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Clinical and Experimental Studies of the Effect of Honey on the Healing of the Oral Mucosa

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Abstract: ***In dentistry, post-extraction infections are diverse and uncertain etiology. The management of these conditions is complicated, painful and often costly for the patient and the practitioner. Because honey is a natural product; abundant in our country and Moroccan people has confidence in his virtues, the question arose on the value of honey in the treatment and prevention of post-extraction infections. A protocol for evaluating the effect of honey on the healing of the oral mucosa has been developed and honed by two experimental studies by taking the thyme honey as a reference. In vitro study: An evaluation of fibroblast culture proliferation and cell growth in honey. An evaluation of bacterial growth in honey. Clinical study: Immediate honey installation in alveolar wounds, in patients with apparent good condition and good oral hygiene. Clinical inspection and tissue samples will be made after 21days. The results of our study have shown that the thyme honey has a healing and antibacterial activity of several bacterial strains. These results are consistent with data from the literature. As against the in vitro study showed a cytotoxicity of fibroblast cells in the presence of honey (2% and 10%), which is probably due to high osmolarity of honey.***

Keywords: Honey, antibacterial, wound healing, oral mucosa

1. Introduction

Several plant-based products and medicines have recently appeared on the market as an alternative to industrial products that are accused of their toxic effects, the appearance of resistance and their high cost. Research institutes, public and private laboratories and universities are studying and multiplying research to meet the growing demand for so-called "natural" products. Honey has been used as a skin wound dressing for a long time, in the early 20th century clinical studies on the use of honey in medicine had reached their peak, but around the 40s the introduction of antibiotics decreased this interest. Nevertheless, the emergence of resistance to these and the new trend towards natural remedies have promoted the reappearance of interest in honey. In odontostpmatology, post-extracorporeal infections are diverse and uncertain etiologies, whether in healthy patients or patients with altered states. The management of these conditions is complicated, painful, often expensive for the patient and for the practitioner. Given that honey is a natural product and the Moroccan population has confidence in its virtues, the idea has come to take advantage of the qualities of honey in the prevention of post-extracorporeal complications, whether for medical or economic reasons

2. Material and Methods

Honey preparation

The honey used in this study is Thyme extract marketed under the name of pure and natural Thyme Honey (Les Domaines *, Morocco), it is packaged in 250g sterile glass vials. This honey is sterilized by passing through a 0.22 µm micropore filter (Sartorius).

In vitro study : Cellular culture

Fibroblast culture

The fibroblasts were obtained from primo-cultures of a healthy human gingival tissue from a patient in apparent general good condition. It was taken from the maxillary tuberosity after the patient's consent.

The gingival biopsy was transported in a tube containing HAM'S and placed in a petri dish. Then it was cut into small fragments (0.5 mm2) using a sterile knife blade. 4 CC HAM'S were added to homogenize the fragments during manual shaking of the tubes. The culture medium was then taken with the fragments and placed in a flask of 50 ml. The cultures were maintained for 3 weeks in an incubator (Jouan IG150) at 37 ° C with a humidified atmosphere containing 5% CO2 and 95% air.

During the 3 weeks of incubation, proliferation and morphology of fibroblasts were observed daily by inverted microscope (SWIFT Instruments International S.A.). The culture medium was changed depending on the state of proliferation of the cells.

When the cultured cells became confluent, trypsin was performed and the cells were placed in a 160 ml flask. (Fig1). Cell proliferation was assessed for 14 days using a 40x magnification optical microscope on a Malassez cell. The initial density at day 0 of the cells was 2 × 10 5 cells / ml.

Evaluation de la croissance cellulaire dans un milieu en miel in vitro :

Pour étudier l'effet du miel naturel sur les fibroblastes en culture, on a utilisé deux boites de culture à 6 puits (Fig 2)

- Dans trois puits : 80µl de miel avec 3920µl (3,920ml) de la solution de fibroblastes ce qui fait un totale de 4ml de

solution dans chacun des 3 puits, avec dans chaque puits une concentration totale de miel de 2%.
- Dans trois autres puits : 400µl de miel avec 3600µl (3,6ml) de la solution de fibroblastes ce qui fait un totale de 4ml de solution dans chacun des 3 puits, avec dans chaque puits une concentration totale de miel de 10%.
- Trois puits témoins négatifs contenant uniquement 4ml de la solution de fibroblastes.
- Les cellules ont été mises en contact avec les milieux puis incubées à 37°C dans l'incubateur. L'incubation a été appréciée durant 1 semaine.

