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Study the bactericidal activity of Propolis in rabbit model

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Study the bactericidal activity of Propolis in rabbit model

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Abstract. This study aims to assess the bactericidal activity of Propolis extract against methicillin resistance *Staphylococcus aureus* (MRSA) in vivo. It included (11 male white New Zealand rabbits) evenly divided into two groups (6 animals in each one). Group I was fed daily with one milliliter of 20% extract Propolis for twenty days, while group II was fed without Propolis to serve as a control group. Ten days after the last dose, all animals were injected in intraperitoneal with single dose viable MRSA antigen at 0.5 x10^8 cfu/ ml. After two days, blood samples that collected from all animals by heart puncture were cultured on blood agar media to assess bactericidal power activity of extract Propolis. The results pointed to effect of this extract on survive viable MRSA bacteria as compared with survive of same bacteria in control group animals. It was concluded Propolis had bactericidal power against pathogenic bacteria that appear resistant to antibiotics like MRSA bacteria.

Key words: Propolis, MRSA, bactericidal, rabbits.

Introduction:

Propolis is a natural resinous product that honeybees collect from several plants and mix it with bees wax and salivary enzymes that is also called bee glue (Silva-Carvalho *et al*., 2015). Bees use propolis on their hives as defense against killers and microorganisms, to repair harm, as a thermal isolator, and to build aseptic locals to avoid infections of microbes (Marcucci, 1995; De Castro and Marcucci, 2000; Bankova, 2005). Since ancient times, propolis has been used by humans to meet the wants of health and food preservation. In the last years the interest in this natural product has better due to its wide spectrum of biological and pharmacological properties (Falcon *et al*., 2013). Propolis is a lipophilic substantial that is firm and fragile when cold however elastic, soft, and very gummy when it heated. Possesses an agreeable aromatic odor and diverse color, including brown, green, and red, among others (Fokt *et al*., 2012; Wagh, 2013). The chemical composition of it, generally composed of 50% resin, 30% wax, 10% essential oils, 5% pollen, and 5% other elements which contain flavonoids, organic compounds like phenolic acids (cinammic and caffeic acid), minerals, fatty acids and esters of phenolic acids terpenes, and alcohols (Barlak *et al*.,2011; Righi *et al*., 2013). Usually, the foremost constituents of propolis are resin and volatiles, which are materials obtained from a variation of botanical processes in different fragments of plants, and beeswax, concealed by the bees (Bankova, 2005). Samples of poplar propolis (from New Zealand, Europe, North America, and Asia) are principally composed of phenolic acids, and their esters as well as flavonoids (De Castro and Marcucci, 2000; Falcao *et al*., 2013). Thus, the characters of different kinds of propolis rendering to its plant source (Silva-carvalho *et al*., 2015). It is stated that pathogenic bacteria have a somewhat large possible for developing antibiotic resistances (Schwaber *et al*., 2006). These resistance bacteria are largely due to the extensive use of antibiotics in agriculture, medicine, and in animal care, and this problem is compounded by the lack of new antibiotics to attack bacteria in different ways to circumvent the resistant genes. Therefore, finding of new antimicrobial agents (of new sources) who are effective against resistant bacteria would be of great importance, consequently propolis is widely and extensively studies all around the world (Silva, *et al*., 2012 b). Many researchers have reported the biological activities of propolis collected from different regions of Iraq (Al-Ammar, 2001) in Najaf city; (Aziz, 2005) in Baghdad and (Al-Shamari, 2006) in Qadysia city, were reported the antimicrobial activities of different sample of Iraqi propolis. Propolis contains substances that are able to inhibit cyclooxygenase and the consequent synthesis of PGE_2 (Araujo *et al*., 2011). Propolis extracts can act on the nonspecific immune response via inhibiting of macrophage activation, H_2O_2 production and nitric oxide production; and this inhibition has been suggested as one mechanism of anti-inflammatory effects of propolis (Orsi *et al*., 2000). The current study was designed to detecting the bactericidal power of blood in rabbits that feeding with Propolis.

1. Materials and methods:

In present study, ethanolic extract was used and prepare by dissolving two grams of crude propolis by ten ml of dimethyl sulfoxide (DMSO) to obtain ethanolic extract propolis solution at 20% concentration, and storage at 4°C up to using for animal administration. MRSA was obtained from microbiology laboratory of Technical Institute in Karbala city that isolated from burned person who has skin lesion, and this isolate re-culture to insure the diagnosis of it. Also, the study included 11 male white New Zealand rabbits) evenly divided into two groups I and II (6 animals in each one). Group I animals were fed with extract Propolis that prepared previously for 20 days, while group II were fed without this extract as control group. Single dose of viable MRSA bacteria was injected in intraperitoneal of animals in both groups and after two days one milliliter of blood was taken from all animals by heart puncture in order to culture on blood agar media to assess the blood clearance activity.

