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In vitro Assessment of Efficacy of Gamma Irradiation on the Antimicrobial Activity of Iranian Honey

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Abstract: Unprocessed honey is well recognized as wound-healing remedy. However, to make use of the honey clinically acceptable, it should be sterilized. It is well-established that the antibacterial activity is heat-labile either by using sterilization by autoclaving, but the effectiveness of gamma-irradiation on the antibacterial activity of honey is unknown. Therefore, an investigation was carried out to assess the effects of the antibacterial activity of Iranian honey using the commercial gamma-irradiation sterilization procedure. The honeys were divided into 4 groups (0, 5, 15 and 25 KGy). Then each group was divided four sub-groups non-irradiated and irradiated (non-heated, 25°C, 35°C, 45°C). Microbiological test of the honeys were carried out against control organisms (*Staphylococcus aureus* , *Escherichia coli* and *Pseudomonas aeruginosa*) and clinically isolated organisms were tested in an agar disk diffusion assay. Results showed that there were no significant changes using the antibacterial activity of gamma-irradiation sterilization of honey, even the radiation was 25 KGy. The results of the study indicate that a better antibacterial activity is derived using the gamma-irradiation technique to maintain sterility and produced undesirable effect on antibacterial activity of honey.

Key words: Gamma irradiation, antibacterial, Iranian honey, 25 KGy

INTRODUCTION

Honey is elaborated by honeybees from sugars present in the nectar of various plants. Besides carbohydrates which are the major constituents (70-80%) of honey contains, in low amounts, various substances such as organic acids, proteins, amino acids, vitamins, enzymes, minerals and different other molecules (pigments, flavonoids, antibacterial factors, etc.) (White, 1979). Honey has been used in wound treatment as long ago as 2000 years (Mathews and Binning, 2002). The ancient Egyptians, Assyrians, Chinese, Greeks and Romans all used honey, in combination with other herbs and on its own, to treat wounds and diseases (Zumla and Lulat, 1989). The use of honey as a medicine has continued into present-day medicine. There is an increasing usage of honey as a dressing on infected wounds, burns and ulcers (Mathews and Binning, 2002; Efem, 1988; Subrahmanyam *et al*., 2001; Tovey, 2000), but there may be a risk of wound botulism from the Clostridial spores sometimes found in the honey. Microbiological studies have demonstrated that there is a significant antiba cterial activity of honey (Cooper *et al*., 1999; Molan, 1992). Physical properties of honey are suggested to be responsible for prevents bacterial penetration and colonization. These properties are included the hygroscopic properties, the high viscosity, acidic pH, inhibine factor, high osmolarity and nutrient contents (Mathews and Binning, 2002; Molan, 1992; Efem *et al*., 1992; Phillps, 1993; Subrahmnyam, 1991) revealed that its antibacterial activity is primarily due to the presence of hydrogen peroxide, generated by the action of an enzyme that the bees add to nectar (Molan, 1996). Likewise, honey is a product that is free of most microbes and those microbes that may be present are likely to be in very low numbers. But it is maybe contaminated during extraction process of honey from honeycomb. Honey is essentially an unsuitable and in fact fatal environment for microorganisms, especially vegetative form of them. However, some bacterial organisms (in particular spore form of them) can be survive in honey such as Clostridium botulinum (Midura *et al*., 1979; Nakano and Sakaguchi, 1991; Nakano *et al*., 1992; Sugiyama *et al*., 1970). From commercial point of view, microbiological contamination

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of bee products has become interesting because of medical and legislative aspects (Fleche *et al*., 1997). There are many methods for disinfection and sterilization of foods and nutritional substances such as honey. Honey is too viscous for sterilization by filtration through microporous membranes. Pasteurization, sterilization and other thermal methods (up to 50°C) are not desirable for this purpose; because they generate some physicochemical changes in nature of honey i.e. decrease viscosity. These changes destruct many nutritional and therapeutic properties in particular antimicrobial effects of honey. The gamma irradiation process seems to be a desirable alternative to pasteurization as it avoids heating. However, there are many reports about efficacy of gamma ray on microbial count and nutritional properties of honey (Matsuda and Sabato, 2004; Migdal *et al*., 2000; Molan and Allen, 1996; Postmes *et al*., 1995) but the effect of gamma-irradiation on the antibacterial activity of honey is unknown. Therefore, an investigation was carried out to assess the effect of the antibacterial activity of the Iranian honey using commercial gamma-irradiation sterilization procedure.

