Effect of Various Daily Consumption Agents on Tooth Extraction Wound Healing: Radiographic and Histological Experimental Study

Walaa Samir[*1], Mushira Mohamed Abdel- Latif Dahaba[2], Mohamed Ayad Abdel- Hamid[3], Gihan Omar[2] and Amal Hassan[2]

[1]Oral Radiology, Misr University for Science and Technology, Cairo, Egypt
[2]Radiology Department, Faculty of Oral and Dental Medicine, Cairo University, Egypt
[3]Surgery, Anaesthesiology, and Radiology, Faculty of Veterinary Medicine, Cairo University, Egypt
[*]walaa8824@yahoo.com

Abstract: The current radiographic and histopathological study was conducted in an attempt to demonstrate the effect of some irrigants and daily used agents on the healing process of sockets following teeth extraction in Mongrel dogs. Thirty two extraction sites were investigated in eight healthy, mature male Mongrel dogs. Extraction sockets were grouped according to the cleaning mode of each socket into, saline, water, and tea-irrigated sockets. A fourth group acted as a control group and was not irrigated at all and left to heal normally. The sockets were studied and analyzed y histopathologically and radiographically using digital densitometric analysis. The results of this study revealed progression in radiographic bone density during healing of extraction wound with the highest value demonstrated at week 3 after extraction for the saline group, then for the control group at week4.The saline – irrigated sockets demonstrated the highest levels of bone tissue at the end of the study period.
[Walaa Samir, Mushira Mohamed Abdel- Latif Dahaba, Mohamed Ayad Abdel-Hamid, Gihan Omar and Amal Hassan. **Effect of Various Daily Consumption Agents on Tooth Extraction Wound Healing: Radiographic and Histological Experimental Study].** Journal of American Science 2011; 7(12):389-399]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: extraction socket; bone density; irritants; healing of socket wound.

1. Introduction

When a tooth is extracted, the socket is left open to the oral environment until the body can heal the wound. In addition to the known complications of tooth extraction (alveolar osteitis, surgical wound infection, oral-antral fistula, bacteremia), leaving an open wound in the oral cavity also may serve as a portal for serious pathogenic vectors. Knowing the damage that ensues and the potential complications of tooth extraction, modern dentistry is moving toward socket protection and regeneration **(Steiner et al 2008).**

Disturbances in the progression of healing from blood clot to granulation tissue, failure or interference in the mechanism of the granulation tissue development to replace the clots results in disintegration of the blood clot by putrefaction rather than by orderly resorption, giving rise to the well-known symptoms of dry socket **(Lupu 2006)**.

Scientists believe that habitual tea drinking could help preserve bone density - one possible reason for this being tea's high fluoride content **(Souchong 2006).**

Other trials concluded that there is no difference in the infection and healing rates between wounds that were not cleansed at all and those that were cleansed with tap water and other solutions **(Sasson et al 2005).**

That's why this study was undertaken in an attempt to throw some light on whether cleansing extraction wounds with certain irrigants would enhance healing rates.

2. Materials and Methods

Thirty two extraction sites were investigated in eight healthy, mature (about 10-12 months old) male Mongrel dogs, weighing about 12-15 kg each. The dogs were clinically examined to rule out the presence of any disease and kept under clinical observation for three weeks preoperatively and were all fed cooked meat, bread, milk and water. The animals were housed in separate cages, supplied with food and a bucket for water and allowed to live in optimal conditions at the Department of Veterinary Surgery, Faculty of Veterinary Medicine, Cairo University. The cages were sprayed with 6/1000 Neocidal Diazinone **(Diazinon, El Nasr Co.for Pharmaceuticals, Giza, Egypt)** and the dogs were bathed in 1/1000 Neocidal Diazinone. The dogs were injected subcutaneously with Ivermectine **(Ivomec :Merck ,Sharp and Dome,USA)** 0.1 mg /kg of body weight .Dogs that died before reaching their scheduled time of sacrifice were excluded and a substitute animal was prepared and operated instead. After induction of general anesthesia, the oral cavity was cleaned with

Povidone Iodine mouthwash solution **(Betadine, the Nile Co.for Pharmaceuticals and chemical Industries, Cairo, Egypt)**.A sterile plastic syringe was placed between the upper and lower canines after cutting its end to keep the mouth opened during the operation .The operative site was scrubbed with betadine **(Providine Iodine 7.5% w/v, THE Nile Co.forPharmaceuticals, Cairo)**.The operation site was isolated with cotton rolls and the animal was draped in the regular surgical manner. Thirty two premolars (first and third premolars) were extracted from the eight dogs (two form each side). Using a number 11 bard parker blade **(Hu-Fraidy, Chicago, USA)**, an envelope incision was made around the first premolar from the distal side of the canine to the mesial side of the second premolar. Another envelope incision was made around the third premolar from the distal side of the second premolar to the mesial side of the fourth premolar. A mucoperiosteal flap was raised using Woodson elevator (**Hu-Fraidy, Chicago, USA)** to expose the bone surface. Decortications of the alveolar bone **(bucally and lingually)** was achieved by an osteotome (mallet and chisel) **(Keystone, Chicago, USA)**. A double-side carborundum disc mounted on a straight hand piece **(Müller, Germany)** attached to a low-speed motor was used for separation of the bi- roots of the premolars under copious cooling with normal saline till the bifurcation area. This was followed by gentle removal of both roots using a lower remaining root forceps by rational movement of the roots **(Hu-Fraidy, Chicago, USA)**. After extraction of both premolars, the surgical wound was inspected and palpated to determine any areas of sharp bony specules or obvious undercuts . If any existed, a bone rongeur and bone file **(Hu-Fraidy, Chicago, USA)** were used to smooth and properly shape the bony ridge.

