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ORIGINAL ARTICLE

Evaluation of Sulfonamide Antibiotic Residues of Honey Samples Produced in Different Regions of Qazvin Province by ELISA

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KEYWORDS

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ABSTRACT: Honey is a unique food product containing bioactive compounds derived from bees and plants Nowadays, animal food products that may contain antibiotic residues have caused a lot of concern for the consumers. The presence of antibiotic residues in honey may be harmful to human health. One of the most important risks of antibiotic residues in food products is the occurrence of drug resistance in pathogenic bacteria in the body. Regarding the complications caused by these residues, the present research has investigated sulfonamide antibiotic residues in the honey samples consumed in Qazvin province. The present research is a cross-sectional study performed in different regions of Qazvin province in 2019. For this purpose, 80 honey samples were collected from different regions of Qazvin. The samples were transferred to the food safety laboratory of the Health Faculty under proper conditions. First, the samples were tested to check the sulfonamide antibiotic residue by the semi-quantitative ELISA assay. The data were recorded in SPSS 23, and data analysis was done by one-way analysis of variance (ANOVA) and (post hoc) Tukey test at the significant level of $P<0.05$. According to the results of the ELISA assay, 10-120 ng of sulfonamide antibiotic residue was found in 23.75% (19 samples) of the samples. The findings showed that the highest and the lowest sulfonamide concentrations were respectively reported as 30.81 and 6.26 ppb, and the average sulfonamide residue was obtained as 14.50 ppb. According to the results, more than 75% of the honey samples collected from Qazvin province are free of sulfonamide or contain a little sulfonamide concentration. The research showed that most of the honey samples collected from different regions of Qazvin province are free of sulfonamide or contain a little sulfonamide concentration. Regarding the important role of honey consumption in health and the wide use of antibiotics in beehives, it is necessary to take the necessary actions to control the quality of this product.

INTRODUCTION

Honey is a naturally sweet food product produced by honeybees gathering the flower nectars, the matters secreted from the living parts of plants, and insect secretions. This product is a complex combination of the

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carbohydrates such as fructose and glucose (about 77-79%) and other compounds (about 3%) such as phenols, organic acids, amino acids, proteins, minerals, vitamins, and lipids. On the other hand, this product contains different types of enzymatic and non-enzymatic antioxidants including glucose oxidase, catalase, L-ascorbic acid, flavonoids, phenolic acid, and carotenoid that can prevent chronic diseases such as cancer, cardiovascular diseases, and diabetes [1]. There are different types of honey compounds depending on their herbal origin, climatic conditions, geographical origin, and apicultural conditions [2]. Iran has the tenth rank of honey production and in terms of the average production, it has got the seventh rank in the world. The highest rate of the annual honey export in Iran has been reported as 2000 tons. However, this annual rate has reached 5000 tons sometimes[3]. Due to its medical and nutritious properties, honey is widely used by people [4]. Honey was the only sweetener and an important source of carbohydrate for a period of time [5]. The average annual consumption of honey in Iran has been reported as 600 g [3]. Nowadays, honey is considered a natural and healthy food product; however, it should be checked in terms of the content of antibiotics and their metabolites [6]. As a natural product, honeybee products are produced in contaminated environments. In recent years, several studies have investigated the sources of contamination of honeybee products and especially honey; one of the most important sources of contamination is the antibiotics used in the colonies [7]. The use of antibiotics in apiculture is not permitted in Europe; while they are abundantly used in most countries [8]. In most of the EU countries, the maximum permitted antibiotic residue has not been specified; i.e. consumption of the honey samples containing antibiotic residues is not permitted in these countries. Nevertheless, some of the European countries such as Switzerland, England, and Belgium have determined a specific range of 0.01-0.05 mg/kg for each of the antibiotic groups[9]. Currently, the antibiotic content of honey products has created a serious problem in the honey business [10]. Unfortunately, different types of antibiotics are currently used in honeybee colonies in Iran; the use of antibiotics in Iran is rarely based on the diagnosis of bacterial diseases in colonies, and more than 60% of the apiarists in different regions of Iran use antibiotics in honeybee products without observing the health principles [11]. Due to the complications caused by the antibiotic residues, international health authorities such as the American Public Health Association (APHA), World Health Organization (WHO), and Food and Drug Administration (FDA) have determined specifically authorized values of antibiotic residues for different products such as honey [8]. Sulfonamides constitute a group of antibiotics that have been widely used in animal products for 70 years; as one of the most widely used antibacterial drugs, the use of sulfonamides has been done with the goal of prevention and treatment of the diseases [12]. Excessive use of antibiotics in animal husbandry and poultry farms without observing the necessary principles, and also the antibiotic residues in meat, milk, and other animal products will cause serious risks, including drug resistance of pathogenic microorganisms to antibiotics, creation of new serotypes with different pathogenicity, causing different allergies, disturbing the fermentation processes in the production of dairy products, and causing diagnosis problems in medical and food safety laboratories in the process of finding the bacterial agents causing the disease [6]. Antibiotics are considered the most important group of antibacterial agents that are widely prescribed for humans and animals. About 100000-200000 tons of antibiotics are annually produced in the world [13]. Nowadays, due to the wide use of antibiotics, antibiotic resistance is considered one of the main challenges of human health. There are different methods for detecting drug residues in food products. Some of these methods include using bacterial screening tests such as Delvo, quantitative methods such as ELISA (Enzyme-Linked ImmunoSorbent Assay) assay, and confirmatory methods such as chromatography. These methods are selected depending on the type of antibiotic, the expected time limitations, the sensitivity of the method, and its costs [14, 15]. This study has investigated the sulfonamide antibiotic residues in honey samples collected from different regions of Qazvin city by ELISA assay.

