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Synergetic effect of Egyptian propolis in immunization of BALB/c mice against bovine cysticercosis

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PEER REVIEW

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Comments
This is a valuable research work in which authors have evaluated the synergetic effect of an ethanolic extract of propolis in immunization of mice with *T. saginata* crude antigen against bovine cysticercosis. They found that propolis extract can enhance the production of antibodies to antigen and recover liver and kidney functions.
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ABSTRACT

Objective: To evaluate the synergetic effect of an ethanolic extract of Egyptian propolis in immunization of BALB/c mice with *Taenia saginata* (*T. saginata*) crude antigen against bovine cysticercosis, with reference to its effects on liver and kidney functions.

Methods: Sixty female mice BALB/c strain weighing 20 to 25 g and 6-8 weeks old were randomly allocated into six groups of ten mice each. Mice in groups 1 and 2 (G1 and G2) were immunized intraperitoneally with 100 μg of *T. saginata* crude antigen in 100 μL phosphate buffer saline emulsified in Freund's adjuvant. Besides, the mice in G2 were administered with propolis extract simultaneously with immunization. Control mice were either administered with propolis extract (G3) or injected with the same volume of phosphate buffer saline emulsified in Freund's adjuvant (G4). The mice in G5 were non-immunized infected control while, those in G6 were non-immunized non-infected control. Two weeks after the last immunization, each mouse was challenged intraperitoneally with 5000 oncospheres except those of G6. Ethanolic extract of propolis was prepared at a dose 50 mg/kg body weight.

Results: After 24 weeks of challenge, the mice in G2 showed the highest level of protection (100%), with no cyst being detected rather than mice in G1 (33.3% protection). Additionally, the ELISA results, in this study, showed higher antibody titer in G2 with reduction the alteration in liver and kidney functions compared to G1.

Conclusions: Egyptian propolis could increase the level of protection against experimental challenge infection with *T. saginata* eggs when administered simultaneously with immunization. Furthermore, it could enhance the production of antibodies to immunized antigen and decrease the alteration in liver and kidney functions.

KEYWORDS
Taenia saginata, Vaccine, BALB/c mice, ELISA, Serum biochemistry

1. Introduction

Taenia saginata (*T. saginata*) is a medically and economically important cestode parasite. Typically, cattle are the intermediate hosts in which the larva or cysticercus encysts in striated muscle. Infection in beef carcases causes economic loss because the carcase is being down-graded and condemned, or special treatment is required to kill the parasite before sale for human consumption[1]. Humans are the definitive host for *T. saginata*, where infection is acquired by ingestion of insufficiently cooked beef meat containing viable cysticerci[2]. Immunity plays an important role in the natural regulation of transmission of taeniid cestodes in their intermediate hosts[3]. Intermediate hosts can be protected against infection by vaccination with nonliving antigens of the parasite, especially

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antigen derived from oncospheres which present in eggs of *T. saginata*[4]. Control of these taeniid parasites essentially focuses on interruption of egg (oncosphere) transmission, with vaccination of the intermediate host which is being a feasible option[5].

Many investigators had reported on the use of immune stimulants for enhancement of the immune response during vaccination[6,7]. Propolis or "bee glue" was a resinous hive product collected by honey bees from exudates and buds of plants and mixed with wax and bee enzymes[8]. The chemical composition of raw Egyptian propolis sample (collected from Dakahlia Governorate) as investigated by gas chromatography-mass spectrometer revealed that 65 compounds were identified, such as aromatic acids: benzoic, cinnamic, trans-p-coumaric, 3,4- dimethoxycinnamic, ferulic and caffeic acids. Of the 19 esters identified, Egyptian propolis contained 11 caffeate esters including two new to propolis, tetradecenyl caffeate (isomer) and tetradecanyl caffeate. Egyptian propolis contained some new triterpenoids including lupeol and alpha-amyrin. It also contained flavonoids, sugar, and aliphatic acids. The investigators stated that Dakahlia propolis sample was a typical popular propolis[9]. The composition of the propolis depended upon the time, vegetation and the area of collection[10]. Propolis had been reported to have immunostimulator and immunomodulator activities, in addition to many different biological and pharmacological properties of its different preparations[11-13]. Therefore, the present work was adopted to evaluate the synergetic effect of an ethanolic extract of Egyptian propolis in immunization of BALB/c mice with *T. saginata* crude antigen against bovine cysticercosis, with reference to its effects on liver and kidney functions.

2. Materials and methods

This study was carried out according to guidelines for animal experimentation and approved by the Institutional Animal Care and Use Committee, National Research Center, Animal Care Unit, Dokki, Giza, Egypt.