Wound closure:

Flap edges were approximated, trimmed and sutured using interrupted 3/0 chromic cat gut **(ProMed, Surgical Suture, Cttrel Ltd, Englewood)**.

Sample Grouping and Application of Irrigants:

The extraction sockets were randomly divided and separated into four groups; each group included eight sockets. One group referred to as Group S was irrigated with1.5 ml of physiologic saline solution (Sterile Nonpyrogenic Sodium Chloride 0.9%) ;this group included the extraction sites of the first left premolars of all dogs, another group was irrigated with 1.5 ml of tap water and referred to as Group W ;this group included the extraction sites of the first right premolars of all dogs, the third group referred to as Group T was irrigated with 1.5 ml of black tea (3 g of black tea; two black tea bags 1.5g each was added to 50 ml of boiling water and was steeped for 15 minutes, then filtered to free the extract from insoluble material; infusion was left at room temperature till it was warm and then used as a wound irrigant) this group included the extraction sites of the third right premolars of all dogs, while the third left premolar sockets were not irrigated at all for all dogs and acted as the control group; Group C. In each group, each alveolus was irrigated once immediately following extraction using a sterile syringe.

Immediate and Follow-up Radiographic Examination

Immediately after surgery and application of irrigants, intra-oral periapical and occlusal radiographs (baseline images) were taken while the animal was still under general anesthesia. Both periapical and occlusal **(Eastman Kodak Ultra-speed films, USA)** films were exposed using the long –cone paralleling technique, i.e. the occlusal film was also used as a periapical film. Fisher **(Fisher Model, Germany)** x-ray machine was adjusted at exposure factors of 60 kVp, 10 mA with exposure time 0.5seconds .The central ray was directed at 90 degrees to the film which was placed parallel to the sockets using a special film holder **(Rinn XCP-film holders, Rinn Co., USA)** which was connected to the x-ray machine by an extension arm and a ring to fix the target-film distance at 75cm during all follow-up periods throughout the study.

A specially fabricated and designed pure aluminum step wedge was constructed of 6 steps with thicknesses of 0.5-3 mm respectively for the periapical radiographs and 8 steps with thicknesses of 0.5-4 mm respectively for the occlusal radiographs .The wedge was attached and fixed on the film during exposure coronal to the teeth using double faced stickers . Radiographs were taken for sedated animals while they were lying on their sides. The animal's heads were positioned 90 degrees to the beam. All films were coded and stored in a refrigerator and then processed together at the end of the sessions using an automatic processor with freshly prepared processing solutions.

Using the same procedures and exposure parameters, consecutive periapical and occlusal radiographs were taken for the immediately pre-sacrificed animals(immediate pre-sacrifice radiographs) and post-sacrifice for the resected mandibles immediately after the sacrifice time (according to the scheduled sacrifice dates assigned

for each group of dogs) (immediate post-sacrifice radiographs) .Two dogs were operated /day (a total 4 surgeries per day).

Postoperative Care:

After operation, the dogs were carried carefully to the recovery room and were kept within confinement and watched carefully till recovery. Then the animals were returned back carefully to their cages. Animals received a combination of Penicllin Strept (2 gram Vet preparation**) (Penicillin G,) (Al Nasr Pharmaceuticals Chemical Co.,Vetwic,Egypt)** and Gentamycnie Sulphate 10% (in dose of 0.5 ml/kg) **(Al Nasr Pharmaceuticals Chemical Co.,Vetwic,Egypt)** which were injected every 12 hours for 7 successive days. Dogs were kept on soft diet for the first postoperative week. On the second postoperative week till the date of sacrifice, they were able to eat the regular diet. All dogs were clinically evaluated on regular daily basis.

Animal Sacrifice:

Eight dogs; two from each group, were sacrificed at day zero (immediately after the first radiographic exposure), 14 days, 21 days, and 30 days after surgery respectively.

Specimen Preparation and Histopathologic Examination:

i- Block samples of the mandibles at the sites of extraction sockets as well as adjacent bone tissues were dissected and resected, and then fixed in 10% neutral buffered formalin solution for 2 days.

ii- After fixation, the tissues were demineralized and embedded in paraffin. Then the biopsies were processed for ground sectioning. Consecutive sections 6 mm thick were cut with a microtome longitudinally in a bucco-lingual plane and parallel to the axis of the central part of the extraction sockets.

iii-The fixed specimens (control and experimental samples) were decalcified in 20% ethylenediamine-tetra-acetic acid **(EDTA)** PH 7 with a change per week for ten weeks until decalcification was completed. Neutral EDTA decalcifying solution consisting of : EDTA (di-sodium salt) 26 gm , distilled water 1750 cm^3 and 26 gm sodium hydroxide was added gradually to adjust the PH of the solution .

iv-The specimens were dehydrated in ascending grades of ethanol, infiltrated in xylene, embedded in paraffin blocks which were sectioned at a 5µm thickness and stained heamatoxylin and eosin stain (H.&E.) by the standard technique (Lilhe and Fulmer 1976) and also by Monson's trichrome .

v-The histological examination was performed with a steriomicroscope (100 X magnification).

Digitization and Analysis of Radiographic Images

Image digitization:

All radiographs were digitized using a digital camera **(Canon, EOS350D, Digital camera 8.0 Megapixels, Japan).** For standardization, all shooting distances and camera settings were kept constant throughout the digitization process. After digitization, all images were stored in a computer's memory to be analyzed at the end of the study period. The obtained images were displayed on the computer's monitor as an array of 512 x 512 pixels with 256 gray levels.

