Materials and Methods

This study is a prospective study including 65 recently extracted teeth from patients attending outpatient clinic (O.P.C) of the National Institute of Diabetes and Faculty of Oral and Dental Medicine (Cairo University) from orthodontic and maxillofacial surgery departments. Patients selected were of age ranging between (20-40) years old (mean age 30 years).

The choice of the groups carried no gender, occupational, social or residential predilection. All patients signed a consent sheet (an approval to be included in this study).

The teeth that had been selected had the following inclusion criteria:

- Sound (no crown or root caries).
- Having no cracked or broken crowns.
- With non-cracked or broken roots.
- Having no root resorption.
- Not restored by filling.
- Not reduced for crowns construction.
- Having no attrition on occlusal surfaces.
- Having no chemical or mechanical erosion in any of their surfaces.

The teeth had been divided into 2 groups:

Group A: (diabetic group)

Forty five (45) teeth extracted from patients having diabetes for at least (8-10) years, taken from the National Institute of Diabetes. The patients selected were not suffering from any other local or systemic disease known to affect the pulpal structure such as advanced periodontal disease or cardiovascular disease.

Group B: (control group)

Twenty (20) sound teeth extracted from healthy persons indicated for extraction, for example orthodontic purposes, taken from orthodontic department in Faculty of Oral and Dental Medicine and from the economic clinic of the National Institute of Diabetes.

Before extraction full medical and dental history had been taken from the patients. Every patient was asked about having habits:

- Smoking
- Bruxism

Thorough clinical and oral examinations were done for each patient. Blood pressure was measured. Teeth were extracted gently using proper forceps, under efficient local anesthesia.

A blood sample was taken from every patient to be tested for:

- Glycated hemoglobin HbA1c (non-diabetics 4.8-6.2%), (controlled diabetics up to 7%)

- Fasting blood sugar (N 70-110 mg/dl)

- Ionized Calcium level (N 1.1-1.3 mmol/l)

- Serum alkaline phosphatase ALP (N 25-135 U/L)

- White blood cells WBC (N4.0-12.0 K/UL)

- Red blood cells RBC (N4.0-6.20 M/UL)

- Platelets PLT (N150-400 K/UL)

- Prothrombin time PTT

- INR

- Hemoglobin HGB (N11.0-17.0 g/dl)

Each test was performed with different technique and device as demonstrated in table (1).

Table (1): Different devices and techniques used to test blood samples

Test	Technique	Device
Glycated hemoglobin	high performance liquid chromatography (HPLC)	D10 device
Ionized calcium in blood	Ion Selective Electrode	Techno system
Alkaline phosphatase	Enzymatic Kinetic	Architect
Complete blood picture (WBC, RBC, PLT, PTT, INR and HGB)		Drew D_3 Line Trade

Decalcification of extracted teeth was done by first widening the apical foramena immediately after extraction with high speed tapered fine ended stone[1], and immersing in 10% formaldehyde solution[2] for 7-10 days; for complete fixation. After that teeth were washed under running water. Decalcification was performed by immersing teeth in 10% formic acid[3] for 3 months. Each tooth was cut longitudinally in cuts of 5µm thickness, and stained using a Hematoxylin and Eosin stain[4].

Histopathological examination of pulp chamber and root canals was done by light microscope[5] under the power 40 and 100 to evaluate:

- Inflammation and fibrosis
- Calcification and stone existence
- Necrosis
- Angiopathy

Inflammation was graded as no, mild, moderate and severe according to **Yaltirik et al (2004)**. Fibrosis was defined as increased fibroblasts and collagen fibers. Calcification was graded as diffuse, complete and stone existence. Necrosis was graded as no necrosis, partial or complete. Presence of vaculation or any combination of findings was noticed. If none of the above mentioned findings were seen, dental pulp was considered normal.