Bacteriological test

Fifty different strains of referenced bacteria from the Institut Pasteur du Maroc (IPM) were used for this study. These are Escherichia Coli, Klebsiella Pneumoniae, Staphylococcus aureus, Hemolytic Streptococcus, and Proteus mirabilis. They are stored in Kligler Hajna tubes containing lactose and glucose in a refrigerator at 2-8 ° C.

Honey preparation

A solution of 10% (V/v) Thym honey was prepared with distilled water (Fig 3).

Transplanting

The culture medium specific to each bacterium was inoculated with the corresponding bacterial strain using a swab (Delta Lab s.l.u. 08191 Rubi Euroturbo). Escherichia coli, Klebsiella Pneumoniae and Proteus mirabilis agar plates were stored in a Binder oven at 37 ° C for 18-24 hours. Agar plates seeded with Staphylococcus Aureus and Hemolytic Streptococcus were placed in the agar at 10% CO2 and stored in a Binder oven at 37 ° C for 18 to 24 hours.

Bacterial suspension

For each bacterial strain, colonies were taken from each petri dish (FIG. 4) and placed in a Kligler Hajna tube containing 5 ml of distilled water. The contents were then homogenized by vortexing (Heidolph, Top-Mix 94323, Bioblock Scientific) and the bacterial density measured by a densitometer (Densimat, Biomérieux) was 0.5 µl which corresponded to 108 bacteria / ml. The bacterial suspension obtained was used to seed a microplate of 96 culture wells (12 × 08). (Fig 5)

Susceptibility test

The first well (of the 12) is filled with 40µl of undiluted pure natural honey. The first to the 10th are filled with 40µl of Mueller-Hinton solution and the last two for growth control (bacteria and Mueller-Hinton solution) and sterility control (Mueller-Hinton Solution). 160µl of natural honey suspension (10% V / v) was added to the second well, and the same amount was sequentially transferred from the second well, from one well to another. Then 80 µl of bacterial suspension is added to each tube except the last, which is dedicated to sterility control of the culture medium.

The final concentrations of honey thus obtained are: 3.33%, 2.66%, 2.13%, 1.70%, 1.36%, 1.09%, 0.87%, 0.7%, 0.56% and 25% for the first well after inoculation. The microplates were incubated in micro-aerobic at 37 ° for 24 hours. After 1µl of each tube will be grown on agar (Fig 6), and observed to see the effects of honey on bacterial strains.

Clinical study

This study involved patients in care at the Center for Consultations and Dental Treatment (CCTD) at the Faculty of Dental Medicine of Casablanca, with a sample number of 100 extractions, and a pre-study of 6 extractions.

a) Inclusion Criteria were as follows :
- Patients in good general apparent condition.
- Good oral hygiene.
- Simple extractions.

b) The exclusion criteria :
- Allergy to hive products
- General state altered.
- Use of ATB, anti-inflammatories.
- Poor oral hygiene.
- Smoking.
- Periodontal disease.

c) Surgical time
- Extraction of premolars with orthodontic indications, and a single operator for the realization of the 6 extractions.
- Simple extractions followed by placement of pure honey in 3 cells, using a sterile intramuscular syringe, from the depth to the periphery (Fig. 7), followed by X-sutures with a son of silk. The other three cells were not filled, and the extraction was followed by X sutures with the same type of wires.
- Patients are released with accurate postoperative counseling.
- Clinical control at 1 week:
- Patients were seen again after 7 days for control and removal of sutures.
- Sampling at 21 days :
- A cicatricial mucosa sample was taken at the level of the 6 alveoli and sent to the anatomo-pathological analysis laboratory anonymously. (Fig 8)

3. Results

Clinical study

At the clinical examination at J7 :

The three alveoli treated with honey showed no post-extracorporeal complication, patients reported clinical comfort and operative follow-up almost non-existent. While the three cells remained without dressing: one out of the three had suppurated alveolitis (Fig 9), and another caused post-extracorporeal pain (EVA: 7).