Image analysis:

Digitized images were manipulated using the special software of the Digora system **(Orion Corporation, Soredex, Medical system, Helsinki, Finland).** By this software, radiometric (densitometric) measurements were performed as follows:

1- Densitometric measurements of the sockets:

In an attempt to assess the bone density changes of each of the studied healing sockets before and after sacrifice, a" Region of Interest" (ROI) was chosen to cover the extraction sites. This ROI was assessed for density changes twice using the same software, once as a rectangle (Fig.24) (the dimensions of the rectangle were calculated for the baseline images and the same dimensions were applied for all follow-up images), and another time in the form of successive lines drawn parallel to each other and 1 mm apart starting tangential to the distal lamina dura of the socket under investigation (Fig.25) till the whole socket was covered. The number of lines was determined according to the size of each socket. The mean of the lines was calculated. The mean of the area and line measurements were pooled and included into further statistical analysis

2-Densitometric measurements of the stepwedge:

The procedure was repeated to record the mean gray levels of standardized 16x16 mm area of each step of the two used stepwedges on each periapical and occlusal radiographs.

Measurements were performed by the same radiologist two times at two different sessions with a three-week interval in-between the sessions and the means were then determined and used to calibrate the corresponding density of the investigated sockets.

3-Calculation of bone density:

Using the calibration equation of the step wedge and the mean gray value of ROI, a corresponding gray value was calculated , to present the density of the socket using the following equation (Zlataric et al 2002) :-

Thickness of Step1-X = Density of Step1- Density of ROI

Thickness of Step1-Thickness of Step2 Density of Step1-Density of Step2

Step 1 and step 2 represent: the radiographic density of the two standard steps which correspond closest to that of the osseous tissue, or which exhibited the most consistent results.

X: corresponding value of bone radiographic density.

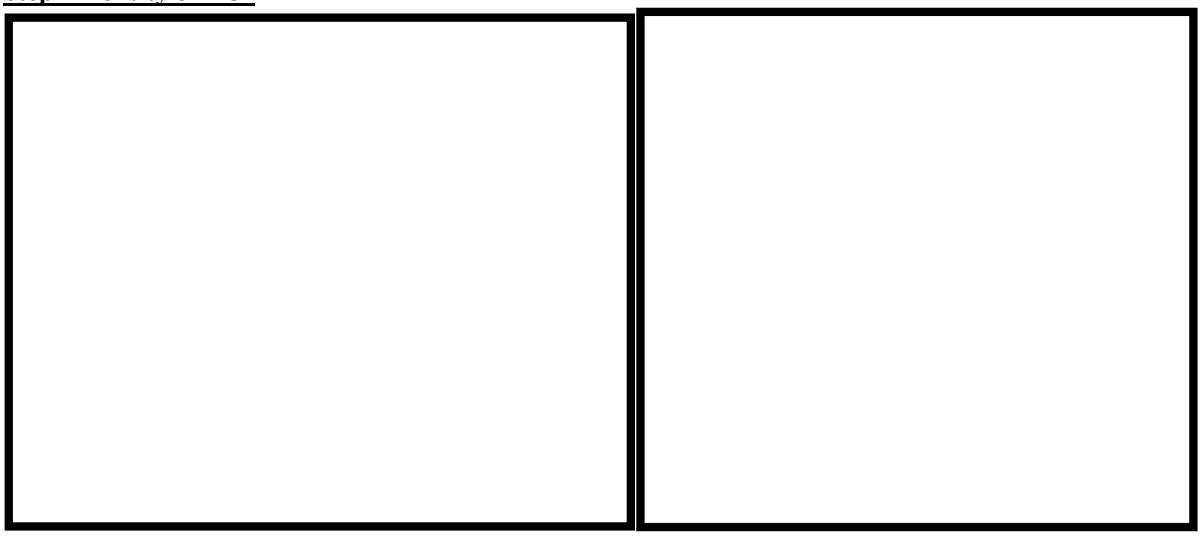

Fig.1: a: A digital radiograph illustrating the area density measurements for one of the investigated extraction sockets and the tool box of the Digora software. b: A zoom –in image for the same case illustrating the density measurements and the calculated mean gray values.

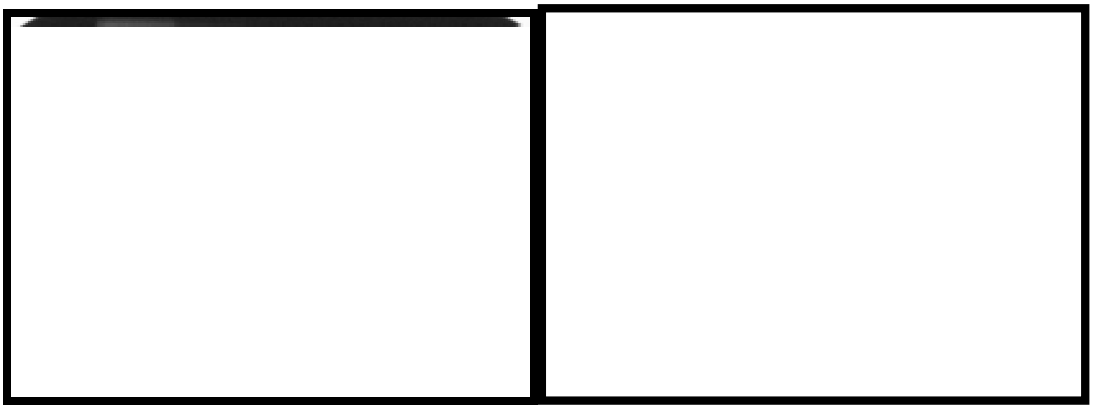

Fig.2 : a:A digital radiograph illustrating the line density measurements for the same extraction socket; upper radiograph is demonstrating 3 lines drawn parallel to each other and starting tangential to the distal lamina dura of the socket of the third premolar. B: radiograph is demonstrating the density measurement along one of these lines.

