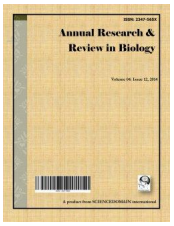

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Effect of Ethanolic Extract of Propolis on Humoral Immunity in Laboratory Animals

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Background: Propolis is a material that collected by bees from different plants and unites with beeswax and enzymes of salivary glands of fly.
Aim: The study was designed to assess humoral immunity activity of propolis against Methicillin Resistant *Staphylococcus aureus* MRSA bacteria.
Materials and Methods: Sixteen male white New Zealand rabbits included in this study, that divided into two groups (8 rabbits at each one), group I received orally Ethanolic Extract Propolis (EEP) at 20% concentration per day for lasting twenty days, while group II have received dimethyl sulfoxide as control group. All animals injected in intraperitoneal (IP) with six doses of 10^8cfu/ milliliter of killed somatic antigen of MRSA bacteria after ten days from last dose of administration of EEP. Blood samples were taken by heart puncture from all animals to estimate the level of immunological parameters immunoglobulin M and G (IgM&IgG) by single radial immune diffusion (SRID) technique.
Results: Results revealed to increase significantly ($p < 0.05$) the mean value of IgM and IgG in group I as compared with group II.
Conclusion: It was pointed propolis induce humoral immunity against bacterial MRSA antigen *in vivo*.

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Keywords: Propolis; MRSA; IgG; IgM.

1. INTRODUCTION

Propolis is a resinous material that has much chemical composition and its principal role to keep up an antiseptic environment in the bee hive and to allow the bee colony health [1,2]. It obtains from several parts of the plant such as floral buds, leaves, branches, buds and barks. The composition varies according to the area visited by the bee. More than 300 constituents have been recognized in diverse propolis sample [3]. Cetin and colleagues [4] demonstrated that addition of propolis at three grams per kilogram in the laying hens' diet resulted in significant increases in the serum IgG and IgM levels and erythrocyte count. The immune modulating activity of propolis has been an object of intensive research for the last years, and it is more than three hindered components affect cellular and humeral immunity by activating different molecular mechanisms such as it is activate natural killer(NK)cells in rats that treated with propolis [5], as well as stimulates macrophages to production of cytokines like tumor necrosis factor (TNF) and interleukin 12(IL-12) in addition to intensify the cytotoxic activity of NKcells [6,7]. Cell-mediated immunity activates by cytokines that released from macrophages after propolis treatment; so it activates the proliferation of Th-cells [8,9]. Immune-stimulation via natural compounds may be considered an alternative tool for the protection and cure of infectious diseases and this stimulation of the immune system by natural substances has already been reported [10].

As soon as stimulated by binding to antigen, such as bacterium or virus, a lymphocyte is multiply into a clone of cells; some of these cells discriminate into plasma cells that generate antibody particles. These are once out into the blood and lymph, they connect to the target antigen and start its damage. Antibody production remains for a numeral of days or months, until the antigen has been rise above, while the other B cells distinguish into the memory B cells that interested to increase but do not distinguish into plasma cells and supply the immune system with long-term memory [11]. The present study was designed to show the effects of Propolis on humoral immunity by assessment the levels of immunoglobulin IgM&IgG in animal's model.

2. MATERIALS AND METHODS

Propolis samples were taken from honey bees hives in Hindia City at Karbala governorate. The samples of propolis were cleaned and cut into small fragments and storage in clean container; and then five grams of propolis were mixed with fifty milliliters of ethanol alcohol in dark-container and left-hand for8-10 days at 37°C with shaking every day. The liquid was filtered and disappear the ethanol in the oven at 45-50°C. Alcoholic extract was weighted and dissolve two grams of it in 10ml of dimethyl sulfoxide (DMSO)to obtain ethanol extract propolis (EEP) solution at 20% concentration, and storage at 4°C up to using for animal administration [12]. Killed somatic antigen of (MRSA) was prepared by streaking of *S. aureus* bacteria on blood agar media at 37°C for 24 hours. The bacterial growth was harvested and introduced into conical flasks that contain hindered milliners of sterilized phosphate buffer solution (PBS) and positioned in water bath at 100°C for two hours to destroy the bacteria. The flask that contains destroyed somatic MRSA antigen was kept in refrigerator up to use to challenge the immune system [13].

