Use of low dose gamma irradiation for honey sterilisation



Aims: To study the effect of low dose gamma irradiation on the sterility of Indian honey. **Methods**: A newly arrived, freshly obtained, raw unprocessed honey, available at Amit Jain's centre for Apitherapy at Amit Jain's Institute of diabetic foot and wound care, Brindhavvan Areion hospital, Bengaluru, was used in this study. This unsterilised honey was cultured in the microbiology laboratory of RRMCH. The honey samples were then subjected to 2.5, 5 and 20KGy gamma irradiation at IIHR, Bengaluru that has the cobalt 60 gamma irradiation chamber facility. **Results**: The unsterilised Indian honey had yielded aerobic spore forming Gram-positive bacilli and bacteriodes species. After irradiation of the three samples with different dose of gamma irradiation, it was noted that honey, which received a dose of 2.5KGy and 5Kgy, grew the same organisms.
Conclusions: This study, which was aimed to determine the sterility with low dose gamma irradiation, found 2.5KGy and 5KGy irradiation doses to be ineffective in achieving sterilisation of Indian honey.

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Most of us who deal with wounds are aware of the role honey can play in wound healing, and today it is used as an accepted antimicrobial agent. Honey has a strong history, in the area of wound healing and it has been used to treat various conditions, including wounds, as early as 2000 years ago (Jalali, 2007; Olaitan and Adeleke, 2007).

Honey has recently regained its popularity as a topical agent in infections, burns and other wounds (Jogdande and Nitave, 2020). The main reason for its resurgence is the increased prevalence of drug resistant microorganisms (Alam et al, 2014). This led to the re evaluation of therapeutic properties of honey (Deshpande and Kulkarni, 2010). Honey is known to have antiinflammatory, antioxidant and antimicrobial activity (Khalil et al, 2012; Alam et al 2014). The antimicrobial activity of honey is believed to be due to its low pH, osmotic effects, as well as, the presence of phytochemical substances and hydrogen peroxide (Pajor, et al 2002; Alam et al).

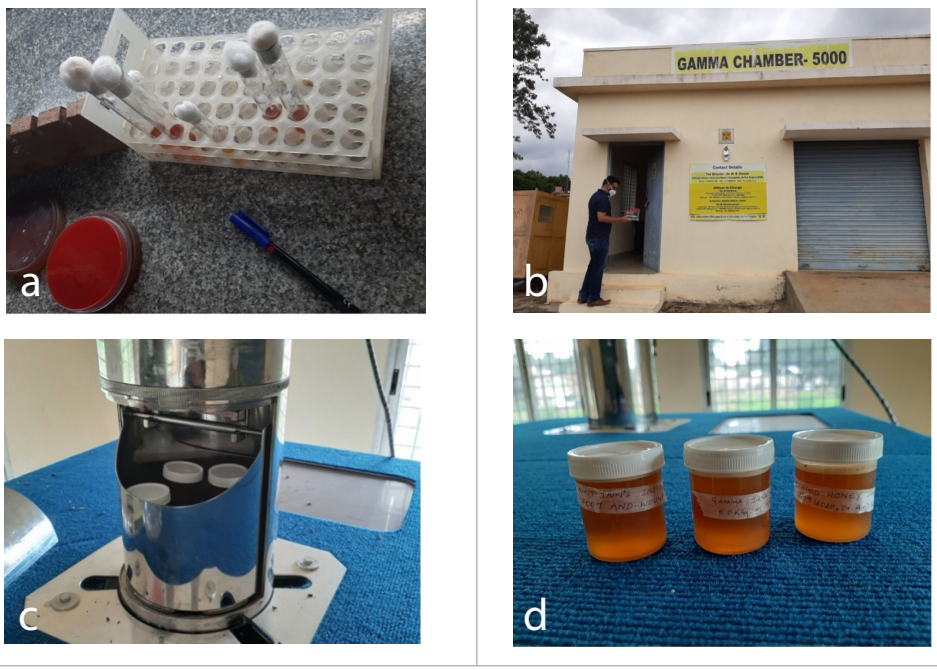
In spite of having strong antimicrobial activity, honey does have few microorganisms in it and it is often advisable to sterilise it before use on wound to render it as a medical grade honey (Cooper, 2017; Jain and Apoorva 2020). Although various methods of sterilisation, such as refrigeration, freezing, pasteurization have been used, gamma irradiation is often recommended for honey that is to be applied to wounds (Postmes et al, 1995; Jain and Apoorva, 2020).

We conducted this study to determine the effect of low dose gamma irradiation in the sterilisation of Indian honey.