2.1. T. saginata worms, eggs and oncospheres

T. saginata worms and eggs were obtained from infected patients with taeniasis in Assuit hospital, Assuit governorate, Egypt. Tape worms were washed thoroughly in tap water and kept at room temperature overnight. They were identified as *T. saginata* according to Verster[14], and kept in antibiotic saline (0.85% sodium chloride containing 1 000 IU/mL penicillin, 1 000 μg/mL sterptomycine, and 100 IU/mL mycostain) at 4 ℃ prior to isolation of eggs. Eggs were isolated from mature proglottides and kept in antibiotic saline at 4 ℃ for up to 4 weeks prior to challenge infection of mice. *In vitro* hatching of oncospheres was carried out by sodium hypochlorite at 56 ℃ (0.5% in normal saline) according to Ito *et al*.[15] and Wang *et al*[16]. The viability of the oncospheres was assessed by a microscopic examination using 0.4% Trypan blue solution[16].

2.2. Preparation of T. saginata crude antigen

Crude antigen of whole *T. saginata* worms was prepared by homogenization and sonication of worms in phosphate buffer saline (PBS)[4]. The protein content was estimated[17].

2.3. Propolis

Propolis sample was collected from beehives located in Dakahlia Governorate, Egypt. The sample was kept in the dark and stored at -20 ℃ up to its processing.

2.4. Ethanolic extract of propolis

Twenty-five grams of the resinous material of Egyptian propolis was cut into small pieces and extracted at room temperature with 100 mL of 80% ethanol. The extraction was performed twice with 24 h interval. The alcoholic extract was evaporated under vacuum at 50 ℃ until dryness. Dried ethanolic extract (yield 7 g) of propolis was suspended in PBS (pH 7.2)[9]. The dose of propolis used was 50 mg/kg body weight.

2.5. Vaccination trial

2.5.1. Animals

Sixty female mice BALB/c strain weighing 20 to 25 g and 6-8 weeks old were purchased from the Animal House, Theodor Bilharz Research Institute, Giza, Egypt. Animals were housed in a well-ventilated animal room under standardized conditions of 24 ℃; relative humidity (50 ± 5)% and 12 h light/dark cycle at the Animal House, National Research Center, Giza, Egypt. All nutrients including water were supplied *ad libitum* to meet the requirements of the NRC[18]. Mice were acclimatized for 15 d before the start of the experiment. According to our pilot study, the BALB/c mice were susceptible to the experimental infection.

2.5.2. Vaccination protocols

Sixty female BALB/c mice were randomly allocated into six groups of ten mice each. Mice in groups 1 and 2 (G1 and G2) were immunized intraperitoneally with 100 μg of *T. saginata* crude antigen in 100 μL PBS emulsified in Freund's adjuvant for three times (first immunization in Freund's complete adjuvant at zero week and followed by two booster immunizations in Freund's incomplete adjuvant at the 2nd and the 4th weeks). Besides, the mice in G2 were administered with propolis extract simultaneously with immunization. Control mice were either administered with propolis extract (G3) or injected with the same volume of PBS emulsified in Freund's adjuvant (G4). The mice in G5 were non-immunized infected control while, those in G6 were non-immunized non-infected control. Two weeks after the last immunization, each mouse was challenged intraperitoneally with 5 000 oncospheres except those of G6. All mice were killed at the 24th week post challenge

and the internal organs were examined for cysts. The protection was calculated as follow:

$$\text{Protection\%} = 1 - \frac{\text{Mean No. cysts in test group}}{\text{Mean No. cysts in control group}} \times 100$$

2.6. Blood sampling

At the end of the experiment of the vaccination trail (24 weeks), blood samples were collected by retro-orbital venous plexus puncture from each mouse (n=5) in the early morning before the diet was administered. Each blood sample was placed in a plain centrifuge tube for serum separation. The serum samples were stored at -20 ℃.

2.7. ELISA

For detection of anti-*T. saginata* IgG at 24 weeks post challenge, the ELISA was done as described by Kandil *et al*.[1]. The wells of polyvinyl plates were coated with 5 μg/mL of antigen diluted in 0.1 mol/L carbonate buffer (pH 9.6) at rate of 100 μL per well and incubated overnight at 4 ℃. Plate was washed three times with PBS containing 0.01% Tween-20. Blocking of excess-binding sites was performed by incubation with 0.1% bovine serum albumin in 0.01 mol/L PBS for 1.5 h at 37 ℃. After washing, 100 μL of mice sera (diluted 1:100 in PBS) were added, followed by incubation for 1.5 h at 37 ℃. After washing, 100 μL of 1:1 000 peroxidase conjugate anti-mouse IgG (Sigma) were added to each well and incubated at 37 ℃ for 2 h. Finally, the ortho-phenylene diamine substrate was added to each well and after 15 min incubation in the dark at room temperature, the reaction was stopped with 1 mol/L H_2SO_4. The plates were read at 490 nm by ELISA reader.