Statistical Analysis:

The histopathological and radiographic data were recorded and tabulated on an investigative report sheet and transferred to an IBM card ;4Mb RAM, 3Mb free hard disk space " GraphPad InStat ,using IBM pc with windows " Version 3 serial number ("GTA-23456-789") GraphPad Software, Inc., San Diego California USA (Plat et al 2003)

3. Results and Discussion

All experimental extraction sites belonging to the four groups were examined clinically,

radiographically for bone density changes and histopathologically.

Clinical observations of experimental extraction sites

All experimental sites healed uneventfully; showed signs of soft tissue inflammation; swelling and redness during the first two weeks of healing .At the end of the study period, the mucosa covering the extraction sites and the gingival tissues at adjacent teeth appeared to be clinically healthy without depicting any signs of inflammation.

Radiographic observations of experimental extraction sites

Statistical analysis of the radiographic bone density data was performed to compare the bone density changes by time within and between the four studied groups (inter and intra-group comparisons).

Comparison between bone density changes of the four groups (intra-group comparisons) throughout the study period is shown in Table 1.

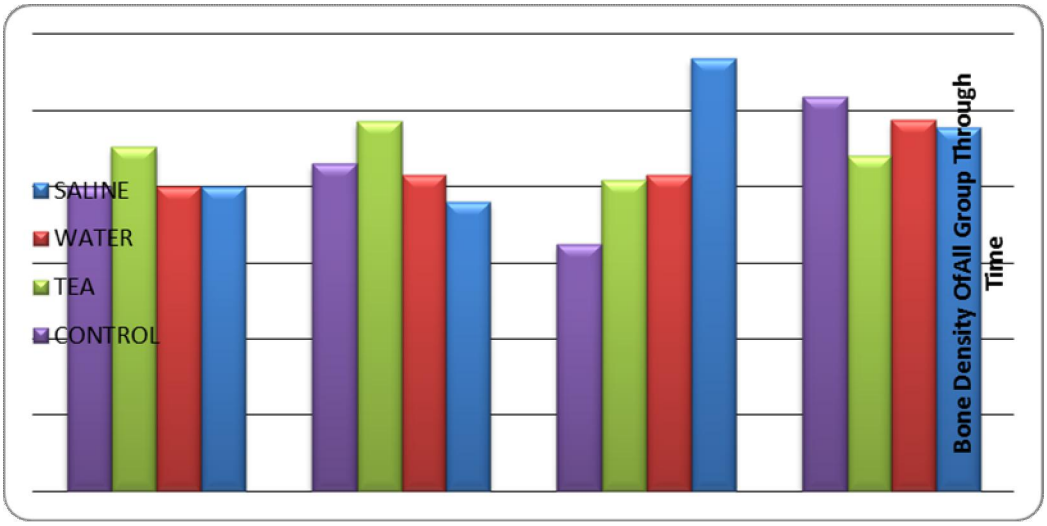

Fig.3: Bar-charts illustrating the intra-group mean bone density changes by time (D= Decrease) (I=Increase) (G= Group) (W=Week).

Table 1. Comparison between bone density changes of the four groups (intra-group comparisons) throughout the study period

Group	Imm-w2	Imm-w3	Imm-w4	W2-w3	W2-w4	W3-w4
Saline	NS-D	**I	**I	***I	***I	NS-D
Water	NS-I	NS-I	***	0	***	***
Tea	***	***	*	***	***	***
Control	*	***	***	***	***	***
Time	**GS-GW**	**S-GT**	**GS-GC**	**GW-GT**	**GW-GC**	**GT-GC**
Imm	NS	NS	NS	NS	NS	NS
W2	***	***	***	***	NS	***
W3	***	***	***	NS	***	***
W4	NS	***	***	***	***	***

From this bar-chart, it could be noticed that the highest mean radiographic bone density at the immediate and W2 intervals was for the tea group. The saline group showed the highest density at W3, while at W4 both saline and control groups showed high density values.

Tooth extraction is a common procedure in dentistry. Wound Cleansing involves the use of a solution that is non-toxic to the tissue to remove debris, wound exudates and metabolic wastes and to promote wound healing (**Fernandez et al 2004**).

Despite significant advances in wound care technology in recent years, very little attention has

focused on the use of cleansing solutions. Since wound care accounts for countless health care, money and practitioner hours each year, it was the main target of this work to assess the effects of certain daily and widely used irrigants on the healing process of the alveolus, bone density of the socket and stability of blood clot of extraction wounds.

With the advent of modern life, the consumption of all kinds of drinks increases but the consumption of tea; specially black tea and tap water shows a much more marked increase among Egyptians. Black tea and tap water are two of the most widely consumed beverages worldwide and local wide in Egypt. Hence, since water and black tea are ubiquitous in the modern diet and inexpensive, the aim of this study was to evaluate the effect of intake of these agents on the early and late stages of bone healing and bone density in extraction wounds.