MATERIALS AND METHODS

Natural honey was obtained from beehives in Urmia, (West Azarbaijan province of Iran) and no additional procedures were performed. The samples of honey were prepared by filtration in order to remove debris and stored at 2-8°C until used. The average composition of the honey is given in Table 1. These honey samples were divided into two groups which include non-irradiated and irradiated groups. The samples were irradiated in closed flasks containing 1 kg each, in normal atmosphere and at room temperature. Irradiation was performed in a 60Co Gamma cell 220 (AECL), at a mean dose rate of 5.53 KGy and dose uniformity factor of 1.13, with doses of 0, 5, 15 and 25 KGy. Dosimetry was done using Amber routine dosimeter (Harwell, UK) and dose rate was established using Fricke reference dosimeter to plot calibration curves. The whole dosimetry system is in IDAS program from the International Atomic Energy Agency. Irradiation process was performed in the Atomic Energy Agency of the Islamic Republic of Iran.

Each sample was heated at 0°C (non-heated), 25°C, 35°C, 45°C and was checked for purity on blood agar plates. Three control organisms, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used to determine the antimicrobial activity of each sample of honey. Three colonies (1.1×10^6 organisms mL^{-1}, equivalent to Brown's opacity tube 3) of each standard organism were emulsified in 4mL of distilled water and used to swab Mueller Hinton sensitivity agar plates. An agar disk diffusion assay was carried out to evaluate the antibacterial activity of each honey sample. Each honey sample was done in triplicate. The plates were left at room temperature till the honey seeped into the agar. After incubation, the inhibition zones were measured in millimeters (mm) and the average of the inhibition zones recorded. The end point of antimicrobial activity of each honey sample was defined as the highest inhibition zone with the control organisms.

Table 1: Average composition of Urmia honey

Average (%)	Component
70.38	Reductant sugars
2.12	Sucrose
0.93	Fructose/Glucose
+	Diastase
-	Commercial glucose
0.05	Mineral components
15.08	Moisture
82.92	Concentration
12.5	Total acid
3.96	pH

+:Existance of activity of Diastase enzyme, -:Lake of Commercial glucose

Using Stokes method (Stokes *et al*., 1993), some multiresistant organisms isolated from hospital patients (Hospital of college of veterinary medicine of Urmia University) *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes, Proteus mirabilis* and *Candida albicans* were subjected to sensitivity test. Organisms showing inhibition zones equal to or greater than that of the control organisms were regarded as sensitive to honey samples.

The results of inhibition zones were analyzed with one way Analysis of Variances (ANOVA) test. A *P* value of less than 0.05 was considered statistically significant. (SigmaStat for Windows, version 2.03, Jandel Corporation, San Rafel, CA).

RESULTS AND DISCUSSION

The inhibition zones of non-irradiated and irradiated (5, 15, 25 KGY) samples of the honey with the standard organisms were showed in Table 2. These values for both honey samples with the clinical isolated organisms at temperature 0 (non-heated), 25, 35 and 45°C were comparatively observed in Table 3 and 4. With pay attention to results of this study, the zones of inhibition on control and clinical isolated organisms were affected significantly at different temperatures ($p<0.05$). The inhibition zones measured at 45°C presented lower level values than those at other temperatures for both types (irradiated and non-irradiated) of honey. This finding is in

Table 2: Zones of inhibition of irradiated and non-irradiated honey samples with control organisms

	Staphylococcus aureus zones of inhibition (mm)				*Escherichia coli* zones of inhibition (mm)				*Pseudomonas aeruginosa* zones of inhibition (mm)			
Honey samples	Non heated	25 °C	35 °C	45 °C	Non heated	25 °C	35 °C	45 °C	Non heated	25°C	35 °C	45 °C
NI-honey	28.42±6.50	21.52±4.22	16.46±2.40	11.22±3.26	22.52±5.62	18.24±3.12	13.82±2.60	10.12±3.22	19.25±5.24	16.44±2.06	10.22±3.04	6.00±2.42
5KGy-honey	26.04±4.28	20.22±5.32	15.00±3.06	10.84±1.50	20.00±2.32	16.68±4.06	12.41±3.66	8.30±1.72	18.42±2.05	15.20±3.42	10.05±2.24	4.04±1.05
15KGy-honey	27.22±5.04	22.04±2.82	14.48±3.25	10.72±2.02	19.24±3.42	15.75±3.42	11.00±2.52	9.50±2.05	18.00±3.24	14.55±3.02	9.05±2.06	5.20±1.22
25KGy-honey	26.32±4.66	20.04±2.14	13.20±3.05	11.92±1.54	20.28±4.64	17.44±4.54	11.48±4.92	8.42±1.66	17.46±4.60	14.46±2.82	9.42±3.54	4.08±1.64

NI-honey: Non Irradiated Honey, 5KGy-honey: Honey was irradiated with 5KGy of gamma ray,15KGy-honey: Honey was irradiated with 15KGy of gamma ray, 25KGy-honey: Honey was irradiated with 25KGy of gamma ray

Table 3: Zones of inhibition of irradiated and non-irradiated honey samples with clinical isolated organisms