MATERIALS AND METHODS

For this study, 80 honey samples were collected from different regions of Qazvin (Eastern Alamut, Western Alamut, Takestan, and Abyek) in summer 2019. The samples were transferred to the food safety laboratory of the Health Faculty of Qazvin University of Medical Sciences. First, the sulfonamide content of the honey samples was assessed by ELISA assay. For this purpose, the honey samples were completely homogenized. Then, 1 g of the homogenized samples was removed and mixed with 2 ml of 15% methanol solution by using a vortex for more than 15 min. The resulted solution was centrifuged at 2000 rpm for 10 min. Then, 45 µl of the surface solution was diluted by 255 µl of phosphate buffer solution (PBS). Then, based on the kit instruction, 50 µl of the sample solution was poured into each well. After that, 50 µl of the mixture (sulfonamide-HRP) was also added to all the wells except H1 and H2. Then, the microplate was shaken for several minutes and incubated at 20-25 ˚ C in a dark place for 30 min. The microplate was discharged and washed by the special buffer three times. In the next step, 100 µl of the substrate solution was added to each well and the microplate was again shaken for some minutes. Then, it was incubated at 20-25 ˚ C in a dark place for 15 min. Finally, 100 µl of the stop solution was added to the wells to terminate the reaction. The absorption was recorded at 450 nm, and finally, 50 µl of the resulted solution was analyzed by the ELISA reader based on the kit brochure [16]. The sulfonamide concentration was assessed by Europeroxima kit (Made in The Netherlands). The data were recorded in SPSS 23, and data analysis was done by one-way analysis of variance (ANOVA) and (post hoc) Tukey test at the significant level of $P<0.05$.

RESULTS

According to the findings, out of the 80 studied samples, 19 samples (23.75%) contained sulfonamide antibiotic residues. About 10-120 ng/g of antibiotic residues were found in all of the 19 samples. The minimum and maximum concentrations of antibiotic residues were obtained as 6.26 ppb and 30.81 ppb. Also, the average sulfonamide residue of the studied honey samples was reported as 14.50 ppb. According to the Iranian standards, the maximum authorized sulfonamide residue of honey is 50 ppb. Table 1 presents the results of the sulfonamide assessment. Moreover, the results of the comparison of the samples collected from different regions show that the highest contamination has been observed in the honey produced in Abyek region, and there was a significant difference between Abyek and Eastern Alamut in terms of the antibiotic contamination of the collected samples ($P<0.05$). Meanwhile, the antibiotic residue was not observed in any of the samples collected from Takestan region (Table 1) (Figure 1).

