

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. It accounts for approximately 25% of all childhood cancers and almost 75% of childhood leukemias (*Pui and Evans, 2006*). The treatment of cancer is associated with nausea and vomiting, oral mucositis, constipation, xerostomia and food aversion and it thus play an important role in decreased food intake, nutrient loss, energy expenditure alterations and weight loss, particularly lean body mass (*Andrassy and Chwals, 1998*).

Clostridium difficile (*C. difficile*) is a gram-positive, anaerobic bacteria, spore-forming bacillus that produces enterotoxin A (TcdA) and cytotoxin B (TcdB), which cause diarrhea (*Pituch, 2009*).

Clostridium difficile infection is a hospital-acquired infection, and its prevalence has increased (*Ananthakrishnan, 2011*). According to a report by the U.S. Centers for Disease Control and Prevention in 2013, *C. difficile* infection has been considered as an urgent threat and requires immediate attention (*CDC, 2013*).

The major risk factors for *C. difficile* infection include the use of antibiotics, the use of proton pump inhibitors (PPIs), hospitalization, aging, and conditions that may affect the colonic flora (*DuPont, 2011*).

It has been reported that cancer patients have a higher risk for *C. difficile* infection as compared to noncancer patients (**Chopra et al., 2010**). Malignancy is the most common underlying chronic comorbidity in children with *C. difficile* associated diarrhea (CDAD) (**Tai, 2011**).

Anticancer therapies result in widely varying degrees of mucocutaneous toxicity and immune system compromise and can substantially influence the outcomes of *C. difficile* associated diarrhea (CDAD). Traditional chemotherapy agents have been shown to perturb fecal microbiota, leading to conditions that promote the incidence and severity of CDAD and simultaneously hinder its resolution (**Zwielehner et al., 2011**). Additionally, chemotherapeutic agents promote inflammatory changes in the gut, intestinal necrosis, and anaerobic conditions, allowing proliferation of *C difficile* (**van Vliet et al., 2010**).

Patients with hematologic disease are susceptible to *C. difficile* associated diarrhea (CDAD) because of their frequent antibiotic use, prolonged duration of hospital stay, and chemotherapy-induced disruption of the intestinal mucosa (**Freeman et al., 2010**).

The human intestinal microbiota is involved in many host functions, such as food processing, regulating intestinal epithelium growth, immune system development, synthesis of essential vitamins, or protection against pathogens (**Montalto et al., 2009**).

Prophylactic and empirical use of broad spectrum antibiotics is the common treatment for neutropenic fever patients with hematologic disease (*Alonso et al., 2012*).

Prebiotics are now defined as selectively fermented ingredients that allow specific changes both in composition and or activity in the GI microflora that confers benefits upon host wellbeing and health (*Gibson et al., 2004*).

Honey is natural substance formed from nectar by honeybees. It is composed primarily of sugars glucose, fructose and its greatest component is water, honey also contains numerous other types of sugar as well as acids, proteins, vitamins and minerals (*Bogdanov et al., 2008*). Honey can fight microbial infection by its immuno-activating, anti-inflammatory and prebiotic activity. Honey inhibits the growth of microorganisms and fungi. The antibacterial effect of honey, mostly against gram-positive bacteria (*ALWaili et al., 2011*).

Important honey effects on human digestion have been linked to honey oligosaccharides. These honey constituents has a prebiotic effect, similar to that of fructooligosaccharides (FOS) (*Corzo et al., 2005*). FOS may help in the prevention and treatment of *C. difficile* infections by aiding in the restoration of microbiota and the strengthening of intestinal barrier integrity (*Jie, 2002*).

AIM OF THE WORK

Evaluation effect of honey supplementation on the frequency of clostridium difficile infection and gastrointestinal complications in pediatric patients undergoing chemotherapy.

Chapter One

HONEY

Honey can be classified based on the source of nectar. These include floral and non floral honeys. Honey can be either be unifloral or multifloral, depending whether the honey collected is from the nectar of the same flower or from nectar of flowers of various types (*Subrahmanyam, 2007*).

Types of honey

Polyfloral honey known as wild flower honey is derived from the nectar of many types of flowers (*Kamal and Klein, 2011*).