2.8. Biochemical assessments

Albumin levels[19] as well as the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT)[20], were determined. The levels of total cholesterol[21], triglycerides[22], blood urea[23] and creatinine[24] were also determined. The used test kits were supplied by bioMérieux, France.

2.9. Statistical analysis

All data were subjected to statistical analysis including the calculation of the mean ± SE. Differences between data of biochemical parameters in different groups of mice were tested for significance using One-way ANOVA followed by Duncan's multiple range test. Differences were considered significant at P<0.05 level[25] using Statistical Package for Social Science (SPSS) for windows version 15 computer program.

3. Results

3.1. Postmortem findings

After 24 weeks of challenge, the non-immunized infected control mice (G5) and those received only adjuvant (G4) showed cysts of *T. saginata*, as well as enlarged and congested liver and spleen (Figure 1). While, immunization with crude antigen and adjuvant simultaneously

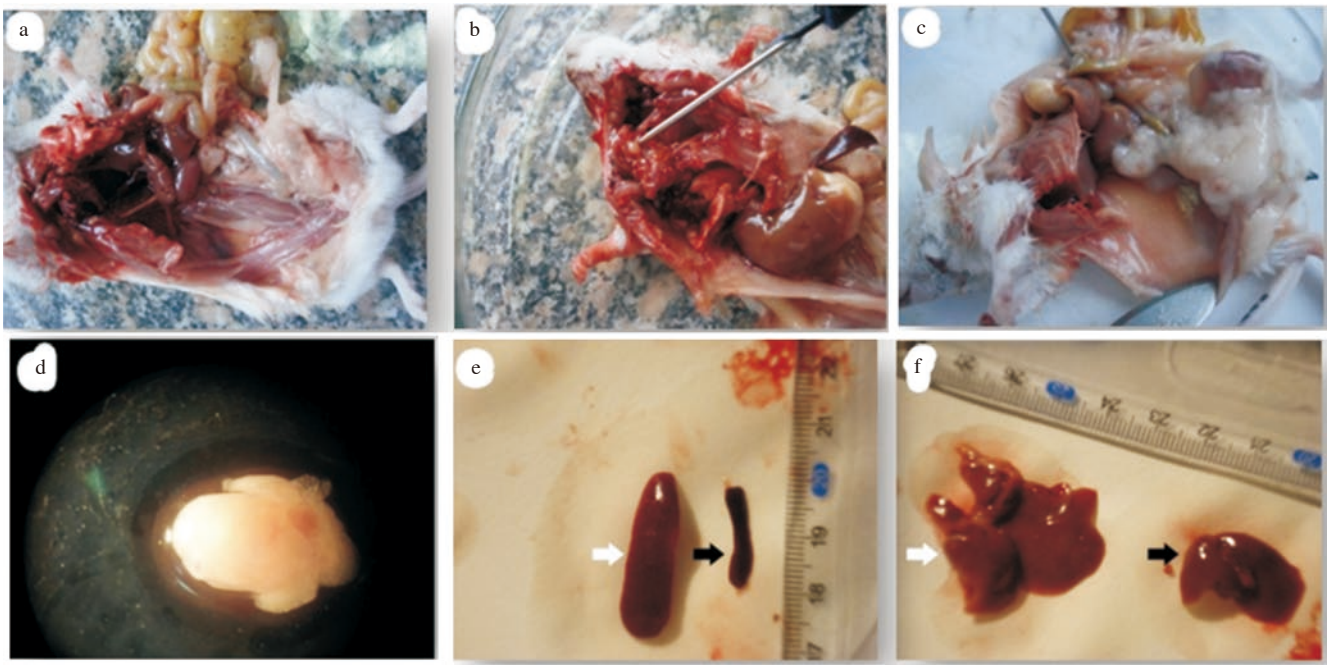

Figure 1. Macrographs of internal organs of immunized and non-immunized BALB/c mice at the 24th week post challenge infection with *T. saginata* eggs. a: Non-immunized non-infected control; b: Immunized with crude antigen of *T. saginata* and adjuvant (G1) showing cyst; c: Immunized with crude antigen and adjuvant simultaneously with propolis administration (G2) showing normal internal organs; d: Light micrograph of cyst (×4); e: Enlarged spleen of mice in G1 (white arrow) compared to those of mice in G2 (black arrow); f: Enlarged liver of mice in G1 (white arrow) compared to those of mice in G2 (black arrow).

with propolis administration (G2), achieved the highest level of protection (100%), with no cyst being detected rather than antigen with adjuvant (33.3% protection). At the same time, G3 that received only propolis extract had fewer cysticerci compared with the infected control group and showed moderate level of protection (58.4%) against experimental challenge infection with *T. saginata* eggs. All groups and challenge infection were shown in Table 1.

