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Original article

Beneficial Effects of *Capparis Spinosa* Honey on the Immune Response of Rats Infected with *Toxoplasma Gundii*

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Key Words

Capparis spinosa honey, cytokines, Saudi Arabia, *Toxoplasma gundii*

Abstract

Objectives: The *Toxoplasma gondii* (*T. gondii*) is an intracellular opportunistic protozoan parasite that infects approximately one-third of the human population worldwide. Honey has long been used for treatment of many diseases in folk medicine. Honey has exhibited significant anthelmintic, nematicidal and anti-protozoal activities.

This study was conducted to investigate the immunological patterns in rats infected with *T. gondii* who were treated orally with supplemented 15% *Capparis spinosa* honey (Saudi Arabia) for a period of 28 days.

Methods: Immunoglobulin M, immunoglobulin G, and cytokines were detected by using enzyme-linked immunosorbent assays (ELISAs). In addition, the mortality and the morbidity rates were assessed.

Results: Oral administration of *Capparis spinosa* honey as a natural food additive was experimentally shown to increase the antibody titer; furthermore, compared with the rats in the control group, the levels of the sera cytokines (IFN-γ, IL-1 and IL-6) were consistently higher at day 7 post-infection in the infected rats treated with oral supplements of *Capparis spinosa* honey.

Conclusion: Orally administered supplements of *Capparis spinosa* honey increased both the antibody titer and the cytokines (IFN-γ, IL-1 and IL-6) levels in rats infected with *T. gondii*.

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1. Introduction

Toxoplasma gondii (*T. gondii*) is an intracellular opportunistic protozoan parasite that infects approximately one-third of the human population worldwide [1, 2]. It forms tissue cysts in the brains of warm-blooded animals and manipulates the behavior of infected rodents [3, 4]. Some researchers have reported that urban rats are important for the epidemiology of toxoplasmosis because they act as a source of infection for domestic cats, as well as other carnivores and omnivores, such as dogs and pigs [5-8].

Honey has been used over the past 2,500 years in many civilizations, especially Egypt. It is considered to be an important element in traditional medicines, and scientists have been, and still are, researching its benefits in relation to modern medicine. In folk medicine, honey has long been used for the treatment of patients with many different diseases [9]. The antimicrobial activities of honey against bacteria [10, 11] and fungi [12-19] have been extensively reported. Moreover, honey has exhibited significant anthelmintic activity at concentrations as high as 300 mg/mL [20], as well as nematicidal [21, 22, 23], and anti-protozoal [24] ac-

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tivities. This study was conducted to investigate the beneficial effects of *Capparis spinosa* honey on the immune response of rats infected with *T. gondii*.

2. Material and Methods

Fresh Saudi honey samples (1 kg) were kindly provided by Alnahal Aljwal Company, during the 2015 flowering season). The mono floral honey harvested from apiaries (authorized apiary farm of Alnahal Aljwal, Saudi Arabia) is vented as "monofloral", meaning that the honey must derive from at least 55% of the pollen from a single floral source. The collected honey sample was Shafallah honey (*Capparis spinose*). The honey samples were collected in sterile universal glass containers and kept at 2 - 8°C until tested. Physiological saline phosphate buffer solution (PBS), pH 7.2, was used for all dilution steps under aseptic conditions.

The study was carried out on 60 males, Albino Wistar rats ranging in weight from 250 to 280 g, which had been obtained from Laboratory Animal House, National Research Center, Egypt. Throughout the study, these animals were housed in standard environmental conditions, a temperature of 24°C and a relative humidity of 50%, with a 12-h:12-h light: dark cycle. They had free access to a standard commercial diet and water.

The *T. gondii* strain used in the present study, the RH strain, was maintained and secured in the Zoonotic Diseases Department, National Research Center, Egypt. Tachyzoites of *T. gondii* (RH strain) [25] were maintained in mice by passage every 3 - 4 days. The tachyzoites obtained from the ascitic fluid of the rats were diluted to adjust the tachyzoites count to 10^3/mL and were used for intraperitoneal acute infection after counting and dilution as necessary [26, 27]. Rats infected with *T. gondii* were treated orally with supplements of 15% *Capparis spinosa* honey (Saudi Arabia) for a period of 28 days.

The experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals, and Ethical Approval for this research was granted by the Committee, National Research Centre, Egypt. The experimental design was as follows: The rats were divided into six groups of 10 rats per group (Table 1). The healthy control group was treated with saline solution (group 1). The groups two and five were treated for 28 days with oral supplements of 15% *Capparis spinosa* honey (Saudi Arabia), groups 4 and six (Drug group treated with the standard treatment with sulfadiazine (tablet: 500 mg) + pyrimethamine drug (tablet: 25 mg) as the treatment of choice for toxoplasmosis [28] while groups 3 (infected control group), 5 and six (infected group treated with the standard treatment) were

Table 1 Groupings of the rats used in this study and the treatment for each group

Group Name	No. of animals	Treatment					
		Saline	Honey	Toxoplasma	Sulfadiazine + Pyrimethamine	Toxoplasma + Honey	Toxoplasma + Sulfadiazine + Pyrimethamine
Healthy Control	10	+	-	-	-	-	-
Honey Control	10	-	+	-	-	-	-
Toxoplasma (Infected Control)	10	-	-	+	-	-	-
Sulfadiazine + Pyrimethamine (Standard Treatment Control)	10	-	-	-	+	-	-
Toxoplasma + Honey (Infected, Treated with Honey)	10	-	-	+	-	+	-
Toxoplasma + Sulfadiazine + Pyrimethamine (Infected, Treated with Standard Treatment)	10	-	-	+	-	-	+

infected with toxoplasma. The rats in all groups were monitored for mortality daily, and at days 7, 14 and 28, some rats were sacrificed, and their weights were measured.

Blood samples were collected *via* the tail vein into heparinized capillary tubes, and the serum portion was separated by centrifugation at 3,500 rpm and was then kept in a deep freeze at - 70°C until the IgM and the IgG levels were determined at days 7, 14 and 28 by using enzyme-linked immunosorbent assays (ELISAs) [29]. Complexes including antigen and antibody couples were tracked. All control and infected rats were examined for infections by using ELISA kits designed in our laboratory. Toxoplasma lysate antigen (TLA) was prepared from tachyzoites of the *T. gondii* RH strain [30]. The RH strain (about 2×10^9 tachyzoites) harvested in PBS was filtered and centrifuged at 750 g three times for 15 min each time. The pellet was solubilized by adding distilled water, after which the solution was supplemented with the protease inhibitor, 5-mM phenylmethylsulphonyl fluoride. The suspension was freeze-thawed five times. The protein content of TLA was determined using the Bradford method [31], after which the TLA was stored at - 20°C until used.

The ELISAs were carried out using a procedure described by Ref [32]. Ninety-six-well, flat-bottom microtiter plates were coated overnight at 4°C with a 10-μg/mL solution of TLA in carbonate buffer, pH 9.6 (100 μL per well). Plates were washed with phosphate buffered saline Tween (PBST) (PBS, pH 7.4, containing 0.05% Tween 20) for three times for 3 min each time. The ELISA plate was blocked for 1 h by using 100 μL of 3% skim milk powder in PBS 0.05% Tween 20 and washed. Sera samples diluted in 3% skim milk in PBS were added to a volume of 100 μL and at a concentration of 1 : 100. After the plate had been washed, it was incubated with peroxidase labelled rabbit anti-rat IgG (Sigma-Aldrich Company, St. Louis, MO, USA) diluted 1 : 10,000 in PBST plus 3% skim milk and incubated for 1 h at 37°C. Finally, the enzymatic activity was revealed using the substrate tetramethylbenzidine (Sigma). After 20 min of incubation at room temperature, the reaction was stopped by adding 50 μL of H2SO4 (1.25 M), and the optical density (OD) was measured at 450 nm by using an ELISA reader. A sample was considered positive when the mean OD for infected rats was higher than the mean of control rats by three standard deviations (cut-off). Titer was defined as the reciprocal of the highest dilution that produced OD readings of more than 0.1 OD unit above background. The absorbance was measured at 405 nm, and an IgG anti-Toxoplasma level < 15 UI/mL was reported as negative while a level ≥15 UI/mL was reported as positive. In regard to IgM, levels lower than 1 UI/mL were reported as negative, and levels equal to or higher than 1 UI/mL were reported as positive.