Monofloral honey is made primarily from the nectar of one type of flower. In order to produce monofloral honey beekeepers keep beehives in an area where the bees have access to only one type of flower (*National Honey Board, 2008*).

Non floral honey Instead of taking nectar, bees can take honeydew, the sweet secretion of aphids or other plant sap-sucking insects (*National Honey Board, 2007*).

Classification of Honey by Packaging and Processing

Honey is sold in liquid and other forms, and are subjected to a variety of processing methods and packaging: Honey can be categorized regarding the mode of processing given below (*Joshi, 2008*).

Crystallised honey is honey where some of the glucose content has crystallised from the liquid form.

Pasteurised honey is honey that has been heated in a pasteurisation process of temperatures of 161 °F (72 °C) or higher. Pasteurisation destroys yeast cells.

Raw honey is honey as close as possible the state it is when extracted from the beehive without adding heat. Raw honey contains some pollen and may contain small particles of wax.

Strained honey has been passed through a mesh material to remove pieces of wax and propolis without removing pollen, minerals or enzymes.

Filtered honey is honey that has been filtered to the extent that all or most of the fine particles, pollen grains, air bubbles, wax and propolis have been removed. The process requires heating the honey to 150–170 °F (66–77 °C) to more easily pass through the filter.

Ultrasonicated honey has been processed by ultrasonication, a non-thermal processing alternative for honey. During ultrasonication, most of the yeast cells are destroyed.

Creamed honey, also known as whipped honey, spun honey, churned honey, honey fondant, and set honey, has been processed to control crystallisation. Creamed honey contains a large number of small crystals, which prevent the formation of

larger crystals that can occur in unprocessed honey. The processing also produces a honey with a smooth, spreadable consistency.

Dried honey has the moisture extracted from liquid honey to create completely solid, non-sticky granules. This process may or may not include the use of drying and anticaking agents. Dried honey is used for baking and to garnish desserts.

Comb honey is honey still in the honeybees' wax comb. It traditionally is collected by using standard wooden frames in bee yard. The frames are collected and the comb is cut out in chunks before packaging.

Chunk honey is packed in wide-mouth containers consisting of one or more pieces of comb honey immersed in extracted liquid honey.

Honey decoctions are made from honey or honey by-products which have been dissolved in water. The water content is then reduced by boiling. The resulting product may be similar to molasses.

Chemical composition of honey

Honey is a natural food, composed of sugars and other constituents such as enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances. It is

rich in flavonoids and phenolic acids that exhibit a wide range of biological effects and act as natural antioxidants (*Alqarni et al., 2012*).

Carbohydrates

Honey is mainly made up of carbohydrates (82.3%) which is a highly complex mixture of sugars. The major sugars in honey are monosaccharides consisting of fructose (38%) and glucose (31%) (*Alvarez-Suarez et al., 2010*). Monosaccharides represent about 75% of the sugars found in honey, along with 10–15% disaccharides and small amounts of other sugars (*Kamal and Klein, 2011; Da Silva et al., 2016*).

Moisture and water in honey

The moisture in honey or water content is the second largest constituent of honey. Its content may vary from 15 to 21 g 100 g⁻¹ (*Yücel and Sultanoglu, 2013*). pH of honey between 3.2 and 4.5 and the natural acidity of the honey inhibit the growth of micro-organisms, as the optimum pH for most organisms is between 7.2 and 7.4 (*Karabagias et al., 2014*).

Proteins, enzymes and amino acids

Honey naturally contains small amount of enzymes that are introduced into honey by the bees during various phases of the honey manufacturing process. The three main honey enzymes are amylase, invertase, and glucose oxidase (*Ahmed et al., 2013*). Other enzymes present in lesser amounts are

catalase and acid phosphatase (*The National Honey Board 2010*).

The most abundant amino acid in honey is proline (*Iglesias et al., 2006*). Besides proline, other amino acids present in honey include glutamic acid, aspartic acid, glutamine, histidine, glycine, threonine, b-alanine, arginine, a-alanine, c- aminobutyric acid, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, tryptophan, phenylalanine, ornithine, lysine, serine, asparagine and alanine (*Keckes et al., 2013; Da Silva et al., 2016*).