Table 1

The average number of *T. saginata* cysticerci in the internal organs in each group of mice.

Groups	Average number of *T. saginata* cysticerci						Infection%	Protection%
	Thoracic cavity	Heart	Spleen	Lung	Liver	Total		
Group 1	0	4	Congested	3	1	8	66.7	33.3
Group 2	0	0	0	0	0	0	0.0	100.0
Group 3	0	2	0	2	1	5	41.6	58.4
Group 4	Necrotic foci	4	Congested	5	2	11	91.7	8.3
Group 5	Necrotic foci	5	Congested	3	4	12	100.0	0.0
Group 6	0	0	0	0	0	0	0.0	-

3.2. Humeral immune response

The anti-*T. saginata* antibody titers (ELISA optical density (OD) at 490 nm) in all groups at the 24th week post challenge were shown in Figure 2. The greatest antibody titers were seen in the group immunized with crude antigen with adjuvant simultaneously with propolis extract administration (Group 2) followed by those received only propolis extract (Group 3). While, sera from non-immunized control animals (Group 4 and Group 5) and animals immunized with crude antigen with adjuvant gave equivalent OD values in the ELISA.

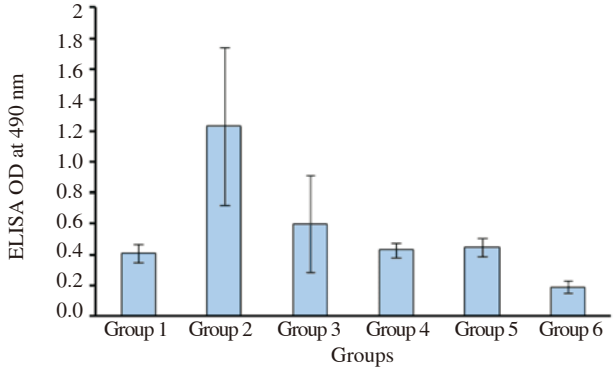

Figure 2. Anti-*T. saginata* antibody titers in all groups at the 24th week post challenge.

3.3. Biochemical changes

The mean values of serum biochemical parameters for all groups of experimental vaccination trial were shown in Table 2.

3.3.1. Serum proteins

After 24 weeks of challenge, total serum proteins and globulins concentrations had a marked (P<0.01) increase in the all immunized and infected groups of mice compared to normal control (non-immunized and non-infected) group (G6). However, there was a significant decrease (P<0.01) in the serum albumin level in the mice received both antigen and adjuvant (G1), or adjuvant only (G4) besides, non- immunized group (G5) compared to normal control group (G6). The values of albumin/globulin (A/G) were markedly decreased (P<0.01) in all infected groups (G1 to G5) in comparison with normal non-infected control group (G6) (Table 2).

3.3.2. Serum enzymes

The activity of serum AST significantly (P<0.01) increased in groups of mice; immunized with adjuvant and antigen (G1), immunized with adjuvant only (G4) as well as non- immunized (G5) in comparison with groups of mice; immunized with antigen and adjuvant simultaneously with propolis administration (G2), those received only propolis extract (G3) and normal non-infected control (G6). While the ALT activity was markedly (P<0.01) increase in two groups of mice (G4, G5) compared to the other groups (G1, G2, G3 and G6) (Table 2).

3.3.3. Serum lipids

Serum total cholesterol was significant decrease (P<0.05) in G2, G3 and G5 compared to the other groups (G1, G4 and G6). However, serum triglycerides exhibited a gradual but significant decrease (P<0.01) in all infected groups compared to the normal non-infected control group (G6) (Table 2).

3.3.4. Serum urea and creatinine

The immunized groups (G1, G2, G4) and non-immunized infected group (G5) exhibited a marked increase (P<0.01) in serum urea level

Table 2

Serum biochemical changes of BALB/c mice after challenge infection in different groups of vaccination trail with *T. saginata* antigen (Mean ± SE, N=5).