The alveolitis was treated locally with prescription antibiotic (Amoxicillin) and level II analgesic.

On histological examination :

Description 1 is similar for 4 samples, out of the 6 samples submitted anonymously to the laboratory :

Description 1: Squamous mucosa with papillomatous, hyperplastic, acanthotic, moderately orthokeratotic coating. The dermis is fibrous richly vascularized with vessels with well-designed walls and is home to a moderate inflammatory

infiltrate made of lymphoplasmocytes mixed with some histiocytes (Fig 10).

One of the specimens presented (**description 2**) : Squamous mucosa papillomatous, acanthotic, hyperplastic, orthokeratotic. The chorion is fibrous, inflammatory, dissociated by lymphocytes mixed with rare granulocytes around the vascular structures (Fig 11).

The remaining sample showed (description 3) : papillomatous, acanthotic, orthokeratotic, papillomatous, squamous mucosa, locally exulcerated in relation to a highly inflammatory fibrous chorion with an infiltrate made of lymphoplasmocytes and histiocytes. (Fig 12)

By raising the anonymity the description 1 corresponded to the samples at the levels of the three cells with the honey and a control cell. Descriptions 2 and 3 correspond respectively to sites with post-extraction pain and alveolitis.

-Bacteriological test

The bacteria: Escherichia coli, Proteus mirabilis, Staphylococcus Aureus and Hemolytic Streptococcus proliferate in the same way in all concentrations, apart from the first concentration of honey (25%) where they all have an absence of colonization at day 1 . (Fig 13) Klebsiella Pneumoniae shows identical proliferation for all concentrations, the first included.

-Cellular culture

The cells cultured in the presence of honey in six wells: three with a concentration of 2%, and three with a concentration of 10%, have not shown cell proliferation since J1 (Fig 14). While the three control wells (without honey) showed J1 fibroblast proliferation, and it was 5 × 10 5 cells / ml at day 7 (Fig 15)

4. Discussion

In the literature, honey is mainly used as dressing skin wounds, it has an antibacterial effect, antiseptic, anti-oedematous, analgesic superficial; thanks to its high sugar content, its low water concentration, and its consistency in gluconic acid and hydrogen peroxide (1). Honey is also used as a local anti-tumor agent in gastroenterological oncology. (2)

In odontostomatology, post-extracorporeal infections are diverse and uncertain etiologies, whether in healthy patients or patients with altered states. The management of these conditions is complicated, painful, often expensive for the patient and for the practitioner. Thyme honey (Thymus vulgaris), used in this study, is traditionally used to promote sleep. Recognized antiseptic, it is used for the prevention and treatment of infectious, respiratory or digestive diseases. Studies at Limoges University Hospital have shown that it has remarkable properties when used for wound healing. In addition it is very rich in copper and boron. (3)

In the first place we proceeded to the sterilization of the honey by a passage in a micropores filter of 0,22µm. (Sartorius *) The honey thus obtained was used first of all for a clinical study which consisted of the immediate establishment of a dressing with natural honey, in healthy patients having undergone a simple dental extraction. Clinical controls and tissue samples were taken after 21 days.

Our clinical results showed that the honey-treated cells did not show any post-extracorporeal complication, and patients reported clinical comfort and almost non-existent operative follow-up. While the cells remained without a dressing: one presented suppurated alveolitis, and another caused post-extracorporeal pain.

On histological examination: the extraction sites treated with honey had papillomatous, hyperplastic, squamous mucosa. The fibrous dermis richly vascularized with vessels with well-defined walls, seat of a moderate inflammatory infiltrate, which showed a physiological tissue healing.