The choice of tap water as one of the investigated agents in this study is similar to the choice of **Schremmerand Robert 2004** who examined its use to cleanse wounds. They justified their choice to the facts that tap water is advantageous because it is highly accessible, inexpensive, can maintain high pressures right out of the faucet, is chlorinated and monitored for bacterial content through local governments, and has been used throughout the years for minor cuts in homes around the world

Reasons for choosing tea to be one of the target investigated drinks in this work are the reported beneficial effects of tea on the immune function and inflammation. **Hamer and Mark2007** performed a review evidence search on in vitro, animal, and human research .He reported that the habitual tea drinking over several years preserves bone density in both men and women. The key could be the high fluoride content in tea, especially green tea. They believe that other ingredients such as flavonoids and phytoestrogen may also help preserve bone density. Other ingredients in tea may inhibit bone resorption and boost metabolic creation of bone.These reports influenced us to investigate these effects of tea; specially black tea which is much more consumed in our country by most Egyptian citizens in comparison to the green tea which is limited to certain classes of the Egyptian population.

Normal saline is most often preferred for wound care as it is relatively inexpensive, nontoxic to tissues, and does not affect normal skin flora. Sterile 0.9% saline is an isotonic solution. It neither donates fluid nor draws it away from the wound bed . Isotonic solutions do not impede normal healing, damage tissue, cause allergy or alter the normal bacterial flora of the skin that would allow the growth of more virulent organisms (**Griffiths et al 2001**). That's why sterile saline was also investigated as an effective wound cleansing agent in this study.

If water or saline are chosen as wound irrigants, they should be used at body temperature as it usually takes 40 minutes for a wound to return to normal temperature and three hours for leukocyte activity to recover after a dressing change (**Fletcher 1997**). Experiments on humans demonstrated that wound temperature drops significantly at dressing changes, mitosis is inhibited and it takes 40 minutes for a freshly cleansed wound to return to normal temperature and three hours for cell mitotic division to restart **(Lock 1997).** Wound healing occurs at normal core body temperature and a body surface temperature above 33ºC; below this temperature or when it is above 42ºC, wound healing is delayed **(McGuinness 2004)**.Consequently, both water and saline applied in the current work were administered to the animals at normal body temperature of the dog which ranges from 39-40 degrees.

On the contrary, we intentionally administered warm tea during the experiment to the dogs. This choice was undertaken in an attempt to simulate and maintain the temperature of the irrigant to be close to the temperature of the regular daily tea consumed by most Egyptians.

A control group was allowed to heal normally following teeth extraction without the application of any irrigants in this study. Therefore, the purpose of the inclusion of a control group was not to assess the normal healing process of extraction wounds but to investigate whether the use of certain wound cleansing irrigants in the other three groups would enhance or delay the healing process in comparison to the non-irrigated control wounds.

The use of animal models to study phenomena which occur in man is based on the ability of the animal to respond in a manner similar to that observed in humans. Moreover, animal studies expand the capabilities of obtaining and investigating histopathological dynamics of bone healing following tooth extraction.

Few researches in the literature performed quantitative histological measurements to assess bone dynamics of extraction socket healing. For this reason and due to the lack of quantitative data regards bone formation following tooth extraction, both qualitative and quantitative histological observations and measurements were done in the current work.

Durate et al 2009 stated that among various clinical situations, bone density and bone healing

may directly affect bone formation following a dental extraction. In agreement to these reports, radiographic bone density was also assessed in the current work .All calculated density data were correlated to the histopathological quantitative results to reveal the impact of saline, tap water and black tea on healing of extraction sockets.

For assessment of radiographic bone density, the extraction sockets were imaged twice,once using periapical size 2 periapical films and size 4 occlusal films with a step wedge to act as a reference image on the radiographs for standardization of any processing variations during developing of the films. The ranges of the optical densities (OD) of all the steps of the used stepwedge were designed to match with the ranges of optical densities of the bone structures of regions of interest (ROIs) measured on the digitized images. The same method was previously applied by **Bodner et al 1993** who performed a radiologic densitometric study to investigate extraction site healing in rats. They measured socket density with a digital densitometer and related the measurements to the equivalent density of aluminum. They reported that this method was simple, objective and reliable for demonstrating radiographic bone density changes.

Histopathological observations of experimental extraction sites

Qualitative histopathological observations were demonstrated from the heamatoxylin and eosin (H.& E. × 100) and Manson-stained (MASON'S ×100) sections while quantitative results were revealed from the H&E sections only.

Figure (4) Mesio-distal sections illustrating the extraction socket after different intervals of healing: (a) 0 day, (b) 14 day, (c) 21 day, (d) 30 day. (H&E staining ×100) at left side while at (MASON'S ×100) at right side of saline group .