Groups parameters	Complete adjuvant + Antigen (G1)	Complete adjuvant + propolis + Antigen (G2)	Propolis only (G3)	Complete adjuvant only (G4)	Control infected (G5)	Control uninfected (G6)	Significance
Total proteins (g/dL)	6.60 ± 0.12^{ab}	7.15 ± 0.10^{a}	7.00 ± 0.07^{a}	5.69 ± 0.31^{cd}	6.13 ± 0.27^{bc}	5.38 ± 0.06^{d}	**
Albumin (g/dL)	3.15 ± 0.05^{bc}	3.65 ± 0.06^{a}	3.73 ± 0.09^{a}	3.01 ± 0.09^{c}	3.26 ± 0.08^{b}	3.51 ± 0.06^{a}	**
Globulins (g/dL)	3.45 ± 0.10^{a}	3.50 ± 0.05^{a}	3.28 ± 0.08^{ab}	2.68 ± 0.27^{c}	2.88 ± 0.10^{bc}	1.87 ± 0.01^{d}	**
A/G ratio	0.91 ± 0.03^{c}	1.04 ± 0.02^{bc}	1.14 ± 0.05^{bc}	1.16 ± 0.10^{bc}	1.19 ± 0.15^{b}	1.89 ± 0.04^{a}	**
AST (IU/L)	164.52 ± 4.60^{c}	155.86 ± 7.75^{cd}	135.24 ± 4.97^{e}	188.50 ± 6.18^{b}	209.78 ± 7.13^{a}	141.15 ± 0.27^{de}	**
ALT (IU/L)	65.96 ± 3.33^{c}	51.43 ± 1.26^{d}	41.03 ± 1.04^{e}	127.24 ± 5.41^{a}	76.79 ± 1.71^{b}	64.68 ± 1.52^{c}	**
Total cholesterol (mg/dL)	92.18 ± 5.79^{a}	66.32 ± 4.05^{cd}	57.64 ± 1.70^{d}	75.20 ± 2.41^{bc}	59.84 ± 3.16^{d}	82.15 ± 2.07^{ab}	*
Triglycerides (mg/dL)	122.83 ± 9.50^{b}	88.22 ± 2.03^{de}	78.96 ± 2.71^{e}	95.89 ± 5.06^{cd}	106.12 ± 0.54^{c}	156.23 ± 4.90^{a}	**
Urea (mg/dL)	58.06 ± 2.11^{b}	45.18 ± 1.31^{c}	36.49 ± 1.70^{d}	43.54 ± 1.99^{c}	64.46 ± 2.70^{a}	34.90 ± 1.94^{d}	**
Creatinine (mg/dL)	0.72 ± 0.03^{a}	0.65 ± 0.01^{a}	0.46 ± 0.11^{a}	0.54 ± 0.03^{a}	0.62 ± 0.06^{a}	0.59 ± 0.02^{a}	NS

Means with different superscripts in the same row are significantly different at P<0.05; *: P<0.01; **: P<0.01.

in comparison with G3 and G6. On the other hand, there was no significant difference in serum creatinine levels between all groups of mice (Table 2).

4. Discussion

An alternative approach for the control of cysticercosis due to *T. saginata* is the use of vaccines in cattle. In order to perform vaccination studies against *T. saginata* there must be enough eggs accessible to challenge infected cattle, eggs that can only be obtained from human beings with taeniosis. Probably this has been the greatest difficulty for performing such studies and the reason why there are so few publications in this respect. Also, because of the difficulty in handling large animals, immunesuppressive mice were first introduced for studying this infection by Machnicka and Smyth[26]. Cysticercosis of *Taenia solium*, *T. saginata* asiatica and *T. saginata* was successfully established in severe combined immune deficiency mice[27,28]. A previous study showed that BALB/c mice were suitable for developing an experimental model of oncosphere infection[29]. Viable cysticerci were found in the BALB/c mice from the 8th week to 20th week post-infection. Immunity played a central role in the regulation of transmission of taeniid cestodes through their intermediate hosts. Concomitant immunity was a prominent feature of infection with all taeniid cestodes which had been investigated. Furthermore, in most species immunity was transferred with colostral antibody from the dam. These features had favored the development of practical vaccines against *Taenia* in their intermediate hosts[30]. In the present study, immunization of mice with crude antigen of *T. saginata* worms induced level of protection against experimental challenge infection with *T. saginata* eggs; up to 33.3% protection was achieved. Indeed, there were many studies in rodents, ovine and bovines that demonstrated that it was possible to acquire protection against cysticercosis by vaccination. In most studies crude antigens had been used which were obtained from oncospheres, cysticerci or tapeworms[30,31]. Various degrees of protection had been reported; it had been demonstrated that living oncospheres and oncospheral antigens were the most effective[32]. Attempts were done to increase the level of protection against cysticercosis. The use of recombinant proteins and DNA as vaccines against rodent, ovine and bovine cysticercosis could induce high degrees of immunity[33-35]. While, the present study could increase the level of protection against experimental challenge infection with *T. saginata* eggs via immunization with crude antigen of whole *T. saginata* worms simultaneously with propolis administration, so that no cysts could be detected. Several articles had provided information of propolis influence on the immune system[36]. As to the immunomodulatory action of propolis, the administration of ethanolic extract of propolis (200 mg/kg) to mice for 3 d enhanced the innate immunity, activating the initial steps of the immune response by up regulating TLR-2 and TLR-4 expression and pro-inflammatory cytokines (IL-1 and IL-6) production by macrophages and spleen cells, contributing to the recognition of microorganism and to lymphocytes activation by antigen presenting cells[36]. Propolis (2.5 and 5.0 mg/kg) also increased hydrogen peroxide generation, favoring the microorganisms killing[37]. Additionally, the ELISA results, in this study, showed higher antibody titer in group of mice immunized with crude antigen of whole *T. saginata* worms simultaneously with propolis administration compared to the other group immunized with crude antigen alone. Indeed, there was evidence that administration of propolis (extracts or ingredients) affected both specific and nonspecific arms of the immune system in mouse, including an increase of antibody response[38,39].