2. Results and Discussion:

The animals that injected with viable MRSA (11 animals), were divided into two groups as described in table (1). Blood samples were cultured to assess the clearance percentage of blood to detect the effects of Propolis on survive the viable bacteria (MRSA) in blood stream. The percentage of bactericidal power in group I was higher (83%), and in group II was (60%) as illustrated in the table (1). The result indicates Propolis has defense mechanisms that involve in the clearance the blood stream from pathogenic bacteria (MRSA) were injected in the animals. The gastrointestinal tract serves as an interface between the gut and immune system, with the intestinal lining functioning as a barrier; lactobacilli colonization decreases the passage of bacteria from the gut into the bloodstream (Mandeep et al., 2013).

Table (1): activity of blood bactericidal effect on MRSA in testing groups

Testing groups	No.	Number of Blood culture +ve	% of +ve blood culture	MRSA Clearance %
Group II	6	1	17	83
Group III	5	2	40	60
Total	11	3		

The inhibiting activity of Propolis may be attributed to indirect effect on DNA replication that lead to inhibition of bacteria cell divisions, so present of many products in Propolis such as phenolic and flavonoids products which may cause this inhibition of bacterial activity (Salmani and Hassan, 2011), as well as disorganizing the cytoplasmic membrane and so inhibits protein synthesis (Hegazi *et al*.,

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2014). Enzyme RNA-polymerase plays important role in DNA replication is also inhibits by Propolis and this mechanism lead to inhibition of bacterial activity (Wojtyczka *et al*., 2013). Propolis and some of its derivatives were reported to be responsible for uncoupling the energy transducing cytoplasmic membrane inhibiting bacterial motility, which might contribute to the antibacterial action (Bankova *et al*., 2000). In a study done by (Rahman, *et al*., 2010), were showed the Propolis inhibit a Gram's positive bacteria better than Gram's negative. The antimicrobial activity of Propolis varied depending on dosage, sample, and the extraction solvents, the DMSO extracts of all Propolis samples were more active than the acetone extracts of the same samples, against *Brucella melitensis* (Orsi, *et al*., 2005). The usage of natural products has been one of the greatest fruitful in modern medicines. Propolis has attracted increased attention because of antimicrobial activity against a varied range of pathogenic germs (Onlen *et al*., 2007). Also the increasing of phagocytic activity by propolis in a study by (Abd Al-Sahib, 2011; Tao *et al*., 2014) who mentioned propolis can significantly enhance the phagocytic function. The increased phagocytic activity against *S. aureus* bacteria may be due to the presence of glucose which assists in the destroying action of macrophages (Chepulis, 2007). Zinc plays important role in immune responses, in innate immune response it act as chemo-attractants for immune cells such as PMNs chemotaxis, and so promotes monocyte adhesion to endothelial cells as well as affects the maturation of DCs. On the other hand, the adaptive immune system is also affected by zinc levels, like T-cells are especially vulnerable to zinc deficiency that cause thymic atrophy and subsequent T-cell lymphopenia (Bonaventura *et al.*, 2015). Zinc also regulates metallothionein which is a family of cysteine-rich protein, that has roles in free radical scavenging and inflammatory process (Choi *et al*., 2015). Haase and Lother, 2009 were mentioned the zinc deficiency cause impaired innate immune cells functions by many pathways such as oxidative burst of neutrophil granules, in contrast of high levels of zinc that induce chemotaxis. In a study done by AL-Greti 2016 who stated levels of Zinc in the serum of animals were fed with Propolis as compared with control groups, and for this fact can be attributed the bactericidal power of blood against MRAS bacteria in animals were feeding with Propolis. Park *et al.* (2014) were reported that dietary zinc-methionine enhanced mononuclear phagocytic function against *Salmonella enteritidis* and influences clearance of *E.coli* from blood in young's Turkey. Zinc is also required for phagocytosis activity of macrophages (Rao *et al*., 2014), for example, high level of zinc prompts chemotaxis of PMNs. On the other hand, zinc deficiency causes decreased phagocytosis, and oxidative burst of neutrophil and monocytes (Prasad, 2000; Haase and Rink, 2009). Nicotinamide adenine di nucleotide phosphate (NADPH) which is regulates superoxide anion production that is responsible for the destruction the pathogen after phagocytosis, is also affected by zinc deficiency (Hasegawa *et al*., 2000).

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