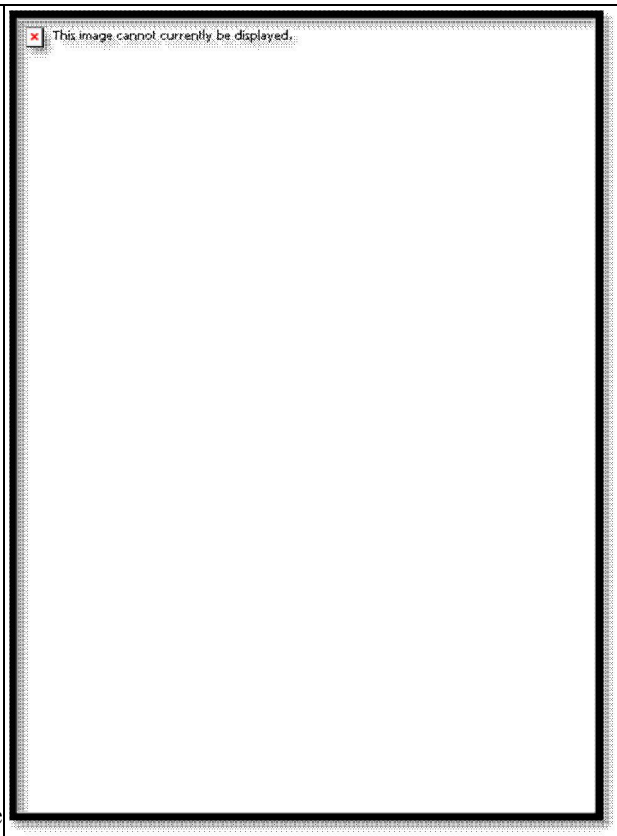

Figure (5) Mesio-distal sections illustrating the extraction socket after different intervals of healing: (a) 0 day, (b) 14 day, (c) 21 day, (d) 30 day. (H&E staining ×100) at left side while at (MASON'S ×100) at right side of water group .

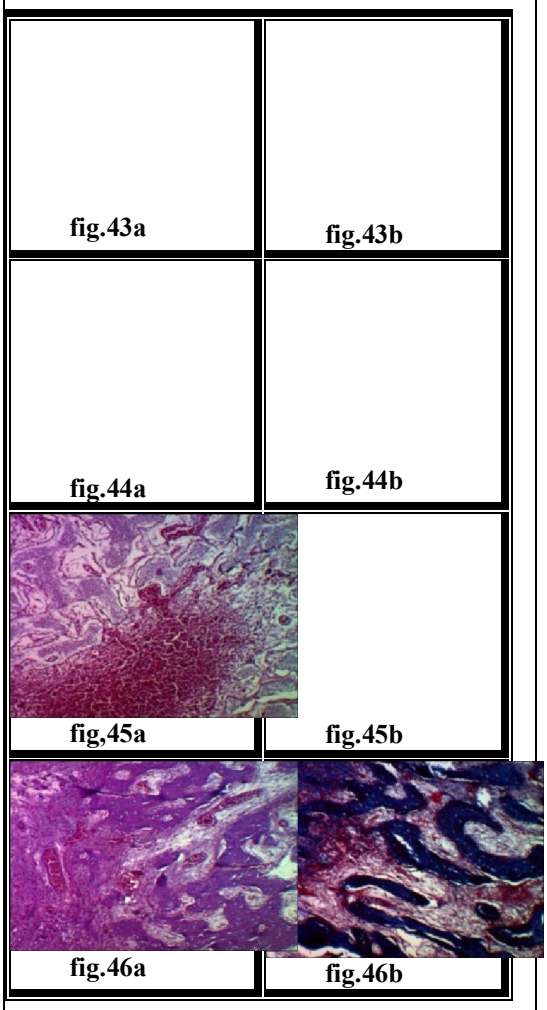

Figure (6) Mesio-distal sections illustrating the extraction socket after different intervals of healing: (a) 0 day, (b) 14 day, (c) 21 day, (d) 30 day. (H&E staining ×100) at left side while at (MASON'S ×100) at right side of tea group.

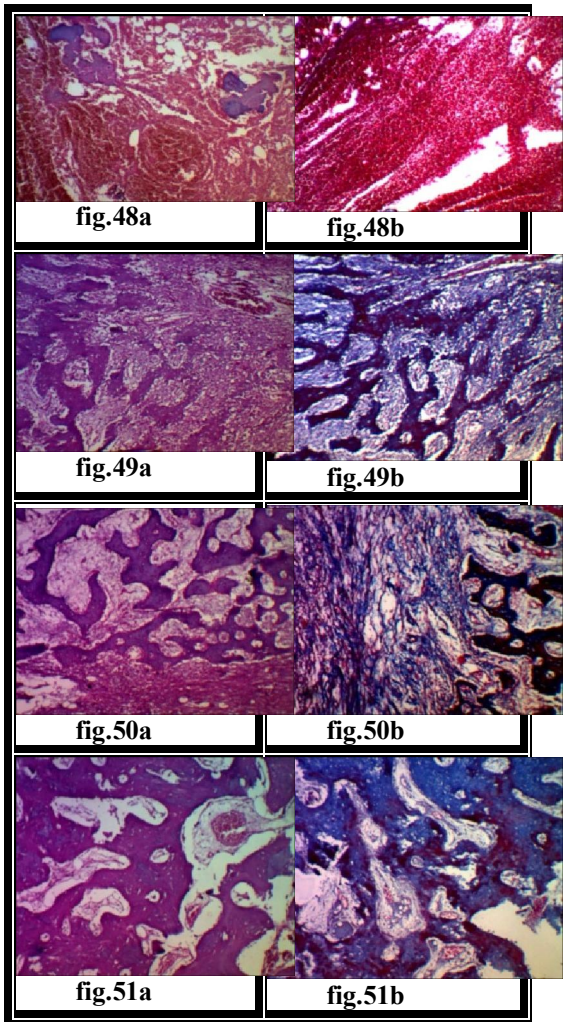

Figure (7) Mesio-distal sections illustrating the extraction socket after different intervals of healing: (a) 0 day, (b) 14 day, (c) 21 day, (d) 30 day. (H&E staining ×100) at left side while at (MASON'S ×100) at right side of control group .

Upon analyzing the histopathological results in the current study, at zero day healing (immediate sacrifice) of the saline group, a well organized blood clot with a bony fragment was demonstarted.The clot showed a prominent aggregation of granulation tissue, formed of premature collagen of bony fragment.In same date specimens for water group, condensing aggregation of granulation tissue in blood clot with bone fragment was seen with minute bony spicules and vascular dilation.