When glucose (honey) is degraded in the presence of water and oxygen by gluco-oxidase, gluconic acid and hydrogen peroxide (H_2O_2) are formed. Hydrogen peroxide formed has a very important role in the healing process. Indeed, as mentioned above, it is a very good antiseptic. In contact with tissues and blood, it decomposes into water and oxygen ($H_2O_2 \rightarrow H_2O + O_2$), which creates a "microeffervescence" and mechanical cleaning of the wound (debridement). In addition, hydrogen peroxide appears as a real stimulus for cell multiplication as well as for the response to the evolution of normal inflammation during healing. In particular, it stimulates the growth of fibroblasts and epithelial cells that will participate in tissue repair. At the same time, it also stimulates the development of neovascularization in scar tissue. (Descottes B., 2009) (1, 4, 5). Honey also induces collagen synthesis, activates transforming growth factor-1 (which has a powerful healing power); to this is added antioxidant and anti-inflammatory powers. The application of honey on the wound generates, thanks to its hygroscopic properties, a moist environment favorable to all the processes mentioned above. All these mechanisms very favorably activate healing, and make honey a highly effective bio-active dressing (many observations obtained in the service of Professor Descottes, CHU Dupuytren), (Assie B., 2004). (1, 6, 7, 8)

In the context of the in vitro study, the use of priming cultures of human gingival fibroblasts following sampling at the level of the maxillary tuberosity, allowed us to develop a biological test model closer to clinical reality. for a better understanding. In our experience our results showed that honey, even at 2% and 10%, is cytotoxic for gingival fibroblasts, this phenomenon could probably be explained by the important osmolarity of the honey which remains cytotoxic and leads to the bursting of the membranes. cytoplasmic cells in cultures. Other researchers, including Du Toit et al. (2009), introduced absorbent paper disks impregnated with pure honey in petri dishes containing cutaneous fibroblast cultures, the results showed a visible cytocompatibility from 5-7 days around disks, then more and more close to the discs (9). The other part of our experimental in vitro study focuses on the antibacterial activity of honey. Our results showed an antibacterial action, in vitro, of thyme honey on: Escherichia Coli, Staphylococcus Aureus, Hemolytic Streptococcus and

Proteus mirabilis; Klebsiella pneumoniae is resistant to thyme honey. All honeys do not have the same antibacterial activity. At the Laboratory of the Department of Analysis and Research of Haute-Vienne, many studies were carried out, including antibiograms (four strains of bacteria were tested: Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus) to determine more precisely the antibacterial activity of different honeys (1, 10). The principle is to place a culture medium (agar) containing the bacterium to be tested in the presence of one or more honeys and to observe the consequences on the development and survival of it. Depending on the diameter of destruction of the germs, the different bacteria tested were classified: sensitive if the zone of inhibition is greater than 12 mm, moderately sensitive if the zone of inhibition is between 6 and 11 mm, resistant if the zone inhibition is less than 5 mm. In view of the results, the four bacteria tested are all sensitive or moderately sensitive to the various honeys tested. (11,12, 13)

Much research has been done to determine an antibacterial spectrum of honey for application to wounds contaminated with susceptible organisms (1, 12). The most sensitive species are: Streptococcus pyogenes, Staphylococcus aureus, and Escherichia coli. Other species such as Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis, Proteus species, Clostridium welchii, Pseudomonas aeruginosa and Clostridium tetani are also sensitive to honey, but to a lesser extent. While Clostridium oedematiens does not seem to be inhibited by honey. (Assie B., 2004) (14)

In the same direction Lusby et al in 2005 showed that different types of honey have anti-bacterial effects in vitro against several species: Alcaligenes faecalis, Citrobacter freundii, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Mycobacterium phlei, Salmonella california, Salmonella enteritidis, Salmonella typhimurium, Shigella sonnei, Staphylococcus aureus, and Staphylococcus epidermidis. (14, 15)

5. Conclusion

The results obtained in our study proved to be interesting since they make it possible to use honey as a medical product in the preventive and curative treatment of alveolar infections. These results are in agreement with several authors who have shown the antibacterial, analgesic, and healing effects of honey, in addition it will enrich the medical field with new molecules of natural origin.