Table1. The results of the analysis of different honey samples by ELISA assay

Region	Number	Mean± Std. Deviation*	Min (ppb)	Max (ppb)	MRL (Maximum residue level) (ppb)	Samples containing erythromycin with values above the kit's LOD (%)
Abyek	22	18.32±8.28^{a}	6.25	30.81	50	45.4
Western Alamut	22	14.78±4.56^{ab}	6.25	23.42	50	13.63
Eastern Alamut	32	12.55±5.82^{b}	6.25	28.03	50	18.75
Takestan	4	11.46±1.94^{ab}	9.26	13.97	50	0
Total	80	14.69±6.57^{ab}			50	77.78

* The different letter in the column shows that two group were significantly different ($P<0.05$).

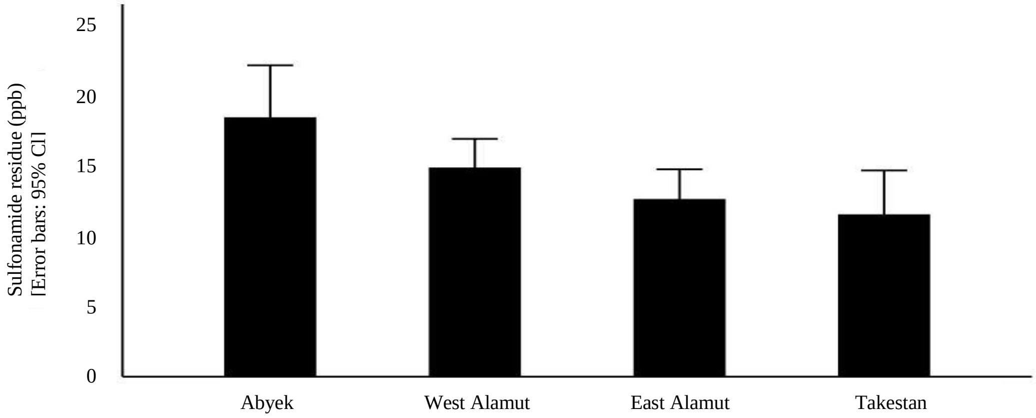

Figure 1. The sulfonamide residue in the honey samples collected from different regions.