At zero days healing of the tea group, blood clot filled with RBCs and granulation tissue with very minute bony fragment and spicules were seen. Meanwhile, in specimens representing the immediate sacrifice (zero days) of healing for the control group, a well formed and condensed blood clot with many dilated vessels and bony fragment was seen with a condensing aggregation of RBCs and granulation tissue in socket area.

From these results, it could be noticed that the immediate sacrifice specimens confirmed that the sockets were filled with blood coagulum. The clot was highly vascular in the control group followed by the tea group. Bony fragments were demonstrated in the three experimental sockets in comparison to the control sockets which did not show evidence of

bony fragments at zero healing. These results are in concordance with the results of **Sato and Takeda 2007** who reported; from their 12- hours after extraction histological specimens, formation of blood clot due to the bleeding from the remaining periodontium and the gingival tissue.

Two weeks after extraction, the saline sockets showed a newly formed bony trabeculation full with wide osteocytic lacini and condensing aggregation of granulation tissue with marked vascularity. The 2 weeks water sockets showed mild persistence of blood clot aggregation together with thin bone matrix. On the otherhand, the 2 weeks tea sockets demonstrated a newly formed bone matrix aggregation within the persisting blood clot with mature collagen condensation and vascular dilation.Finally ,the control 2 weeks sockets revealed thick bony trabecular aggregation with narrow osteocytic lacini having a calcified center with evidence of new bone formation and granulation tissue aggregation.

These results show histological similarity between the control and saline sockets as both showed evidences of new bone formation which was thicker in the control sockets. Persistance of the clot after 2 weeks was only shown in the water and tea sockets with evidence of thin new bone matrix formation in both sockets. These findings are in agreement with the work reported by **Steiner et al 2008** who found that 7 days after extraction in normal healing sockets, the clot was replaced with granulation tissue which was further replaced after 20 days by collagen and bone at the base and periphery of the extraction socket.

Three weeks after extraction, the saline sockets showed a very thick and uniform network of newly formed bony trabeculae with a very narrow marrow space around center nidus of calcified structure .At the same time interval ,the water sockets demonstrated thick separate bony trabeculae with condensed bone matrix with osteocyte and present aggregation of granulation .After 3 weeks ,the tea sockets showed thick bone matrix trabculate, thin bony trabeculae with newly formed bone with condensed premature collagen formation with persistent condensation of blood clot showing area of congestion .The control sockets after 3 weeks showed thick bony trabeculation with less wider bone marrow space and highly condensing granulation tissue together with premature collagen aggregation (red color) and condensed old collagen.

Our data describes the formation of thick bone and thin bone marrow spaces in both saline and control groups after 3 weeks. Similar results were shown in the water sockets but in addition to formation of granulation tissue which was seen in the control and water groups only. On the contrary, the tea group showed a striking finding which is the persistence of the blood clot together with thick bone matrix and thin bone trabeculae formation.

The saline results at this time interval in our study go in accordance with those of **Kreuger et al 2007** who reported that 21 days after extraction, the sockets treated with saline presented a dental alveolus filled with thick mature trabecular bone in the peripheral area while the central portion of the alveolus showed portions of connective and granulation tissue. The currently presented observation is in agreement with the results of **Kreuger et al 2007** who found that the normal healing process has proceeded similarly in the control and saline groups.

Four weeks after extraction (end of study period), the saline sockets showed a prominent healing of bone with condensed matrix of newly formed bone with prominent number of osteocytes around calcified areas. The water sockets also demonstrated condensing aggregation of highly calcified bony trabculate with normal bone marrow space .The tea sockets again showed different features from the other groups as they revealed aggregation of bone matrix tissue showing newly formed and old formed bone matrix integrated with blood clot tissue. Finally, the control sockets showed the most highly thickened bony trabeculation with narrow marrow space among the four groups.

When our specimens were quantitatively analyzed and compared regarding their composition by time, the area percent through time of all histopathological findings (clot, GT, BM and MB) for the four groups were similar regarding the clot, GT and MB. Greatest values of granulation tissue were recorded at week 2, clot records had the greatest value immediately after sacrificing animals, and mineralized bone recorded the greatest value at week 4. The only difference between the groups was demonstrated with bone marrow which recorded the greatest value at week 4 for the water and control groups and at week 3 for the other two groups. The qualitative and quantitative results were highly correlated .

Cardaropoli et al 2003 reported; according to their histological findings, that the healing of an extraction socket involved a series of events including the formation of a coagulum that was replaced by provisional connective tissue, woven bone, lamellar bone and bone marrow. Further in the process of healing, a hard tissue bridge of bone formed and closed the socket. These findings were similar to previous documented histopathological

studies about normal bone healing. When our quantitative data were statistically analyzed to reveal the effect of each irrigant on the healing process, the saline group results were consistent with Cardarpoli's results. In this group, bone mineralization at the end of the study period was significantly higher than the water group which revealed the second highest values after saline. Tea irrigation resulted in the least bone formation values after 4 weeks from teeth extraction.

Rhea 2009 stated that using water to cleanse wounds may be detrimental because adding water to human cells involved in wound healing results in diffusion that removes dissolved substances from the intracellular fluid. Based on this idea, we hypothesize that the studied water-irrigated sockets in our work that revealed less amounts of bone was due to destruction of the bone tissues by osmosis resulting from water application. Previous results by **Magson-Roberts 2006** are in accordance with our results. They reported that water is a hypotonic solution which under osmosis causes cells to swell and rupture within the tissue. That's why; they recommended the use of saline for cleansing of wounds because it is an isotonic solution that has similar osmotic pressures to interstitial fluid.