	Staphylococcus aureus zones of inhibition (mm)				*Escherichia coli* zones of inhibition (mm)				*Pseudomonas aeruginosa* zones of inhibition (mm)			
Honey samples	Non heated	25 °C	35 °C	45 °C	Non heated	25 °C	35 °C	45 °C	Non heated	25°C	35 °C	45 °C
NI-honey	27.24±4.20	20.44±5.45	15.26±3.25	10.94±2.04	21.26±5.04	18.20±3.50	13.22±3.42	9.01±2.45	19.05±4.32	16.04±3.22	10.00±3.25	6.00±1.50
5KGy honey	25.32±5.33	21.02±4.02	15.02±2.44	10.22±1.22	20.04±3.54	16.60±3.25	12.50±3.50	8.42±1.06	18.40±3.22	15.12±3.05	10.02±2.20	4.02±1.25
15KGy-honey	27.4±4.15	20.00±4.55	14.00±3.62	10.05±1.05	19.62±2.33	15.05±3.33	11.00±2.22	9.04±2.33	18.00±3.45	14.02±2.44	9.00±2.52	5.11±1.42
25KGy-honey	26.05±4.02	19.95±3.32	13.92±3.22	10.96±1.25	20.09±3.04	16.42±4.62	11.02±2.54	8.94±1.08	17.06±2.40	14.00±2.05	9.22±2.33	4.05±1.06

NI-honey: Non irradiated honey, 5KGy-honey: Honey was irradiated with 5KGy of gamma ray, 15KGy-honey: Honey was irradiated with 15KGy of gamma ray, 25KGy-honey: Honey was irradiated with 25KGy of gamma ray

Table 4: Zones of inhibition of irradiated and non-irradiated honey samples with clinical isolated organisms

	Staphylococcus aureus zones of inhibition (mm)				*Escherichia coli* zones of inhibition (mm)				*Pseudomonas aeruginosa* zones of inhibition (mm)			
Honey samples	Non heated	25 °C	35 °C	45 °C	Non heated	25 °C	35 °C	45 °C	Non heated	25°C	35 °C	45 °C
NI-honey	10.09±4.63	8.04±2.12	5.24±3.25	3.42±1.44	8.44±4.24	6.40±4.52	3.41±4.22	1.22±1.05	2.00±4.20	1.02±2.22	00.00	00.00
5KGy -honey	9.45±5.02	7.22±2.62	5.00±3.24	3.04±2.05	7.26±3.50	5.42±3.22	3.22±2.50	1.06±2.22	2.52±3.33	1.42±3.05	00.00	00.00
15KGy -honey	9.52±4.38	7.45±5.33	5.22±3.25	3.62±1.15	7.50±2.33	5.04±4.15	3.06±3.16	1.04±1.51	2.95±2.52	1.00±3.12	00.00	00.00
25KGy-honey	8.95±6.54	6.05±4.23	4.81±2.10	2.42±3.33	7.86±5.22	5.00±3.24	3.45±2.50	1.05±3.15	2.42±5.14	1.00±2.00	00.00	00.00

NI-honey: Non irradiated honey, 5KGy-honey: Honey was irradiated with 5KGy of gamma ray, 15KGy-honey: Honey was irradiated with 15KGy of gamma ray, 25KGy-honey: Honey was irradiated with 25KGy of gamma ray

agreement with some reports, which were showed higher temperatures have the greatest destructive effects on antimicrobial properties in contrast with non heated honey (Munro, 1943; Morse, 1986). Heat can destroys the hydrogene peroxide as one of the important antimicrobial agent of honey and also the viscosity as another antimicrobial property of honey can be impaired with the heat up to 25°C (Dustmann, 1972; White and Stubers, 1964). However, Bogdanov (1997) suggested that the heat up to 70°C and 15 min duration does not negative effect on antimicrobial properties of honey. The honey samples of both types (Non-irradiated and irradiated) have high antimicrobial potency with *Staphylococcus aureus* and low antimicrobial potency with Candida albicans ($p<0.05$). These ranking were stay in different temperatures (non-heated, 25, 35, 45°C) of the honey samples. From the viewpoint of gamma irradiation, it did not impair the antimicrobial properties in the studied doses (5, 15 and 25 KGy) and they did not differ significantly from the control for both types (irradiated and non-irradiated) of honey ($p<0.05$). These results are in agreement with Molan and Allen (1996) finding, which reported gamma-radiation in commercial amount (25 KGy) does not affect bactericidal properties of Manuka honeys. Matsuda and Sabato (2004) evaluated effect of gamma irradiation on Brazilian honeys' consistency. The viscosity of this kind of honey was not impaired in low doses of gamma irradiation (5, 10 KGy). According to the results of this study, the antibacterial activities of the Iranian honey were not impaired by gamma irradiation treatment in the doses studied (up to 25 KGy). With attention to these results and other scientific literatures of this field, gamma irradiation can be considered as an available and useful sterilization technique without any undesirable effect on antimicrobial activity of honey.

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