All honeys have a slight acidity, as a result of approximately 0.57% organic acids, the predominant acid in honey is gluconic acid (*Karabagias et al., 2014*).

Vitamins and minerals

Honey contains small amounts of vitamins, especially the vitamin B complex, which are from the pollen grains in suspension. Vitamins found in honey include thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B8 or H) and folic acid (B9). Vitamin C is also present (*Bonté and Desmoulière, 2013; Da Silva et al., 2016*).

Potassium is the most abundant element, corresponding one third of the total mineral content found in honey (*Yücel and Sultanoglu, 2013*). In smaller quantities, honey also

contains macro elements (such as potassium, calcium, and sodium) and trace minerals (such as iron, copper, zinc, and manganese) perform a fundamental function in biological systems (*Alqarni et al., 2012; da Silva et al., 2016*).

Polyphenolic compounds

The main functional components of honey are flavonoids. They can significantly contribute to the total antioxidant activity of honey, bringing beneficial effects for human health (*Alvarez-Suarez et al., 2012*).

Nutritional benefits of honey

Honey is an important source of carbohydrates and the only widely available sweetener which provides strength and energy to our body. The carbohydrates in honey are easily digested and quickly transported into the blood to be utilized for energy requirements by the human body. Thus, honey is particularly recommended for children and sportsmen because it can help to improve the efficiency of the energy system of the elderly (*Alvarez-Saurez et al., 2010*).

Antimicrobial and antiviral activities

Antibacterial activity of honey is one of the most important findings that was first recognized in 1892; by van Ketel (*Dustmann, 1979*). Honey has a broad-spectrum antimicrobial activity on gram-negative and gram-positive bacteria (*Katrina and Calvin, 2014*). Several studies have

revealed that honey is effective against Methicillin resistant *Staphylococcus aureus* (MRSA) (**Patel and Chauhan, 2016; Neerajarani et al., 2016**), β -hemolytic *Streptococci* and Vancomycin-resistant *Enterococci* (VRE) (**Jenkins et al., 2012; Almasaudi et al., 2017**).

Due to its antibacterial activity, honey has carioprotective effect by inhibiting the growth of bacteria causing caries (**Molan, 2001b**). It was reported that Manuka honey has a positive effect against dental plaque development and gingivitis (**English et al., 2004**) making it a useful substitute of refined sugar in the manufacture of candy (**Molan, 2001b**).

Honey can also inhibit the growth of a wide range of fungi, protozoa and viruses (**Blair and Carter, 2005**). It was shown to have inhibitory effect on the Rubella virus (**Zeina et al., 1996**) and *Leishmania* parasite (**Zeina et al., 1997**) as well as fungi such as *Candida* spp and *Trichosporon* spp (**Koc et al., 2009**).

Pathogens found sensitive to honey

Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives. Various results are in favor of its activity against *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Pasteurella*

multicoda, *Yersinia enterocolitica*, *Proteus species*, *Pseudomonas aeruginosa*, *Acinetobacter spp*, *Salmonella diarrhoea*, *Sal. typhi*, *Serratia marcescens*, *Shigella dysentery*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Strep. mutans*, *Strep. pneumoniae*, *Strep. pyogenes* and *Vibrio cholerae* (**Mundo et al., 2004; Mandal and Mandal, 2011**). Natural honey had an antimicrobial activity against *MRSA*, *E. coli* and *Pseudomonas aeruginosa* in vitro condition (**Maeda et al., 2008; AL-Waili et al., 2012; Neerajarani et al., 2016**).

Antibacterial activity of honey is mostly reliant on its peroxide activity and non-peroxide mechanisms (**Mohd et al., 2013**). Non-peroxide honey (e.g. manuka honey), displays significant anti-bacterial effects even when the hydrogen peroxide activity is blocked. Its mechanism may be related to the low pH level of honey and its high sugar content (high osmolarity) that is enough to hinder the growth of microbes. Honey traditionally has an acidic pH, between 3.2 and 4.5, which is low to be inhibitory for many bacteria (**Mandal et al., 2011**). A study examining the antimicrobial properties of honey *in vitro* found that H_2O_2 , methylglyoxal and an antimicrobial peptide, bee defensin-1, are distinct mechanisms involved in the bactericidal activity of honey (**Khan et al., 2007**).