Ogur et al 2007 reported that caffeine can cause increased fractures and decreased bone mineral density, and that the acid load due to consumption of cola drinks may have a negative influence on the calcium and bone metabolism. Using this data as a base line reference for the present investigation, the significantly lower values of bone demonstrated in the black tea-irrigated sockets could be attributed in part to the fact that it is caffeine–containing product. **Durate et al 2009** demonstrated in their study that a high daily caffeine intake may disturb the early stages of bone healing.

When the radiographic bone density results were analyzed, they showed similar results to the histopathological results and were highly correlated till week 3 after extraction. At 3 weeks, the saline group showed the highest bone density, a finding which is explained by the highest bone tissues at the histopathological specimens at the same time interval. Yet, at 4 weeks, though the saline group showed a high density value, yet, the control group revealed highest radiographic density values, this finding is not correlating with the histological analysis. This result is attributed to the fact that radiographic analysis provides two-dimensional analysis for the resected mandible; hence the obtained results are collective and relative to the underlying composition and not determining the exact bone content at this specific area as the histological specimens which analyze the tissues in sectional manner not a collective manner.

Conclusion:

It could be concluded that there was a histopathological evidence to support the use of sterile saline over water and black tea in extraction wound healing. The evidence relies on the highest values of bone tissue formation in the saline-irrigated sockets in comparison to the other three tested irrigants. Within the limitations of the present study, this evidence is not strong enough to stick to saline. There is a need for more research in this area to further demonstrate the efficacy of the irrigant.Further evidence from human studies ,which control for potential confounding from lifestyle and biological factors, is therefore required before strong conclusions could be made regarding the association between sterile saline and promotion of bone healing.

Corresponding author
Walaa Samir
Oral Radiology, Misr University for Science and Technology, Cairo, Egypt
walaa8824@yahoo.com

References:

Bonder I, Littner MM., and Jerry Cohen. "Extraction site healing in rats: A Radiologic densitometric study." Pmid Elsevier Inc, 1993: 75 (3) :367-72.

Cardarpoli G, Araujo M, and Lindhe J. "Dynamics of bone tissue formation in tooth extraction sites (an experimental study in dogs)." Journal of The Clinical Periodontology, 2003: 30, 809–818.

-Duartea, poliana mendes, marcelo rocha marquesb, joyce pinho bezerree, and marta ferreira bastosa. "The effects of caffeine administration on the early stage of bone healing and bone density: A Histometric study in rats." Archives of Oral Biology, 2009: 54 (8) 717-722.

Fernandez R, Griffiths R, and Ussia C. "Effectiveness of solutions techniques and pressure in wound cleansing." J.B.I, 2004: 2(1) : 231-270.

Fletcher J: Update: wound cleansing. Professional Nurse, 1997; 12: 11, 793-796.

Griffiths F R and Ussia C: Effectiveness of solutions, techniques and pressure in wound cleansing. JBI Reports.2004; 2, 1. 231-270.

Hamer, and Mark. "The beneficial effects of tea on immune function and inflammation: a review of evidence from in vitro, animal, and human research." Nutrition Research, 2007: 27(7): 373-379.

Kreuger M, Ternes CE , Mello LL , Cruz AB , Leite SNand T ames DR, Kreuger M, Ternes CE, Mello

LL, Cruz AB, and Leite. "The influence of the essential oil of melaluca alternifolia on the healing of infected dental alveoli : a histological study in rats." Brazilian Journals of Pharmacology, 2007: 17(3) : 349-355.

Lock PM."The effects of temperature on mitotic activity at the edges of experimental wounds. Kent: Lock Laboratories Research ,1997 ;1:1-3.

Lupu Alexandera. "Cinical evaluation of post-extraction site wound healing." J Contemp Dent Pract, 2006: 7(3):40-9.

Magson-Roberts S." I s tap water a safe alternative to normal saline for wound cleansing?"J .C ommunity Nursing .2006;20(8):19-24.

McGuinnes W : Influence of dressing changes on wound temperature. Journal of Wound Care, 2004; 13: 9, 383-385.

Ogur, Recai, Uysal B, Ogur T, and Yaman H. "Evaluation of the effect of cola drinks on bone mineral density and associated factors." Journal Compilation Nordic Pharmacological Society, 2006: 100 ,334–338. .

Rhea Paone. "Does tap water decrease wound infection rates as compared to sterile saline?" The Internet Journal of Academic Physician Assistants, 2009: 6(2).

Sato, Hirotaka, and Takeda Y. "Proliferative activity, apoptosis, and histogenesis in the early stages of rat tooth extraction wound healing." Cells Tissues Organs, 2007: 186:104-111.

-Sasson, Comilla, Kennah A, and Diner B. "Wound cleansing- water or saline?" 5, 4 (2005).

Schremmer and Robert. "New Concepts in Wound Management". Clinical Pediatric Emergency Medicine ,20045(4):239-245

Souchong, Llapsang. "Make time for tea." www.allaboutyou.com. 10 31, 2006. (Accessed 5 27, 2011).

Steiner, Gene G, Francis W, Burrell R, and Kallet MP. "The healing socket and socket regeneration." Research Update, 2008: 29(2).

Stübinger S, Leiggener C, Sader R and Kunz C."Intraorbital abscess. a rare complication after maxillary molar extraction". Journal of the American Dental Association 2005; 136, 921-925.

11/21/2011