Methods and materials

A freshly obtained 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, was used in this study. The Amit Jain's centre for Apitherapy is one of the dedicated eponymous wings within the Institute that deals with the acquisition, sterilisation, storage and use of honey on different types of wounds. This dedicated wing is first of its kind that is run by the diabetic foot and wound care specialist, aimed at propagating the use of honey on wounds in India. The honey used in this study was Indian honey from *Apis dorsata* bee (Indian rock bee). The new raw honey samples were collected in three sterile containers and were labelled as sample A, B and C at Amit Jain's centre of Apitherapy. It was then sent to Department of microbiology of Raja Rajeswari medical college located around 15km from our centre and where it was cultured for aerobic bacterial, anaerobic bacterial and fungal growth.

Figure 1. *a) Unsterilised honey being inoculated in different culture media's at microbiology laboratory. b) Prof Amit Jain taking the Indian honey to the gamma chamber. This facility is located far from the city as it involves using radiation under licence. c) honey samples placed in the box to be gamma irradiated and d) Gamma irradiated honey*



The honey samples were inoculated onto 5% sheep blood agar, chocolate agar and Mcconkey agar ***(Figure 1a)*** to look for bacterial aerobic growth at 37ºC overnight, Robertson's cooked meat broth was used for anaerobic bacterial growth and sabourauds dextrose agar for fungal growth at 37ºC and 25ºC, respectively, for 48 hours.

These honey samples were then taken to IIHR, Bengaluru that was located at periphery of city by the primary author and team as this government institute has gamma chamber and the honey was subjected to gamma irradiation at different irradiation doses. The gamma radiation treatment of the honey was carried out in Cobalt 60 gamma chamber-5000 ***(Figure 1b–d)***. Sample A honey was irradiated at 2.5KGy, sample B honey was irradiated with 5KGy and sample C was irradiated with 20KGy irradiation. The irradiation was done after pre-booking our schedule at the gamma irradiation facility in different batches. These samples were then assessed for bacterial and fungal growth as described above.

Results

Unsterilised Indian honey yielded aerobic spore forming Gram-positive bacilli and bacteriodes species. There was no growth in fungal culture in the honey samples.

Post irradiation, it was observed that sample A and sample B that received a dose of 2.5KGy and 5KGy grew the same organisms whereas sample C honey, which received a dose of 20KGy, did not grow any organisms and was rendered completely sterile.

Discussion

Honey has an excellent antimicrobial activity against wide variety of organisms (Alam et al, 2014; Cooper, 2017; Jain and Apoorva, 2020a). However, raw honey is a reservoir for microbes (Olaitan et al, 2007). The contamination of honey can occur due to different sources (Al-Waili et al, 2017). The primary source of microbial contamination could be due to pollens, dust, air, nectar and bee's intestine, (Hussein et al, 2014). The secondary source of contamination can occur from post-harvesting from humans and equipment (Pajor et al, 2018). The organisms isolated from honey include fungi and bacteria, such as *Penicillium*, *Bacillus, Aspergillus, Clostridium spp*., etc (Olaitan et al, 2007). The microorganisms obtained from honey differed in various geographical areas (Pajor, et al 2002). One worrying organism is *clostridium* spores, which have been reported in many countries (Al-Waili et al 2012). Therefore, it is often recommended to sterilise honey before use. The most accepted method of sterilisation is gamma irradiation as it does not affect the properties of honey (Jalali et al, 2007).

Different doses of gamma irradiation have

been recommended. Postmes et al, in their study, found 25KGy irradiation was needed to render honey sterile of *Clostridium* spores (Postmes et al, 1995). Another study found that gamma radiation of 10KGy achieved microbial decontamination in most of the honey samples they studied (Saxena et al, 2014).

We found 15KGy of gamma irradiation good enough to sterilise Indian honey in our earlier work which rendered it medical grade honey and we had started using on different wounds (Jain and Apoorva, 2020a;2020b; Jain et al, 2020). In this study, the low dose irradiations were not effective for honey sterilisation. One needs to aware that gamma sterilisation of honey is expensive.

Conclusion

Honey is currently one of the agents of choice in wound care. Although different honey has different characteristic and antimicrobial properties, they need to be sterilised before rendering it as medical grade honey to be used on wounds. This study found 20KGy of gamma irradiation to be effective in achieving complete sterility and low doses of gamma irradiation consisting of 2.5KGy and 5KGy were ineffective in achieving sterilisation. We aimed to conduct further studies on medical grade Indian honey and to determine the effect of different doses of irradiation on its physicochemical properties. WAS

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