Blood samples were obtained from anesthetized animals. Serum samples were stored at - 70°C until analyzed. Sera were diluted 1/10 in PBS; then, the tumor necrosis factor (TNF)-α, IL1β and IL6 levels were measured at 7 and 28 days by using the ELISA technique as described in Ref [33]. ELISA reagent kits (Lucerne Chem AG, Lucerne, Switzerland) were used according to the manufacturer's instructions. All measurements were performed in triplicate. Experiments were repeated three times, with 3 animals per group. The concentrations of cytokines were determined spectrophotometrically. The absorbance was read at 450 nm. A standard curve was constructed by using cytokine standards. The cytokine concentrations in unknown samples were calculated according to the standard curve, and the absorbance readings were converted to pg/mL based upon the standard curves obtained using recombinant cytokine in each assay.

The results obtained in the present study are represented as means ± standard errors of the mean (SEM) and were analyzed using the analysis of variance (ANOVA). Samples were compared using the unpaired Student's *t*-test (two-tailed) for unpaired samples with equal variance, as calculated using Excel (Microsoft, Seattle, WA). The significance of any difference between means at $P < 0.05$ was calculated using the Duncan Multiple Range Test [34].

3. Results

Rat serum immunoglobulin M and G at days 7, 14 and 28 under this experiment was determine by ELISA assy. Reciprocal titers were determined in sera collected from all groups of control rats and from all rats infected with *T. gondii* and treated with oral supplements of 15% *Capparis spinosa* honey or with the standard treatment illustrated in (Fig. 1). Antibody responses after intraperitoneal infection with *T. gondii* reached their maximum levels, in particular in the groups infected with *T. gondii* which represented significant increases, at day 7 (IgM-specific antibody) and at day 28 (IgG-specific antibody) post infection, as detected by using ELISAs (Fig. 1). The immune status of honey normal group showed rising its level while the group infected and treated with honey showed rising titer. While group treated with sulfa showed reduction of the antibody level.

Rat serum samples for determining the cytokine levels by using ELISAs were obtained from the rats in the healthy, the infected, the honey, and the standard treatment control group, as well as from infected rats treated either with honey or the standard treatment of sulfadiazine + pyrimethamine. The results are presented in Fig. 2. After infection with *T. gondii*, the rats in the infected control group showed significantly elevated levels of TNF-α, IL-1β and IL-6 levels. compared to the rats in the healthy control group. Administration of honey to the infected rats significantly reduced the TNF-α, IL-1β and IL-6 levels. Values in Fig. 2 are presented as means ± SEMs ($P < 0.05$).

4. Discussion

T. gondii is an opportunistic intracellular parasite pathogen that infects approximately one-third of the human population worldwide [1]. The rat model was chosen for this research because the natural resistance of this species to toxoplasma infection is similar to that observed in humans [35]. Antibody responses after intraperitoneal infection reached their maximum levels in the infected controls at day 7 (IgM) and at day 28 (IgG) post infection, as detected by using ELISAs (Fig. 1). During the course of acute and

