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Sterilization of Indian Honey with Cobalt 60 Gamma Irradiation

Kobalt 60 Gama Işını ile Hindistan Balının Sterilizasyonu

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Abstract

Honey has been used since ancient times and its use has reemerged in wound care over the past 2-3 decades. Honey has excellent antimicrobial properties. However, fresh raw honey is contaminated with various microbes and it is often advised to sterilize it before use. Gamma irradiation, which is mode of sterilization accepted globally, was preferred in this study. A study was conducted wherein a sample of freshly obtained honey was initially cultured to determine the microorganisms in it followed by sterilization of the same honey sample with gamma radiation. Post gamma sterilization with 15kGy irradiation, it was noticed that there was no growth of any organism in the honey.

Keywords: Honey, Sterilization, Wound, Gamma Irradiation

Özet

Bal eski çağlardan beri kullanılmaktadır ve son 20-30 yılda yara bakımında kullanımı yeniden artmıştır. Balın mükemmel antimikrobiyal özellikleri vardır. Bununla birlikte taze ham bal çeşitli mikroorganizmlar ile kontamine olmakta ve kullanılmadan önce sterilize edilmesi sıklıkla tavsiye edilmektedir. Bu çalışmada dünya çapında kabul gören sterilizasyon modu Gama ışıması tercih edilmiştir. Taze olarak elde edilen balın bir örneğinin, içindeki mikroorganizmaları belirlemek için ilk olarak kültürlendiği ve ardından aynı bal örneğinin gama radyasyonu ile sterilizasyonunun yapıldığı bir çalışma yürütülmüştür. 15 kGy ışınlama ile gama sterilizasyonu sonrası, balda herhangi bir organizma gelişiminin olmadığını fark edilmiştir.

Anahtar kelimeler: Bal, Sterilizasyon, Yara, Gama Işıması

Abbreviations:

1. INTRODUCTION

With the increasing incidence of diabetic foot being infected with a multi-resistant organisms that has resistance to commonly used antibiotics, there has been a resurgence in the use of honey which now is considered by many as a last resort medicine (Alam et al., 2014; Mandal & Mandal, 2011).

Honey has been used since ancient times for wound healing (Mandal & Mandal, 2011). It is believed that its antimicrobial action is due to various factors including low pH, its ability to generate hydrogen peroxide, increased osmolarity due to high sugar content, and presence of several other natural substances (Alam et al., 2014; Haynes & Callaghen, 2011; Mandal & Mandal, 2011;). The pH of honey is acidic ranging from 3.2-4.5, which restricts the growth of several pathogens (Haynes & Callaghen, 2011; Mandal & Mandal, 2011).The osmotic effect of honey is due to its high sugar content (82.4%) and it includes fructose and glucose mainly (Al-Nahari et al., 2015). Another important factor for its antimicrobial activity in the presence of hydrogen peroxide that is generated by glucose oxidase enzyme (Cooper, 2014). Often, the hydrogen peroxide is of low concentrations and its level in honey is influenced by floral source (Cooper, 2014).

Honey is considered monofloral honey if the bee collects nectar from one type of flower and multifloral/polyfloral honey if it is collected from many types of flowers (Park et al., 2020). Nectar collected by bees from flowers of plants, shrubs, and trees are known as blossom honey whereas those collected by bees' from the secretion of injured plants, shrubs and trees are known as honeydew honey (Cooper, 2014).

Honey is effective against a variety of microorganisms including Staphylococcus aureus, Escherichia coli, Enterobacter, Pseudomonas, etc (Mandal & Mandal, 2011). When it is applied topically, honey rapidly clears infection facilitating wound healing (Mandal & Mandal, 2011). Honey's antibacterial activity potency varies up to a hundred-fold (Molan & Betts, 2004). The honey which is sold in super marketed has no antibacterial activity (Deshpande & Kulkarni, 2010).

For honey to be used as a wound care product, it has to be sterilized because honey is a reservoir for microbes (Olaitem et al., 2007). However, heating is known to destroy the peroxidase activity of honey (Cooper & Jenkins, 2009). Honey is often sterilized with gamma irradiation. It is known as medical grade honey. Different doses of gamma irradiation have been used in different studies to achieve sterility in honey. We conducted this study to know the effect of 15kGy radiation in achieving the sterility of honey.