References

[1] ROSSANT A., LE MIEL, UN COMPOSE COMPLEXE AUX PROPRIETES SURPRENANTES, POUR LE DIPLÔME D'ETAT DE DOCTEUR EN PHARMACIE, CHU Limoges, Thèse Avril 2011, 5- Karpelowsky J, Wound healing with honey--a randomized controlled trial. SAMJ; May 2007, Vol. 97, No. 5.
[2] SWELLAM T., MIYANAGA N., ONOZAWA M., Antineoplastic activity of honey in an experimental bladder cancer implantation model: In vivo and in vitro studies International Journal of Urology (2003) 10, 213–219
[3] Khanal B., Baliga M., Uppal N., Effect of topical honey on limitation of radiation-induced oral mucositis: an intervention study, Int. J. Oral Maxillofac. Surg. 2010; 39: 1181–1185
[4] Medhi B, Puri A, Upadhyay S, Topical Application of Honey in The Treatment of Wound Healing: A Metaanalysis, Vol. 10 No. 4, Oct-Dec 2008
[5] Karpelowsky J, Wound healing with honey--a randomized controlled trial. SAMJ; May 2007, Vol. 97, No. 5.
[6] Moore O.A. et coll; Systematic review of the use of honey as a wound dressing; BMC Complementary and Alternative Medicine (2001) 1:2.
[7] Song J., Salcido MD., Use of Honey in Wound Care: An Update, ADVANCES IN SKIN & WOUND CARE & Vol. 24 No. 1, 2011.
[8] Molan P. C., The Evidence Supporting the Use of Honey as a Wound Dressing, LOWER EXTREMITY WOUNDS 5(1); 2006 pp. 40–54
[9] Du Toit D.F., Page B. j., An in vitro evaluation of the cell toxicity of honey and silver dressings, journal of wound care, vol 18, no 9 , september 2009
[10] Noori S., Al-Waili, Mixture of Honey, Beeswax and Olive Oil Inhibits Growth of Staphylococcus aureus and Candida albicans, Archives of Medical Research 36 (2005).
[11] Mohapatra P., Thakur V., Antibacterial Efficacy of Raw and Processed Honey, Biotechnology Research International, Volume 2011.
[12] Asadi-Pooya A. et coll. The Antimycobacterial Effect of Honey: An in vitro Study Biology Forum 96 (2003).
[13] Henriques A. F, Jenkins R. E, Burton N. F, The effect of manuka honey on the structure of Pseudomonas aeruginosa, Eur J Clin Microbiol Infect Dis, 2010
[14] Lin S, Molan P, Cursons R, The in vitro susceptibility of Campylobacter spp. to the antibacterial effect of manuka honey, Eur J Clin Microbiol Infect Dis (2009) 28:339–344
[15] Sherlock O., Dolan A., Athman R, Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin resistant Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, BMC Complementary and Alternative Medicine 2010, 10:47

Figure 1 : Thyme honey sold in supermarkets

Figure 2: The trypsin of cultured cells of the first passage

Figure 3: Use of 6-well plate for culturing fibroblasts in honey

Figure 4: Preparation of honey dilution at 10% V/v

Figure 5: Colonies were taken from each Petri dish

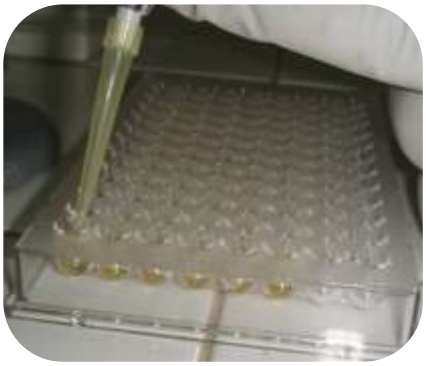

Figure 6: Inoculation of a 96-well microplate with the bacterial suspension and the honey solution.

Figure 7: 1 µl of each well is cultivated on an agar plate.

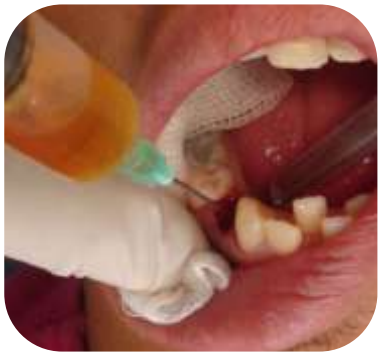

Figure 8: Placement of pure honey in 3 cells, using a sterile intramuscular syringe, from the depth to the periphery.