Regarding the serum proteins; total serum proteins and globulins concentrations had a marked increase in the all immunized and infected groups of mice. In this study the increase in total serum proteins reflected an increase in the globulins, particularly immunoglobulins[40]. It might be due to increase in γ-globulin which appeared to be in response to the antigenic stimulation of the infectious agent, kidney damage, or myocarditis[41,1].

Albumin levels decreased in the serum of mice received adjuvant only (G4) as well as non-immunized infected group (G5), or group of mice immunized with both antigen and adjuvant (G1). This result might be attributed to hepatic dysfunction that was induced by the presence of large amount of *T. saginata* cysticerci in the organs[42].

The AST activity was increased in serum of mice G1, G4 and G5, while ALT activity was increased in serum of mice G4 and G5. The increase in AST indicated soft-tissue damage/necrosis while alterations in ALT indicated liver damage[40]. In the present study, administration of propolis with or without immunization caused a reduction of AST and ALT activities as well as urea, cholesterol and triglycerides levels. This result might be due to hepato- and renal- protective effects of propolis against liver and kidney damaged[6,13,43,44]. This study revealed that there were changes in the ALT activity, total cholesterol, urea and creatinine of infected mice with *T. saginata* viable eggs.

Serum lipids revealed that the administration of propolis extract with or without immunization with *T. saginata* antigen had a lowering effect on serum total cholesterol and triglycerides which might be attributed to the presence of flavonoids, steroids, phenolic acids and their esters among propolis constituents[45,13]. The hypocholesterolemic effect of propolis could be a result of a direct effect on the liver or an indirect effect through thyroid hormones which affected reactions in almost all the pathways of lipid metabolism[46]. Total cholesterol revealed high concentration in serum of infested mice. Cholesterol might have a role in pathogenesis by helping the larvae to survive in the host tissues or it might be due to the break in the liver function and changes the hormone secretion which provoked by the presence of parasite. Cholesterol enhanced larval survival, development and growth[47,1].

In conclusion, the current study indicated that Egyptian propolis could increase the level of protection against experimental challenge infection with *T. saginata* eggs when administered simultaneously with immunization. Furthermore, it could enhance the production of antibodies to immunize antigen and decrease the alteration in liver and kidney functions.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

T. saginata is a medically and economically important cestode parasite. Immunity plays an important role in the natural regulation of transmission. It has been reported that use of immune stimulants for enhancement of the immune response during vaccination. Propolis has immunostimulator and immunomodulator activities. In this work, the authors evaluated the synergetic effect of an ethanolic extract of Egyptian propolis in immunization of mice with *T. saginata* crude antigen against bovine cysticercosis.

Research frontiers

Until recently, there are few reports discussing about the immune function of propolis extract during vaccination.

Related reports

Propolis had been reported to have immunostimulator and immunomodulator activities in several disease models, such as tumor immunity, *Fasciola gigantica* infection, *Cryptosporidium* spp infection, *ect*.

Innovations and breakthroughs

In this paper, the authors describe that propolis extract could increase the level of protection against *T. saginata* infection via enhancing antibody production. This argument is kind of novel.

Applications

T. saginata is a medically and economically important cestode parasite. It is essential to use the immune stimulants for enhancement of the immune response during vaccination. Here, the authors found that propolis extract could increase the level of protection against *T. saginata* infection via enhancing antibody production and recover liver and kidney function.

Peer review

This is a valuable research work in which authors have evaluated the synergetic effect of an ethanolic extract of propolis in immunization of mice with *T. saginata* crude antigen against bovine cysticercosis. They found that propolis extract can enhance the production of antibodies to antigen and recover liver and kidney functions.

References

[1] Kandil OM, Nasr SM, Mahmoud MS, Nassar SA, El-Metanawey TM, Abd El-Aziz MHM, et al. Serological and biochemical studies on cattle naturally infested with *Taenia saginata* cysticercosis. *Glob Vet* 2012; **9**: 571-9.