1: FGDiamond Bur, TAIWAN

2: ADWIC (ElNasr Pharmaceutical Chemicals Co.), Egypt

3: ADWIC (ElNasr Pharmaceutical Chemicals Co.), Egypt

4: Mayr's Hematoxylin

5: Leica Microsystems Wetzler, Germany

Measuring the surface area of several sizes of pulp stones was done by an image analyzer computer device, using a software program*. The image analyzer comprises a light microscope, digital camera, colored monitor and microcomputer capable of performing high speed digital image processing. Tracing each stone was done manually with computer mouse, the image analyzer is calibrated automatically to convert the measurement units (pexils) into actual micrometer units (μm^2) as shown in figure (1).
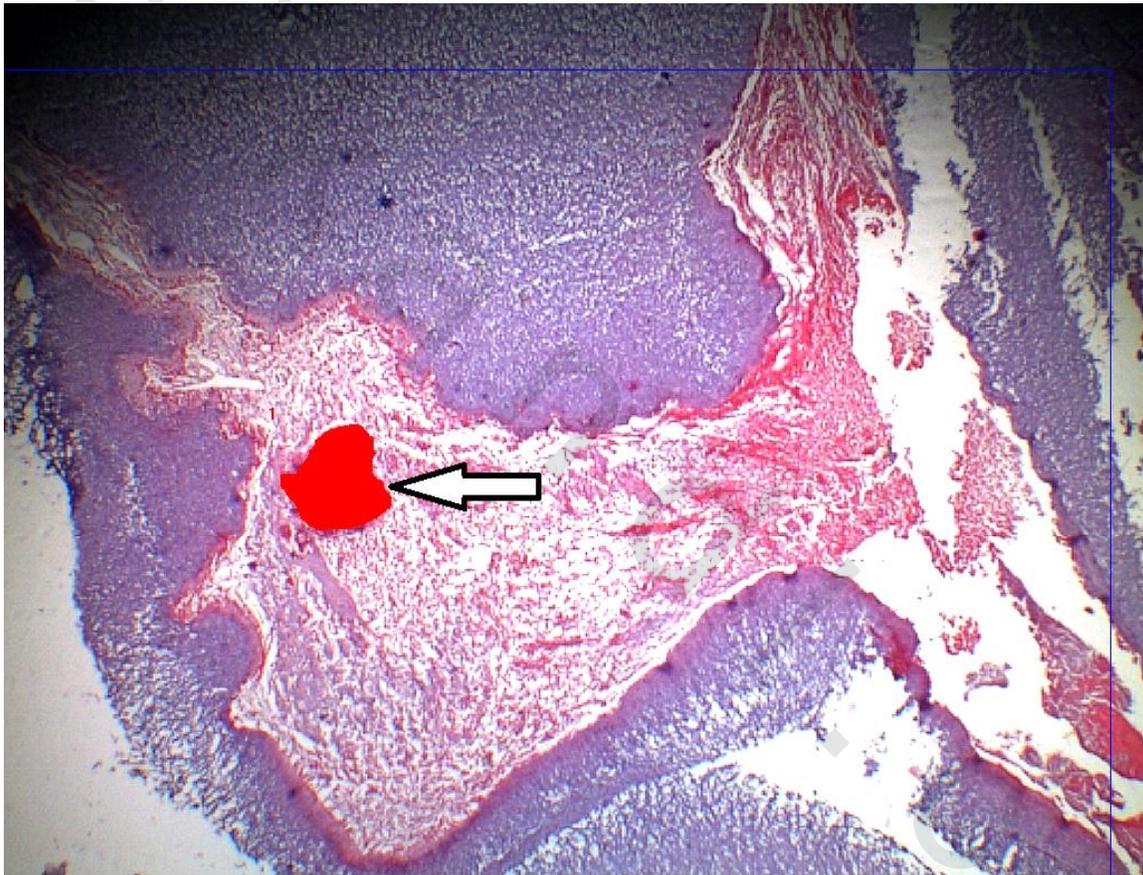

Figure (1): Copy of display seen on the monitor of the image analyzer demonstrating how surface area of stone was done by manual tracing the outline of each stone

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* leica quin 500, Germany

Statistical analysis:

Numerical data were presented as mean and standard deviation (SD) values. All data showed parametric distribution except for ALP and stone areas data which showed non-parametric distribution. For parametric data; Student's t-test was used to compare between the two groups.

For non-parametric data; Mann-Whitney U test was used to compare between the two groups.

Spearman's correlation coefficient was used to determine significant correlations between stone areas and different variables.

Qualitative data were presented as frequencies and percentages. Chi-square (x2) test was used to compare between the groups.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

® IBM Corporation, NY, USA.

® SPSS, Inc., an IBM Company.