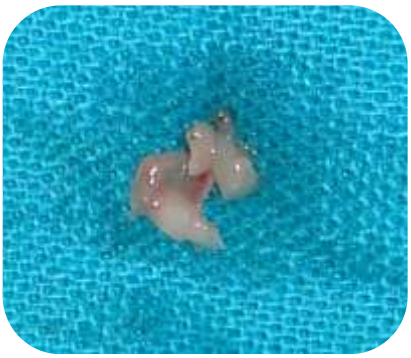

Figure 9: Removal of a fragment of cicatricial mucosa.

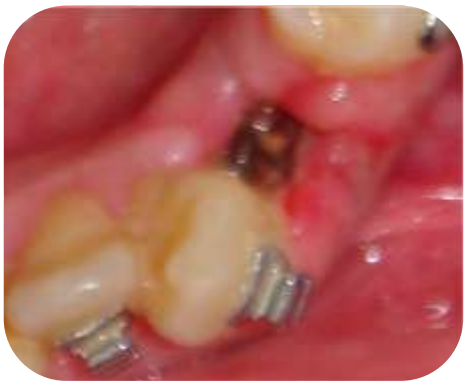

Figure 10: Suppurated alveolitis on an extraction site without honey dressing

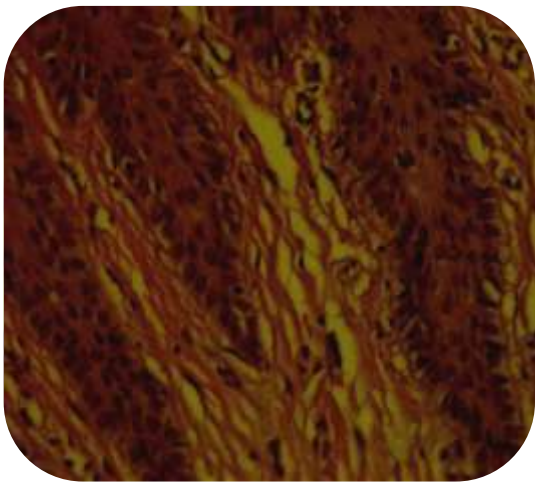

Figure 11: Squamous mucosa papillomatous, hyperplastic, acanthotic, moderately orthokeratotic. The dermis is fibrous richly vascularized with vessels with well-designed walls and is home to a moderate inflammatory infiltrate made of lymphoplasmocytes mixed with some histiocytes. (X40)

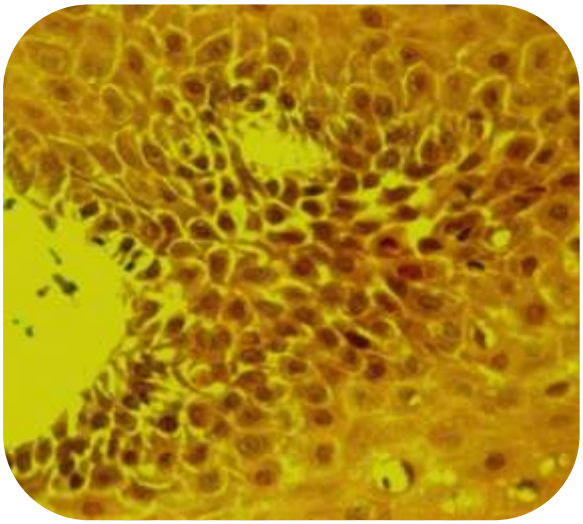

Figure 12: Squamous mucosa with papillomatous, acanthotic, orthokeratotic coating, exulcerated in places opposite a highly inflammatory fibrous chorion seat of an infiltrate made of lymphoplasmocytes and histiocytes. (X10)

