4.4%) and (4.4%) respectively (Table: 3), on the other hand T2 and T3 had decreased this parameter as compared to T0 by about (.5%) (Table: 3).

Mean Cell Hemoglobin Concentration (MCHC %)
Appendix 1 showed that oral application of bacterial cells vaccine was significantly (P<0.05) affected MCHC of albino rats (Appendix: 1).

T2 (32%) treatment had significantly higher mean of MCHC as compared to T0 (31%), T1 (31.9%) and T3 (29.8%) treatments (Table: 3). Moreover T1 had significantly higher mean of this parameter as compared to T0 and T3 (Table: 3). In contrast T3 had significantly lower mean of MCHC as compared to T0 (Table: 3). The percentage increased of MCHC under T2 as compared to T0, T1 and T3 was (3.2%), (.3%) and (7.4%) respectively (Table: 3). While T1 had increased this parameter as compared to T0 and T3 by about (2.9%) and (7%) respectively (Table: 3). But T3 had decreased this character as compared to T0 by about (3.9%) (Table: 3).

Appendix (1): Mean square values showed the effect of oral application of Bacterial cells vaccine on some characteristics of albino rats according to duration of administration.

Source of variation	D.F	B.C	Ig-A	wei	WBCs	Neu	Lym	Mon	Eos	Bas	RBCs	plts	HB	Hcv	MCV	MCH	MCHC
Treatment	3	217.4 **	8.78 **	647 **	17.7 *	6.4 n.s	1.9 n.s	1.9 *	5.8 **	0.3 n.s	0.2 n.s	5213.9 n.s	143.7 **	8.6 n.s	17.6 n.s	0.9 n.s	5.8*
Error	20	3.6	0.013	36	5.3	22.4	23.7	0.5	0.2	0.2	0.2	16442.1	0.3	5.6	11.2	0.9	1.3
C.V%		20	4.8	4.8	29	16	7	34	19	50	5	17	4	6	6	5	4

n.s: N0 significant effect.
*: Significant effect at 5% level of probability.
**: Significant effect at 1% level of probability.

DISCUSSION
In this study, the oral application of killed *Streptococcus mutans* cells is significantly reduced the number of bacteria in saliva of albino rats. Sharply reduce occur after 3weeks of immunization. Although the application of GTF enzyme provide significantly reduce in number of bacteria in saliva of rats, oral application was appear is a good rout. This result was supported by studies done by Mesteky, *et al* (1999),[18], when reported that historical aspects of mucosal immunology and Loesche, (1986),[19], when demonstrated the Role of *Streptococcus mutans* in human dental decay[19] in opposite disagree with that study done by Lehner. (1992),[20], Immunology of dental caries, Immunology of oral diseases.

Immunoglobulin-A was increase after one week and sharply after 3weeks of oral application of killed *S.mutans* cells. In agreement with study done by Smith, and Taubman, (1997), vaccine against dental caries infection.[21]

Also the oral application of killed *S.mutans* cells vaccine increase total leucocytes count after 3weeks of immunization as incase of intranasal application of GTF enzyme which increased leucocytes count after 3weeks and contrary to oral application of GTF enzyme which increased leucocytes count only after 2 weeks.

Neutrophils and lymphocytes count shown no differences in their number during all times of administration while monocytes recorded slightly increased after one week and then fall down. In contrast easinophils count shown reduces in number after 2 weeks and 3 weeks, whilst basophils shown slightly increased after 3 weeks.

In this study the oral application of killed *S.mutans* cells vaccine had no change on the red blood cells and platelets counts. Hemoglobin concentration and MCHC were giving various results that mean they were not influence by killed *S. mutans* cells. PCV and MCV were slightly increased. Whilst MCH was shown slightly increase after one week and then decreased after 2 and 3 weeks.

RECOMMENDATION

- The development of vaccine against dental caries involves identification of appropriate antigens of *Streptococcus mutans* against which protective immune responses can be mounted.
- Selection of the method of immunization that will generate sustained levels of saliva antibodies.
- The next studies should be come to improve this vaccine and search for knowledge if there is any complication from removing normal flora *S. mutans* from the mouth.
- The presence of large number of *S. mutans* as a normal flora in mouth of rats need more studies.
- This study has been never completed yet.

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