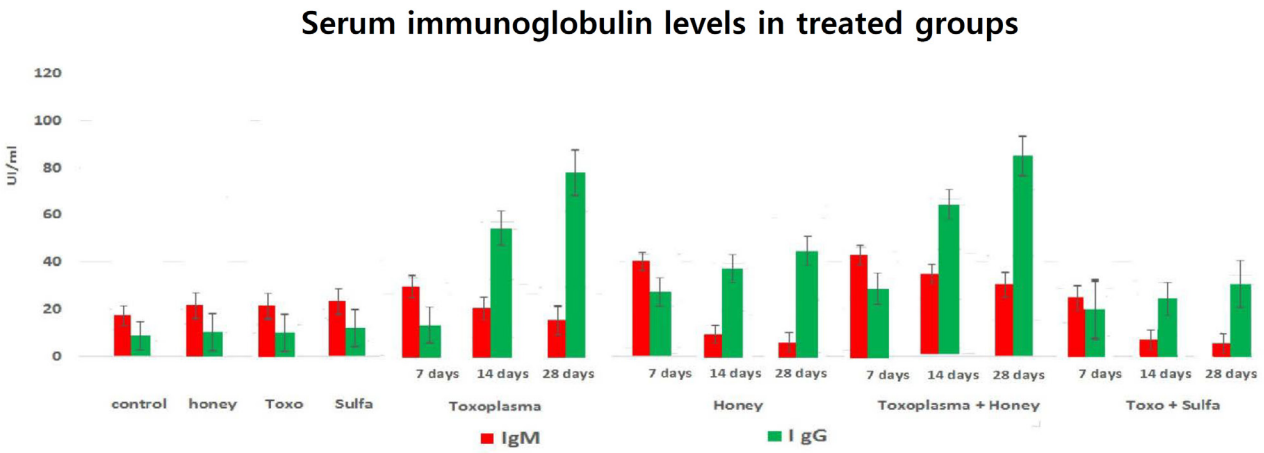

Figure 1 Serum immunoglobulin levels in the control and the treated groups.

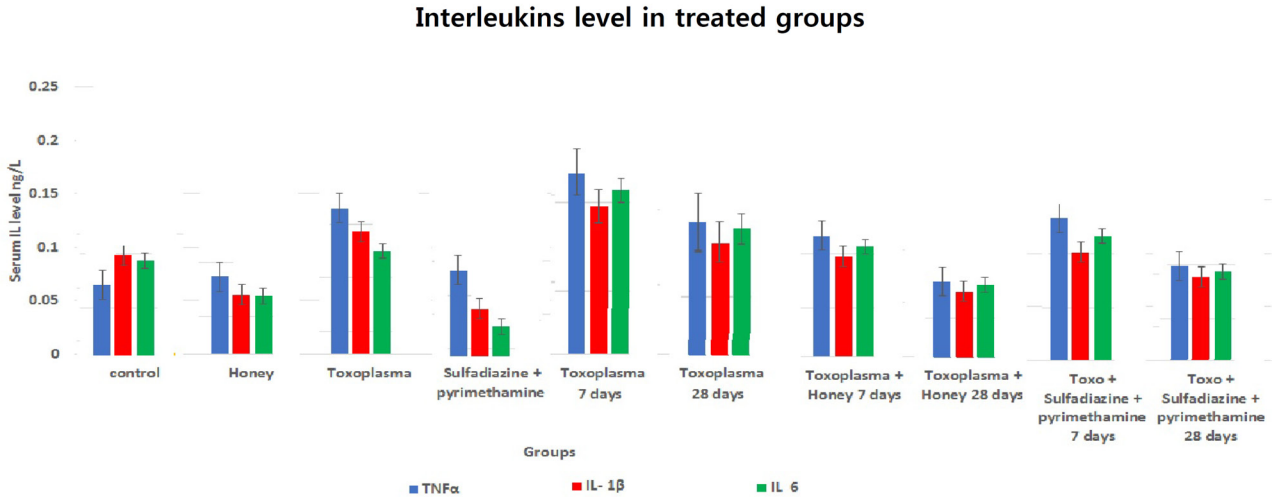

Figure 2 Levels of cytokines level in the control and the treated groups.

chronic toxoplasma infection in mice, the serum levels have been found to be elevated [36, 37]. The IgM- and the IgG-specific antibodies of rats infected with *T. gondii*, but not treated, were increased significantly, as were those of the infected rats treated orally with supplemented of 15% *Capparis spinosa* honey, compared to the values for rats in the healthy control and the honey control groups. Previous findings were observed by. [38] Who reported a rise in IgM first, followed by the IgA response [38]. A contrary to the findings of others, observed simultaneous rises in the IgM and the IgA responses [39]. The results obtained so far show that for orally-induced infection, the IgM and the IgA responses appear concomitantly, with less than a one-day lag between them [40].

A variety of biologically active compounds, such as flavonoids, vitamins, antioxidants and hydrogen peroxides, are present in honey [41]. Muhammad *et al* [42] found that consumption of honey daily had both positive and negative effect on male Wister albino rat. The major components of honey, chrysin, and other flavonoids exerted beneficial effects [43, 44]. The synergistic effects of the wide range of compounds present in honey are due to the antioxidant activity [16], hepatocytes protection [45], and anti-inflammatory activity [46]. Administration of honey to rats significantly reduced the TNF-α, IL-1β and IL-6 levels in rats infected with *T. gondii*. All kinds of honey significantly increased the TNF-α, IL-1β and IL-6 releases from MM6 cells (human monocytes) when compared with untreated and artificial-honey-treated cells ($P < 0.001$). Jelly bush honey significantly induced the maximal release of each cytokine compared with manuka, pasture, or artificial honey ($P < 0.001$). These results suggest that the effect of honey on wound healing may in part be related to the stimulation of inflammatory cytokines from monocytic

cells [58].