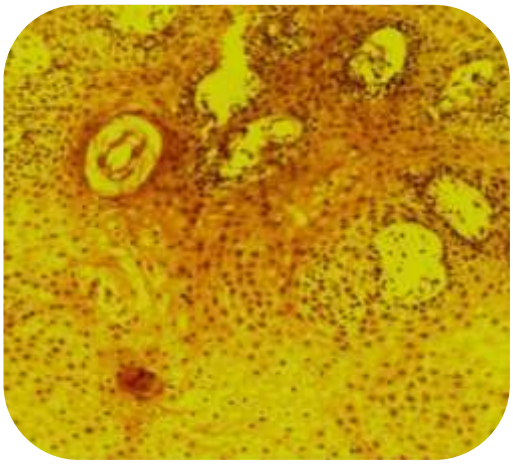

Figure 13: Squamous mucosa papillomatous, acanthosic, hyperplastic, orthokeratotic. The chorion is fibrous, inflammatory, dissociated by lymphocytes mixed with rare granulocytes around the vascular structures. (X10)

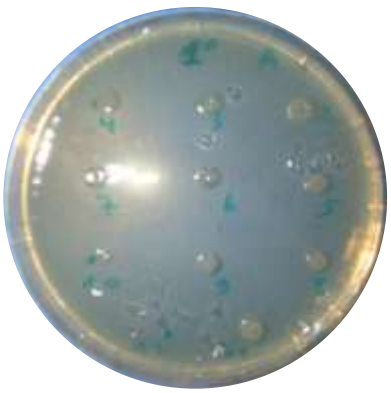

Figure 14 (a)

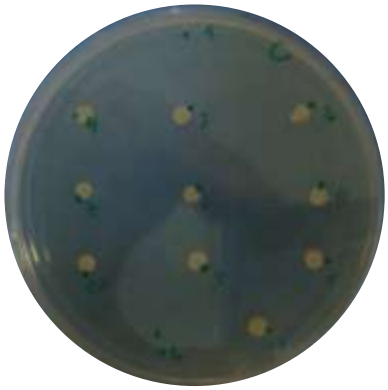

Figure 14 (b)

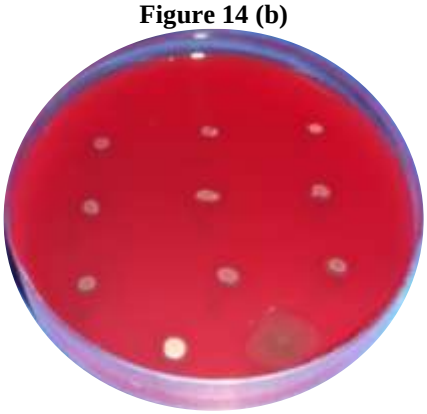

Figure 14 (c)

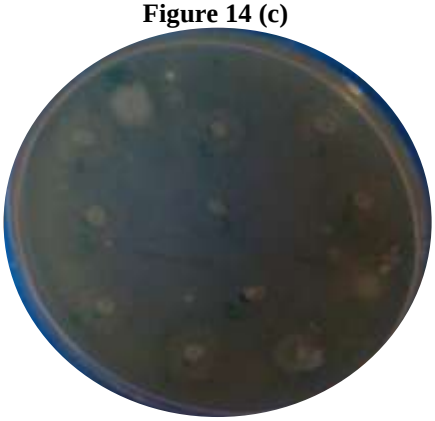

Figure 14 (d)

Fig 14 : Proliferation of a) Escherichia Coli b) Staphylococcus Aureus c) Hemolytic Streptococcus d) Proteus mirabilis, is the same except for the first concentration of honey.

Figure 15: The three control wells showed a proliferation of fibroblasts from 5 × 105 cells / ml to J7.

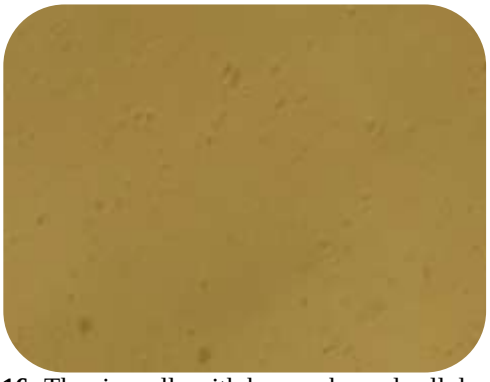

Figure 16: The six wells with honey showed cell death as of Day 1.