[2] Flisser A, Madrazo I, Delgado H. *Cisticercosis humana.* México: El Manual Moderno; 1998.

[3] Rickard MD, Willams JF. Hydatidosis/cysticercosis: immune mechanisms andimmunization against infection. *Adv Parasitol* 1982; **21**: 229-96.

[4] Lightowlers MW, Rolfe R, Gaucci CG. *Taenia saginata*: vaccination against cysticercosis in cattle with recombinant oncosphere antigens. *Exp Parasitol* 1996; **84**: 330-8.

[5] Benitez L, Garate T, Harrison LJ, Kirkham P, Brookes SM, Parkhouse RM. Cloning and sequencing of the gene encoding the principal 18-kDa secreted antigen of activated oncospheres of *Taenia saginata*. *Mol Biochem Parasitol* 1996; **78**: 256-8.

[6] Nassar SA, Mohamed AH, Soufy H, Nasr SM, Mahran KM. Immunostimulant effect of Egyptian propolis in rabbits. *Scientific World Journal* 2012; doi:10.1100/2012/901516.

[7] Nassar SA, Mohamed AH, Soufy H, Nasr SM. Protective effect of Egyptian propolis against rabbit pasteurellosis. *Biomed Res Int* 2013; doi: 10.1155/2013/163724.

[8] Burdock GA. Review of the biological properties and toxicity of bee proplis (propolis). *Food Chem Toxicol* 1998; **36**: 347-63.

[9] Hegazi AG, Abd El Hady FK, El Menway FA, Al Mehdar H. Egyptian propolis: 5 influence of propolis adminestration on some chicken biochemical parameters. *J Appl Vet Sci NRC* 2004; **1**: 43-58.

[10] Banskota AH, Tezuka Y, Adnyana IK, Midorikawa K, Matsushige K, Message D, et al. Cytotoxic, hepatoprotective and free radical scavenging effects of propolis from Brazil, Peru, the Netherlands and China. *J Ethnopharmacol* 2000; **72**: 239-46.

[11] El-khawaga OA, Salem TA, Elshal MF. Protective role of Egyptian propolis against tumor in mice. *Clin Chim Acta* 2003; **338**: 11-6.

[12] Hegazi AG, Abd El Hady FK, Shalaby HA. An *in vitro* effect of propolis on adult worms of *Fasciola gigantica*. *Vet Parasitol* 2007; **144**: 279-86.

[13] Abd El-Aziz TH, El-Beih NM, Soufy H, Nasr SM, Khalil FAM, Sharaf M. Effect of Egyptian propolis on lipid profile and oxidative status in comparison with nitazoxanide in immunosuppressed rats infected with *Cryptosporidium spp. Glob Vet* 2014; **13**: 17-27.

[14] Verster A. A taxonomic revision of the genus *Taenia* Linnaeus, 1758 S. str. *Onderstepoort J Vet Res* 1969; **36**: 3-58.

[15] Ito A, Yamada T, Ishiguro T. Vaccination of rats with frozen eggs, ethanol-fixed eggs and frozen oncospheres with or without embryophoric blocks of *Taenia taeniaeformis*. *Jpn J Parsitol* 1996; **45**: 330-2 .

[16] Wang IC, Ma YX, Kuo CH, Fan PC. A comparative study on egg hatching methods and oncosphere viability determination for *Taenia solium* eggs. *Int J Parasitol* 1997; **27**: 1311-4.

[17] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; **193**: 265-75.

[18] Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council). *Nutrient requirements of laboratory animals*. 4th revised ed. Washington, D.C: National Academy Press; 1995.

[19] Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 1971; **31**: 87-96.

[20] Reitman SMD, Frankel S. A colorimeter method for determination of serum glutamic oxaloacetic acid and glutamic pyruvic acid transferases. *Am J Clin Pathol* 1957; **28**: 56-63.

[21] Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; **20**: 470-5.

[22] Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 1982; **28**: 2077-80.

[23] Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for determination of ammonio. *Anal Chem* 1977; **49**: 464-9.

[24] Siest G. *Interpretation of clinical laboratory tests: reference values and their biological variation*. Oxford: Butterworth-Heinemann Ltd; 1985.

[25] Snedecor GW, Cochran WG. *Statistical methods*. 8th ed. Ames: Iowa State University Press; 1989, p. 503.

[26] Machnicka BS, Smyth JD. The early development of larval *Taenia saginata in vitro* and *in vivo*. *Acta Parasitol Pol* 1985; **30**: 47-52.

[27] Ito A, Ito M, Eom KS, Chung WC, Chen CC, Ma L, et al. *In vitro* hatched oncospheres of Asian *Taenia* from Korea and Taiwan develop into cysticerci in the peritoneal cavity of female SCID (severe combined immunodeficiency) mice. *Int J Parasitol* 1997; **27**: 631-3.