ELISAs were used to measure the serum cytokine levels for the infected and the non-infected rats in the control groups and for the infected rats treated either with honey or the standard treatment of sulfadiazine combined with pyrimethamine, and the results are shown in Fig. 2. From this figure, the serum cytokine levels of rats after infection with *T. gondii* showed significantly elevated levels of TNF-α, IL-1β and IL-6 levels compared to those of rats in the healthy, honey, standard treatment, and infected control groups. Moreover, administration of honey to rats significantly reduced the TNF-α, IL-1β and IL-6 levels compared to the values for the infected rats. The values represent means ± SEMs ($P < 0.05$). IL-lβ is important in the induction and the propagation of the inflammatory response and is intimately involved in the regulation of the acute phase response [36]. IL-6 levels may be intimately involved in the pathogenesis of *T. gondii* infection or be associated with an acute phase response [47, 48].

Neutrophils and inflammatory monocytes are important for the control of toxoplasmosis in the mouse [49]. In comparison to the serum cytokines of the rats in the healthy and the honey control groups, those of the control infected with *T. gondii* were increased significantly, as were those of infected rats treated with an oral supplement of 15% *Capparis spinosa* honey. Infected rats treated with a honey, supplement showed significantly increased cytokine levels compared with the levels in the healthy, the honey, and the standard treatment control groups. Infection with *T. gondii* is characterized by the development of acute hyperinflammation and lethal ileitis [50]. The primary function of the innate immune system is the detection of pathogens and the rapid activation of host defense mechanisms [51, 52]. The effects of honey chrysin and other flavonoids mediated the stimulation of TNF-α release, inhibited lipoprotein oxidation, and induced apoptosis [43, 44].

The authors of Ref [53] stated that macrophages could be able to limit parasite replication and produce cytokines that contributed to immunity, making them important regulatory and effector cells during toxoplasma infection. Neutrophils influence the T-cell response by enhancing the functions of dendritic cells [54] or inflammatory monocytes [55]. Infection of mice by using intraperitoneal (i.p.) inoculation with low amounts of a highly virulent strain of *T. gondii* or with a high inoculum of low-virulence strains resulted in the recruitment of neutrophils to the peritoneal cavity [56].

TNF-α and IL-1β have roles in the pathogenesis of many inflammatory diseases. IL-6 is considered to be an anti-inflammatory cytokine that inhibits the generation of TNF-α and augments the actions of acute-phase proteins and immunoglobulins [57]. Tonks *et al* [58] stated that TNF-α was a pleiotropic, pro-inflammatory cytokine, with the ability to affect almost every tissue and organ system. Both TNF-α and IL-1β stimulate the release of growth factors; these, in particular, PDGF and TGF-b, are chemotactic for monocytes and fibroblasts and maintain the activity of these cells [59].

Cytokines have been shown to play an important role in the pathogenesis of toxoplasmosis. The induction of inflammatory cytokine (IL-12, TNF-α, and IFN-γ) responses is a key event in the initiation of immunity to *T. gondii* [60]. Moreover, this pro-inflammatory context may lead to a modulation of immune responses, either directed against a parasite or unrelated antigens that develop in the host concomitantly with the infection. Early stimulation of macrophages also plays an important role in directing cell- mediated immunity because IL-12 promotes Th1-type acquired immunity, which is essential for controlling toxoplasma infection. A balance between IL-12 and IL-10 is, thus, essential for controlling toxoplasma infection [61]. IL-4 is secreted by type-2 lymphocytes. IL-4 alone does not appear to influence the intracellular growth of toxoplasma in vitro [62]. Pro-inflammatory mediators, such as IL-1β, IL-6 and TNF-α, may also act as amplification signals for immune cells [63]. The anti-inflammatory action of honey has been assessed [64], and cytokines have been found to be regulatory proteins that normally function as part of a complex interactive network [65].