2.1 Experimental Animals

A total of sixteen Male white New Zealand rabbits at age 6-9 months and their body weight ranged between 2.5 – 3.5 kilograms were kept in the same suitable environmental conditions of 25 – 27°C. After six weeks of adaptation, rabbits were separated erratically into two groups (eight animals every group); group I were received one milliliter daily of EEP at 20 % concentration for twenty days, while group II were received one milliliter of DMSO as control group. After ten days from latter dose of EEP, all animals were inoculated intra-peritoneal with killed somatic antigen of MRSA that primed previously. About three milliliters of blood were obtained from all animals to assess the levels of immunoglobulin G and M (IgG and IgM) by SRID technique according to leaflet of manufactured company (LTA Company, Italy). Obtained results were analyzed by SPSS V. 18.

3. RESULTS

The level of IgM in the serum of animals in group I was increased significantly ($p< 0.05$) as compared with group II as illustrated in Table 1. Serum IgG level was increased significantly in group I as compared with IgG level in group II at $p< 0.05$ as illustrated in Table 2.

Table 1. IgM level (mg/dL) in sera of testing groups after immunized with killed MRSA antigen

Testing groups	No.	Mean value	Standard deviation	P value
Group I	8	74.863	3.059	0.002
Group II	8	58.075	3.972	

Group I: Animals feed with propolis and injected with killed MRSA antigen
Group II: Animals feed wit propolis alone and injected with killed MRSA antigen as control

Table 2. IgG level (mg/dL) in sera of testing groups after immunized with killed MRSA antigen

Testing groups	No.	Mean value	Standard deviation	P value
Group I	8	1549.25	376.11	0.001
Group II	8	759.25	260	

4. DISCUSSION

The results of this study indicated to improve humoral immunity during raise the levels of immunoglobulins, the main factors of humoral immunity and this increasing of it may be reflect many factors like activating T- cells practically CD^{+4} cells that produce large amount of interferon gamma and interleukin- 2 (IL-2) which is considered as T-cell growth factor and it plays important role through enhance antibody production as well as generation and proliferation of T-cells [14], in addition IL-2 acts as growth and distinction factor for B-cells, monocytes to yield cytokines such as tumor necrosis factor (TNF-α) [15], in many studies propolis proliferation IL-2 that is may be lead to increase antibodies production [14,16]. The administration of this extract to the laboratory animals increased the levels of immunoglobulin G & M against MRSA antigen as compared to animals that received antigens without propolis, and this prompting in immunoglobulins production may be quickly handled by the cells of the immune system moving a weak humeral immune response, and it increases the Toll-like receptors (TLRs) appearance, as in increased TLR-2 expression in macrophages and spleen cells, as well as increased TLR-4 expression in macrophages as indicated by [16]. TLRs are commonly expressed by different cells of the immune system and these identify conserved pathogen related molecular patterns shared by special microorganisms, such as bacterial components, viral RNA and DNA, among other, playing an critical role in immune response and in the beginning of adaptive immune response [17,18]. TLRs stimulation leads to up regulation of pro-inflammatory cytokines, chemokines, antimicrobial peptides, and additional defense molecules, in addition to rise in the dealing out and giving of antigens to lymphocytes [16,18]. The results in present study agreement with [18,19] who originate "that vaccine with propolis augmented the potency of the humoral immune response when compared to the vaccine without Propolis" as well as [20] who stated administration of Brazilian propolis have helpful effect on both arms of immunity in aged mice. Propolis control the adaptive immune response is maybe due to potent stimulatory influence on unlike cells of innate immune response such as NK and macrophage, these cells influence adaptive immunity by secreting cytokines which controller the role of B-cells and T-cells [21]. In 2012, in a vaccine against *Klebsella pneumonia* was formulated with the alcoholic extract of the Egyptian brown Propolis and injected in white New Zealand rabbits, the results appear lower dose of the antigen to produce similar levels of antibodies in rabbits that injected with the same vaccine but alone, as well as the antibody titer responded earlier and persisted for a longer period [22]. There is a previous study [23] showed that propolis supplementation increased leukocytes count, lymphocytes and plasma IgG and IgM.

5. CONCLUSION

A current study concluded a propolis extract induces of humoral immunity *in vivo* against MRSA antigens.

ETHICAL APPROVAL

As per international standard or university standard Ethical Approval has been taken by the author.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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