DISCUSSION AND CONCLUSIONS

According to the results, 19 honey samples contained sulfonamide residue and no antibiotic residue was found in 61 samples (76.25%). The minimum and maximum concentrations of antibiotic residues were obtained as 6.26 ppb and 30.81 ppb. The results of the comparison of the samples collected from different regions show that the highest contamination has been observed in the honey produced in Abyek region, and there was a significant difference between Abyek and Eastern Alamut in terms of antibiotic contamination of the collected samples ($P<0.05$). Fazlara et al investigated the tetracycline and oxytetracycline antibiotic residues in honey samples produced in Khuzestan province by the HPLC method. They found that 14 samples (23.33%) were free of tetracycline and oxytetracycline antibiotic residues, while 31 samples (51.66%) contained both the mentioned antibiotic residues; overall, 46 samples (76.67%) out of the 60 studied honey samples were found to be positive in terms of containing antibiotic residues [11]. A study in Greece investigated 251 honey samples in terms of containing tetracycline residue by HPLC method; in this study, 29% of the samples were positive [17]. Also in Germany, a study investigated 47 imported honey samples and 30 domestic honey samples and it was found that 22 samples (about 50%) imported from Argentina, China, and Canada contained antibiotic residues and especially sulfamethoxazole; while only one of the domestic honey samples contained antibiotic residues[18]. In Turkey, 50 honey samples were assessed and they were found to be free of sulfonamide and oxytetracycline; this finding is inconsistent with the results of the present research [19]. Sandra et al (2018) investigated the sulfonamide residues of the honey samples in the Czech market by ultrasound liquid chromatography; mass spectrometry revealed three positive samples (6%) containing sulphadiazine and sulfamethoxazole. Among the positive samples, two samples were imported from EU countries and one sample was a combination of the honey samples imported from EU countries and non-EU countries[20]. In Turkey, Gones et al studied 50 honey samples to determine their erythromycin content by LC-MS. They found that 4 samples (8%) contained erythromycin [21]. In another study in Spain, Banuhi et al studied 576 honey samples to check tetracycline antibiotic residue. They found that 24 samples contained tetracycline [22]. Arasou et al studied 66 honey samples collected from different provinces of Italy; out of the 40 studied samples, 366 samples contained tetracycline [23]. In Turkey, Korkmaz et al studied the tetracycline and sulfonamide residues in honey samples, and they found that out of the 59 studied samples, 35 samples contained tetracycline residue and 31 samples contained sulfonamide residue [24]. In another research, Kalarini et al studied the residues of 27 veterinary medicines in honey samples. Out of the 74 studied samples, the existence of sulfonamide was confirmed in 9 samples (12%) [25]. In EU countries, the use of antibiotics is not permitted in apiculture. Furthermore, MRL EU has not determined any MRL antibiotic for honey; it suggests that according to EU regulations, honey should not contain any antibiotic residue and such products cannot be sold [20, 24]. As observed in the mentioned studies, most countries have always investigated and controlled the level of antibiotic residues in their produced and imported honey samples and compared the results with the determined authorized amounts. However, there have been few studies in this area in Iran. As a result, there is limited information in this area [11]. Experimental, epidemiological, and ecological studies have suggested a direct relationship between antibiotic consumption and antibiotic resistance. High consumption of antibiotics has always led to increased antibiotic resistance [26]. The evidence suggests that microbial resistance to antibiotics is considered an important health challenge in Iran. Since studies suggest the excessive consumption of antibiotics in Iran and the food producers' inobservance of avoiding the abstinence, antibiotic resistance is expected to become more severe in the future and it puts the consumers' health at risk. In terms of etiological aspects, the most important causes of the excessive consumption of antibiotics include the poor system of inspection and control of the farms, lack of a distribution control system, the use of livestock antibiotics, inobservance of Good Hygiene Practice (GHP) principles in farms, the food producers' low information, and unprincipled use of medicines in farms especially the prohibited medicines such as chloramphenicol, furazolidone, malachite green, etc. that are imported illegally, lack of rational policy-making in the Ministry of Agriculture for supporting production and balancing the prices with production costs [27]. Every country should determine a specific MRLS. However, in our country, no authorized level has been determined for the use of veterinary medicines such as the antibiotics used in beehives. In Iran, the 7087 Standard of honey sampling for controlling the medicine residuals was approved by the Iranian Institute of Standard and Industrial Research in 2003. Failing to control the drug residues in livestock foods is a serious problem in developing countries such as Iran. In addition to threatening the consumers, this problem is an obstacle to expert the products to other countries such as the EU countries where there is a specific MRLS [11]. Therefore, the following suggestions are proposed for controlling the health factors in such products: Regarding the results of the studies, the relevant authorities must perform comprehensive studies to determine the proportional standards for every country. There should be some programs to promote the livestock raisers' knowledge of the proper use of medicines and the harms caused by excessive use of them. Also, the authorized institutions should develop some programs to control the consumption of medicines in livestock farms; these programs should be applied after clinical tests and examinations, antibiogram test, and prescription of an antibiotic. The medicines should be prescribed by a veterinarian and based on the instruction of the producer company. The samples found to contain an antibiotic content above the kit detection range can be tested by advanced methods such as HPLC and AC for the final confirmation. However, different types of widely used antibiotics should be tested by field studies to control the current situation and plan preventive actions. The manufacturer company's instruction about the residue, tolerance, the way of consumption, amount, and the abstinence period included in the drug brochure should be paid more attention[6]. Meanwhile, there should be more strict control over the produced or imported honey products supplied in the market.

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ETHICAL CONSIDERATION

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Conflict of interests

The authors declare no conflict of interest.

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