5. Conclusion

The experimental observations made during this research clearly demonstrate that the oral administration of honey (*Capparis spinosa*) as a natural food additive increased the antibody titer and the levels of sera cytokines (IFN-γ, IL-1 and IL-6) in rats infected with *T. gondii*.

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Conflict of interest

The authors declare that they have no conflicts of interests.

References

1. Joynson DH, Wreghitt TG. Toxoplasmosis: a comprehensive clinical guide. United Kingdom: Cambridge University Press; 2001. 412 p.
2. McConkey GA, Martin HL, Bristow GC, Webster JP. *Toxoplasma gondii* infection and behaviorr - location, location, location?. J Exp Biol. 2103;216(1):113-9.
3. Dawkins R. The extended phenotype: the long reach of the gene. New York: Oxford University Press; 1999. 336 p.
4. Luder CG, Giraldo-Velasquez M. Sendtner M, Gross U. *Toxoplasma gondii* in primary rat CNS cells: differential contribution of neurons, astrocytes, and microglial cells for the intracerebral development and stage dif-

ferentiation. Exp Parasitol. 1999;93(1):23-32.
5. Vidotto O, Navarro IT, Mitsuka R, Freire RL. [Estudos epidemiológicos da toxoplasmose em suínos da região de Londrina, PR]. Semina Cien Agrárias. 1990;11(1):53-9. Portuguese.
6. Dubey JP. Advances in the life cycle of *Toxoplasma gondii*. Int J Parasitol. 1998;28(7):1019-24.
7. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. Int J Parasitol. 2000;30(12-13):1217-58.
8. Ruffolo BB, Toledo RS, Martins FDC, Bugni FM, Costa LD, Marana ERM, *et al*. Isolation and genotyping of *Toxoplasma gondii* in seronegative urban rats and presence of antibodies in communicating dog in Brazil. Rev Inst Med Trop Sao Paulo. 2016;58:28.
9. Molan P. Why honey is effective as a medicine. 2. the scientific explanation of its effects. Bee world. 2001;82(1):22-42.
10. Hegazi AG. Antimicrobial activity of different Egyptian honeys as comparison of Saudi Arabia honey. Research Journal of Microbiology. 2011;6(5):488-95.
11. Hegazi AG, Abd Allah FM. Antimicrobial activity of different Saudi Arabia honeys. Global Veterinaria. 2012;9(1):53-9.
12. Hegazi AG, FK Abd El Hady, MA El Ansari, F Abd Allah, NAM Saleh. Influence of storage on chemical composition and antimicrobial activity of Coriander (Coriandrum sativum) honey. Egypt J Vet Sci. 2002;36:17-30.
13. Hegazi AG, Moharm NZ, Allah FA, Nour MS, Khair AM. Antibacterial activity of different Egyptian honeys in relation to some bee products. Egypt J Vet Sci. 2002;36:31-42.
14. Al-Waili NS. Investigating the antimicrobial activity of natural honey and its effect on the pathogenic bacterial infection of surgical wounds and conjunctiva. J Med Food. 2004;7(2):210-22.
15. French VM, Cooper RA, Molan PC. The antibacterial activity of honey against coagulase-negative staphylococci. J Antimicrob Chemother. 2005;56(1):228-31.
16. Hegazi AG, Abd El Hady FK. Influence of honey on the suppression of human low density lipoprotein (LDL) peroxidation (*In vitro*): Evid Based Complement Alternat Med. 2009;6(1):113-21.
17. Hegazi A, Al Tahtawy RHM, Allah FA, Abdou AM. Antitumor and antioxidant activity of honey in mice bearing ehrlich ascites carcinoma. AJCR. 2014;7(3):208-14.
18. Hegazi A, Abdou AM. Allah FA. Influence of honey on immune response against newcastle disease vaccine. IJBAV. 2013;2(1):1-5.