Fungal infections

Honey has been reported to have inhibitory effects on fungi. Pure honey inhibits fungal growth and diluted honey

appears capable of inhibiting toxin production (*Al-Waili and Haq, 2004*). An antifungal action of honey has also been observed for some yeast and species of *Aspergillus* and *Penicillium*, as well as all the common *dermatophytes* (*Sampath et al., 2010, Koc et al., 2009*). Candidiasis, caused by *Candida albicans*, may respond to honey (*Irish et al., 2006; Feas and Estevinho, 2011; Moussa et al., 2012*). Cutaneous and superficial mycoses like ringworm and athletes foot are found to be responsive to honey (*Bansal et al., 2005*). In addition, some studies have reported that topical application of honey was effective in treating seborrheic dermatitis and dandruff (*Al-Waili, 2005*).

Antiviral effects of honey

In addition to antibacterial and antifungal effects, natural honey has showed antiviral effect. *Al-Waili (2004)* investigated the effect of the topical application of honey on recurrent attacks of herpes lesions and concluded that topical honey application was safe and effective in the management of the signs and symptoms of recurrent lesions from labial and genital herpes compared to acyclovir cream (*Al-Waili et al., 2004*). Furthermore, commercial and manuka honey seem to act against herpes simplex virus (HSV-1) isolates in vitro (*Hashemipour et al., 2014*). Honey has also been reported to have inhibitory effects on rubella virus activity (*Al-Waili and Haq, 2004*). The manuka honey, revealed to be effective against influenza virus in vitro (*Watanabe et al., 2014*).

Manuka and clover honey were also effective in vitro against varicella zoster virus (*Shahzad and Cohrs, 2012*).

Protective effect in GIT

Infections of the intestinal tract are common throughout the world and affect people of all ages. The use of honey for prevention and treatments of gastrointestinal disorders such as peptic ulcers, gastritis and gastroenteritis has been reported in various publications around the world (*Bogdanov et al., 2008; Manyi-Loh et al., 2010*). Honey has antimicrobial activity against bacteria that can cause diarrhea especially the ones that are caused by *E. coli* (*Adebolu, 2005*) and against many enteropathogenic organisms including those of the *Salmonella* and *Shigella* species (*Molan, 2001a*).

There are several possible explanations for prevention of bacterial adherence demonstrated by honey: (a) non-specific mechanical inhibition through the coating of the bacteria by the honey; (b) some of the fractions, within honey, may alter bacterial electrostatic charge or hydrophobicity which have been reported to be important factors in the interaction of bacteria with host cells or (c) killing of the bacteria due to the previously mentioned antibacterial factors in honey (*Alnaqdy et al., 2005*).

Helicobacter pylori (*H. pylori*) infection is one of the most common bacterial infections worldwide and complications resulting from this infection caused gastritis,

gastric and duodenal ulcers (*Tiwari et al., 2005*). Honey may be useful in the management of *H. pylori* infections (*Manyi-Loh et al., 2010*). A Bulgarian clinical study with dyspeptic patients showed that honey consumption more than once weekly led to lower *Helicobacter pylori* rates of the patients (*Boyanova et al., 2015*). Oral pretreatment of honey (2 g/kg), prevented indomethacin-induced gastric lesions, microvascular permeability, and myeloperoxidase activity of the stomach (*Nasutia et al., 2006*). Also, it has been suggested that natural honey has curative properties for healing of antral ulcers and may be used like sucralfate in the management of peptic ulcer disease (*Ali, 1995*).

Honey has high density, high viscosity, and low surface tension, and therefore, can stay longer in the oesophagus as a coating on the mucus membrane, and can be used against reflux oesophagus, and against heartburn (*Math et al., 2013*).

Honey is a good appetizer and is used for treating disorders related to digestive tract (*Ladas and Raptis, 1999*). It is also used against constipation (laxative agent) (*Shirah and Shirah, 2016*),

Honey in gastroenteritis in children

Diarrhea and gastroenteritis are found to resolve quickly with honey at 5% (v/v) concentration, honey decreased the duration of diarrhea in cases of bacterial gastroenteritis caused by organisms such as *Salmonella*, *Shigella* and *E. coli* as