[28] Ito A, Ito M. Human *Taenia* in severe combined immunodeficiency (SCID) mice. *Parasitol Today* 1999; **15**: 64-7.

[29] Kandil OM, Nassar SA, Nasr SM, Fahmy HA, Mahmoud MS, El Nemaky AH, et al. Biochemical and histopathological changes in Balb/C mice-as an experimental animal model-infected with *Taenia saginata* oncospheres. *Glob Vet* 2013; **11**: 528-33.

[30] Lightowlers MW, Rickard MD. Vaccination against cestode parasites. *Immunol Cell Biol* 1993; **71**: 443-51.

[31] Flisser A, Pérez-Montfort R, Larralde C. The immunology of human and animal cysticercosis: a review. *Bull World Health Organ* 1979; **57**: 839-56.

[32] Lightowlers MW. Vaccination against animal parasites. *Vet Parasitol* 1994; **54**: 177-204.

[33] Johnson KS, Harrison GB, Lightowlers MW, O'Hoy KL, Dempster RP, Lawrence SB, et al. Vaccination against ovine cysticercosis using a defined recombinant antigen. *Nature* 1989; **338**: 585-7.

[34] Harrison GB, Heath DD, Dempster RP, Gaucci C, Newton SE, Cameron WG, et al. Identification and cDNA cloning of two novel low molecular weight host-protective antigens from *Taenia ovis* oncospheres. *Int J Parasitol* 1996; **26**: 195-204.

[35] Rosas G, Cruz-Revilla C, Fragoso G, López-Casillas F, Pérez A, Bonilla MA, et al. *Taenia crassiceps* cysticercosis: humoral immune response and protection elicited by DNA immunization. *J Parasitol* 1998; **84**: 516-23.

[36] Orsatti CL, Missima F, Pagliarone AC, Bachiega TF, Bufalo MC, Araujo JP Jr., et al. Propolis immunomodulatory action *in vivo* on Toll-like receptors 2 and 4 expression and on pro-inflammatory cytokines production in mice. *Phytother Res* 2010; **24**: 1141-6.

[37] Orsi RO, Funari SRC, Soares AMVC, Calvi SA, Oliveira SL, Sforcin JM, et al. Immunomodulatory action of propolis on macrophage activation. *J Venom Anim Toxins* 2000; **6**: 205-19.

[38] Scheller S, Gazda G, Pietsz G, Gabrys J, Szumlas J, Eckert J, et al. The ability of ethanol extract of propolis to stimulate plaque formation in immunized mouse spleen cells. *Pharmacol Res Commun* 1988; **20**: 323-8.

[39] Park JH, Lee JK, Kim HS, Chung ST, Eom JH, Kim KA, et al. Immunomodulatory effect of caffeic acid phenethyl ester in Balb/c mice. *Int Immunopharmacol* 2004; **4**:4 29-36.

[40] Kaneko JJ, Harvey JW, Bruss ML, editors. *Clinical biochemistry of domestic animals.* 5th ed. California: Academic Press; 1992.

[41] Chapman CB, Knopf PM, Hicks JD, Mitchell GF. IgG_1 hypergamma globulinaemia in chronic parasitic infections in mice: magnitude of the response in mice infected with various parasites. *Aust J Exp Biol Med Sci* 1979; **57**: 369-87.

[42] Scandrett B, Parker S, Forbes L, Gajadhar A, Dekumyoy P, Walkagul J, et al. Distribution of *Taenia saginata* cysticerci in tissues of experimentally infected cattle. *Vet Parasitol* 2009; **164**: 223-31.

[43] Eraslan G, Kanbur M, Silici S. Evaluation of propolis effects on some biochemical parameters in rats treated with sodium floride. *Pestic Biochem Physiol* 2007; **88**: 273-83.

[44] Atta AH, Nasr SM, Mouneir SM, Abdel-Aziem SH, Nassar SA. Egyptian propolis alleviates gentamicin-induced nephrotoxicity in rats. *J Adv Chem* 2014; **6**: 1109-19.

[45] Talas ZS, Gulhan MF. Effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicol Environ Saf* 2009; **72**: 1994-8.

[46] Alves MJQF, Mesquita FF, Sakaguti M, Tardivo AC. Hypocholesterolemic effect of propolis' caffeic acids. *Rev Bras Plantas Med* 2008; **10**: 100-5.

[47] Wiedermann U, Stemberger H, Unfried E, Widhalm K, Kundli M, Altenriederer M, et al. Intestinal worm burden and serum cholesterol or lipid concentration in a Shipibo population (Peru). *Zentrabl Bakteriol* 1991; **275**: 279-86.