19. Hegazi AG, Abdel-Rahman EH, Abd Allah F, Abdou AM. Influence of honey on immune status in mice-bearing ehrlich carcinoma. J Clin Cell Immunol. 2015;6(1):1000295.
20. Salkova D, Panayotova-Pencheva MS. Movsesyan SO. Some bee products as antiparasitic remedies. Russian Journal of Parasitology. 2015;75-8.
21. Azim MK, Perveen H, Mesaik MA, Simjee SU. Antinociceptive activity of natural honey in thermal-nociception models in mice. Phytother Res. 2007;21(2):194-7.
22. Mesaik MA, Azim MK, Mohiuddin S. Honey modulates oxidative burst of professional phagocytes. Phytother Res. 2008;22(10):1404-8.
23. Sajid M, Azim MK. Characterization of the nematicidal activity of natural honey. J Agric Food Chem. 2012;60(30):7428-34.
24. Hegazi AG, El-Fadaly HA, Barakat AM, Abou-El-Doubal SKA. *In vitro* effects of some bee products on *T. gondii* Tachyzoites. Global Veterinaria. 2014;13(6):1043-50.
25. Dubey JP. Advances in the life cycle of *Toxoplasma gondii*. Int J Parasitol. 1998;28(7):1019-24.
26. Elfadaly HA, Hassanain MA, Shaapan RM, Barakat AM, Toaleb, NI. Serological and hormonal assays of murine matern fetal *Toxoplasma gondii* infection with emphasis on virulent strains. World Journal of Medical Sciences. 2012;7(4):248-54.
27. Fadaly HA, Soror AH, Barakat AMA, El-Razik KAA. Zoonotic and histopathological aspects of various Toxoplasma gondii strains in femal rats. Afr J Infect Dis. 2015;9(2):32-8.
28. Stuart MC, Kouimtzi M, Hill SR. WHO Model Formulary 2008. Geneva: World Health Organization; 2009. 126 p. 205 p.
29. Hassan SE, Toaleb NI, Shaapan RM, Abdel-Rahman EH, Elmahallawy EK. Diagnosis of toxoplasmosis using affinity purified fraction of tachyzoites local isolate. Research Journal of Parasitology. 2016;11:13-9.
30. Daryani A, Hosseini AZ, Dalimi A. Immune responses against excreted/secreted antigens of *Toxoplasma gondii* tachyzoites in the murine model. Vet Parasitol. 2003;113(2):123-34.
31. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.
32. Voller A, Bidwell DE, Bartlett A, Fleck DG, Perkins M, Oladehin B. Microplate enzyme-immunoassay for toxoplasma antibody. J Clin Pathol. 1976;29(2):150-3.
33. Roberts CW, Cruickshank SM, Alexander J. Sex-determined resistance to *toxoplasma gondii* is associated with temporal differences in cytokine production. Infect Immun. 1995;63(7):2549-55.
34. Steel RGD. Principles and procedures of statistics: a biometrical approach. Michigan: McGraw-Hill; 1997. 666 p.
35. Remington JS, Krahenbuhl JL. Immunology of *Toxoplasma gondii*. compr. Immunol. 1982;9:327-71.
36. Chang HR, Grau GE, Pechere JC. Role of TNF and IL-1 in infection with *Toxoplasma gondii*. Immunology. 1990;69(1):33-7.
37. Beaman MH, Pearce MK, Abrams JS, Remington JS. Serum cytokine profile in lethal murine toxoplasmosis. proceed first. Quebec: Inter Congr Biological Response Modifiers; 1991. p. 61.
38. Favre G, Bessieres MH, Seguela JP. Dosage des IgA sdriques de la toxoplasmose par une methode ELISA. application a 120 cas. Bull Soc Fr Parasitol. 1984;3:139-42.
39. Turunen H, Vuorio KA, Leinikki PO. Determination of IgG, IgM and IgA antibody responses in human toxoplasmosis by enzyme-linked immunosorbent assay (ELISA). Scand J Infect Dis. 1983;15:307-11.
40. Pappas MG, Lunde MN, Hajkowski R, McMahon J. De-