2. METHODS AND MATERIALS

We used fresh, raw, unprocessed honey that was available at Amit Jain's centre for Apitherapy at Amit Jain's Institute of diabetic foot and wound care, Brindhavvan Areion hospital, Bengaluru, in this study. This honey is obtained from a honey collector, who regularly supplies fresh raw honey at Amit Jain's centre for Apitherapy whenever

required. The Amit Jain's centre for Apitherapy is one of the wings within the Amit Jain's Institute for diabetic foot and wound care that deals with acquisition, sterilization, storage and use of different types of honey on different wounds apart from doing research work on honey. The honey that is studied here was obtained from the giant coomb of Apis dorsata bee which is an Indian rock bee. This raw honey sample that was collected in sterile container (50 ml) was sent to the Department of microbiology laboratory at RRMCH for culture. The honey samples from this container was inoculated onto 5% Sheep blood agar, chocolate blood agar and McConkey agar for aerobic growth, Robertson's cooked meat broth for anaerobic growth incubated for 2 days and 7 days respectively. The honey was inoculated on Sabourauds dextrose agar for fungal growth and incubated at 37ºC and 25ºC for 3 weeks.

This raw honey sample in the same container (50ml) was subsequently sent for sterilization using gamma irradiation. The gamma radiation treatment of honey was carried out in Cobalt 60 gamma chamber-5000 (GC-5000) and the dose used was 15kGy. This gamma chamber roughly delivers 6kGy radiation per hour and it took us around 3 and half hours to sterilize the sample.

3. RESULTS

We noticed that the unsterilized raw honey yielded Bacteroides species in anaerobic culture and Penicillium species in fungal culture.

Post irradiation, the honey sample yielded no growths thereby rendering completely sterile and this medical grade honey was ready to be used.

4. DISCUSSION

Honey, though has antimicrobial activity against many microorganisms, is also known to be contaminated with microorganisms (Migdal et al., 2000). Various bacteria's, molds and yeast have been found in honey. The microorganisms include *Bacillus, Bacteridium, Clostridium, Saccharomyces Aspergillus, Penicillium*, etc (Olaitem et al., 2007). Often contamination happens during the process of honey extraction (Jalali et al., 2007).

The primary source of contamination of honey includes dust, air, flowers, pollens, and the intestine of honeybees whereas the secondary source of honey contamination is the human, containers and equipment (Olaitem et al., 2007). The honey bee intestine contains 27% gram positive bacteria's like *Bacillus, Bacteridium* and *Streptococcus*, around 70% gram negative bacteria and 1% yeast (Al-Waili et al., 2012). Often, spore forming microorganisms are known to survive in honey (Olaitem et al., 2007).

It hence becomes imperative to sterilize honey and gamma irradiation is considered the best sterilization procedure as it does not destroy the antibacterial activities of honey (Jalali et al., 2007). Enough studies exist that show that gamma irradiation does not alter the anti-biofilm activity and antibacterial activity of honey (Horniackova et al., 2017; Jalali et al., 2007; Saxena et al., 2014).

Different doses of gamma irradiation were used to achieve sterility. Postmes et al. (1995) used 25kGy (Cobalt 60) of irradiation to achieve honey sterility. Migdal et al. (2000) observed that after irradiation with 10kGy (linear accelerator), there was a 99% decrease in the microbial count. In a study by Saxena et al. (2014), few honey samples required 15kGy to achieve microbial decontamination of honey.

We also sterilized our honey with 15kGy and it achieved no growth post-irradiation. We noticed that it takes more than 2 hours to achieve this and it is also expensive to gamma sterilize the honey.

5. CONCLUSION

Honey has reemerged as a wound care product with good results in various studies. In clinical practice, medical grade honey which is sterilized with gamma irradiation is often recommended. Gamma irradiation maintains sterility and does not affect the antibacterial action of honey. Our study showed that 15kGy of irradiation was acceptable to achieve sterility. Further studies are needed to determine whether lower doses of gamma irradiation can produce the same sterility.

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