termination of IgM and IgG antibodies to Toxoplasma using the IFA test, ELISA, and Dot-ELISA procedures. Vet Parasitol. 1986;20(1-3):31-42.
41. Mohammadzadeh S, Sharriatpanahi M, Hamedi M, Amanzadeh Y, Ebrahimi SES, Ostad SN. Antioxidant power of Iranian propolis extract. Food Chem. 2007;103(3):729-33.
42. Muhammad A, Oyeronke AO, Solomon EO, Michael AG, Muhammad IC, Farooq AD, *et al*. Daily consumption of honey: effects on male wister albino rats. International Journal of Food Nutrition and Safety. 2012;1(2):66-74.
43. Gheldof N, Wang XH, Engeseth NJ. Identification and quantification of antioxidant components of honeys from various floral sources. J Agric Food Chem. 2002;50(21):5870-7.
44. Woo KJ, Jeong YJ, Park JW, Kwon TK. Chrysin-induced apoptosis is mediated through caspase activation and Akt inactivation in U937 leukemia cells. Biochem Biophys Res Commun. 2004;325(4):1215-22.
45. Wilson JI, George BO, Umukoro GE. Effects of honey on the histology of liver in adult wistar rats. BLM. 2011;3(1):1-5.
46. Fiorani M, Accorsi A, Blasa M, Diamantini G, Piatti E. Flavonoids from Italian multifloral honeys reduce the extracellular ferricyanide in human red blood cells. J Agric Food Chem. 2006;54(21):8328-34.
47. Beaman MH, Abrams JS, Remington JS. Serum cytokine profile in acute and chronic toxoplasma infections of mice: evidence for the role of interleukin-6 in pathogenesis. 1994.
48. Hunter CA, Subauste CS, Remington JS. The role of cytokines in toxoplasmosis. Biotherapy. 1994;7(3-4):237-47.
49. Dunay IR, Fuchs A, David Sibley L. Inflammatory monocytes but not neutrophils are necessary to control infection with *toxoplasma gondii* in mice. Infect Immun. 2010;78(4):1564-70.
50. Gaddi PJ, Yap GS. Cytokine regulation of immunopathology in toxoplasmosis. Immunol Cell Biol. 2007;85(2):155-9.
51. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol. 2004;5(10):987-95.
52. Yarovinsky F, Sher A. Toll-like receptor recognition of *toxoplasma gondii*. Int J Parasitol. 2006;36(3):255-9.
53. Masek KS, Hunter CA. Pro-inflammatory responses in macrophages during *toxoplasma gondii* infection [Internet]. Madame Curie Bioscience Database. Available from: https://www.ncbi.nlm.nih.gov/books/NBK5976/.
54. van Gisbergen KP, Geijtenbeek TB, van Kooyk Y. Close encounters of neutrophils and DCs. Trends Immunol. 2005;26(12):626-31.
55. Soehnlein O, Drechsler M, Doring Y, Lievens D, Hartwig H, Kemmerich K, *et al*. Distinct functions of chemokine receptor axes in the atherogenic mobilization and recruitment of classical monocytes. EMBO Mol Med. 2013;5(3):471-81.
56. Mordue DG, Monroy F, La Regina M, Dinarello CA, Sibley LD. Acute toxoplasmosis leads to lethal overproduction of Th1 cytokines. J Immunol. 2001;167(8):4574-84.
57. Wheeler AP, Bernard GR. Treating patients with severe sepsis. N Engl J Med. 1999;340(3):207-14.
58. Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J, Tonks A. Honey stimulates inflammatory cytokine production from monocytes. Cytokine. 2003;21(5):242-7.
59. Slavin J. The role of cytokines in wound healing. J Pathol. 1996;178(1):5-10.
60. Jones LA, Alexander J, Roberts CW. Ocular toxoplasmosis: in the storm of the eye. Parasite Immunol. 2006;28(12):635-42.
61. Denkers EY, Gazzinelli RT. Regulation and function of T-cellmediated immunity during *toxoplasma gondii* infection. Clin Microbiol Rev. 1998;11(4):569-88.
62. R Appelberg, IM Orme, MI Pinto de Souza, MT Silva. *In vitro* effects of interleukin-4 on interferon-gamma induced macrophage activation. Immunology. 1992;76(4):553-9.
63. Thomson A, Lotze M. The cytokine handbook. Boston: Academic Press; 2003. p. 227-80.
64. Subrahmanyam M. A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. Burns. 1998;24(2):157-61.
65. Popa ID, Schiriac EC, Ungureanu D, Cuciureanu R. Immune response in rats following administration of honey with sulfonamides residues. Revista Română de Medicină de Laborator. 2012;20:63-72.