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EFFECT OF ETHANOLIC EXTRACT OF IRAQI PROPOLIS AND HYDATID CYST FLUID IN SOME IMMUNOLOGICAL PARAMETERS IN ALBINO MALE RATS INFECTED WITH PROTOSCOLICES OF ECHINOCOCCUS GRANULOSUS PARASITE

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ABSTRACT

The current study was conducted for the period from October 2014 / until September 2015 /, which aims to assess the effect of ethanolic extract for Iraqi propolis either alone or mixed with hydatid cyst fluid in the some immunological parameters of albino male rats infected with protoscolices of the *Echinococcus granulosus* parasite . Results of the examination of some immunological parameters indicate the impact of the treatments to increase the percentage rate of the of phagocytosis index and is significant at the level of probability of less than 0.05 was the highest in the seventh group, which amounted 36.63% compared to positive control, which amounted to 25.4%. The results also showed a significant rise at the possibility of less 0.01 and 0.05 in the percentage of T-rosette formation coefficient for bone, spleen, lymph nodes and was the highest in the seventh group, which amounted to 18.7%, 27.5%, 15.3%, respectively, compared to positive control, which amounted to 9.2%, 11.1%, 9%, respectively. And the results of the mitotic index showed significant increase at the possibility of less 0.01 and 0.05 in the percentage of mitotic index of bone, spleen, lymph nodes and the highest percentage in the seventh group, which was 15.9%, 32.8%, 28.93, respectively, compared to positive control, which amounted to 18.06%, 20.1%, 9.46% respectively. The results of single radial immunodiffusion assay to estimate the concentration of some immunoglobulins in sera of albino male rats showed that the impact of the treatments led to stimulate the humoral immune response through significant increase at the possibility of less than 0.01 in the immunoglobulin concentration rates IgA, IgG, IgM. The highestconcentrationin seventh group, which amounted to (450.6, 3040.4, 941.7) g /dl, respectively, compared tothe positivecontrol, which was (412.77, 1266.7, 183.43) g /dl, respectively.The conclusion from this study that the ethanolic extract of propolis very effective in stimulating cellular and humoral immunity in albino male rats infected with protoscolices of *Echinococcus granulosus* parasite.

KEYWORDS: Iraqi Propolis, Protoscolices, *Echinococcus Granulosus*, Phagocytosis Index, T-Rosette Formation Coefficient, Mitotic Index

INTRODUCTION

Hydatidosis is a Zoonotic diseases caused by the larval stage of the tapeworm *Echinococcus granulosus* (McManus *et al.* 2003). The life cycle of the parasite going through two different types of life cycles to complete the life cycle are the definitive host who representCanids family members and other carnivores and harbor adult phase in their intestines, and the intermediate host represented in human and herbal animals and is home the cystic larval stage (Dvorak *et al.* 2008). The hydatid cyst consists of a layer of fibrous connective tissue surrounds the cyst from the outside, because of the growth of the cysts and get the reaction of the host these are called adventitious layer (Singh *et al.* 2001), so this

layer protectsthe parasitefrom the host immune response. Laminatedlayer which is the outer layer of white non-cellular and produced by the parasite and not by the host tissue and protects it from the reaction of the host tissue or immune responses against him and allow the passage of food to the parasite (Lin *et al*. 2013). Germinal layer an inner layer is characterized by a thin by being generate laminated layer,protoscolices ,hydatid fluid and Brood capsules and line from the inside by epithelial tissue and generate protoscolices through asexual reproduction by budding (Arora & Arora,2010). Hydatid cyst fluid (HCF) consists of a patient's serum with the help ofgerminallayer that fills the cystcontains salts is this fluid is an important source for the processing of thenecessary food for the growth of protoscolices, as well as the main source of parasite antigens used indiagnostic serological tests and be highly toxic (Bowman, 2009), where extracted two types of antigens from HCF that is a fatty proteins whichan antigen Awith a molecular weight of 400 kD and antigen B with amolecular weight of 160 kD that they have a significant role in stimulating the humoral immune response and the formation of large amounts of antibodies (Siracusano & Vuitton, 1997; Mamuti *et al*. 2002). Propolis also known as bee glue is aresin natural product, colloidal textures, complex installation and contrasting color, combines by honey bee workers*Apis mellifera* from developing parts of the trees and shrubs as the leave buds and bark of trees (Noor Al-Deen *et al*. 2013. The propolisone of the most important bee products from the medical and therapeutic terms as it contains active chemical groups such as flavonoids, terpenes and phenols and back use propolis to ancient times has been used as a medicine in several regions of the world, whether exterior or interior use. Also tended recent studies to use propolis againstmany parasites such as*Trypanosoma cruzi* (Dantas *et al*.2006), *Trichmonas vaginalis*(Starzyk et al. 1977),*Schistosoma mansoni* (Issa, 2007) and *Leishmania tropica*(Ozbilge *et al*.2010).As well as the use propolisas immunomodulatorby increasing the effectiveness of phagocytic cells and activated B cells and T(Zhang *et al*. 2008).The aim of this study is to evaluate the effectiveness of the ethanolic extract for Iraqi propolis alone through the mouth or subcutaneous or mixed with hydatid cyst fluidin some immunological factors in albino male rats infected with*E. granulosus* parasite.

MATERIALS AND METHODS

Iraqi propolis were collected from Barakat Al -Hussein place forraisehoneybeesin the province of Karbala, and attended ethanolic extract for propolis by the method of Bankova *et al*. (2002),and attended a dose of 100 mg / kg of body weight of the ethanolic extract for propolis and used sterile physiological phosphate buffer solution (PBS) as a solvent for the purpose of treatment of experimental animals. The adopted method by Smyth (1985) in the collection of hydatid cyst fluid and protoscolices.(56) healthy male rats were chosen randomly between the ages of 8-6 weeks and with weights ranging from 150-250g, were divided into nine groups, Each group consists of (6) rats. Group I (negative control) is injected under the skin and in the dorsal area behind the neck by 2 ml PBS and only once and the second group (positive control) injected through intraperitonealat 1 ml of protoscolices suspension which contains 2000 ± 20 protoscolex for each ml of sterile PBS, the third group was given each animal 1 ml of the ethanolic extract for propolis by mouth every day for a week, and after two weeks each animal were injected with 1ml ofprotoscolices suspension and the fourth group were injected at 1 mL of ethanolic extract for propolis under the skin to only once in the dorsal behind the neck region and after two weeks injected into each animal with 1ml of protoscolices suspension, and the fifth group injected only with hydatid cyst fluid by 2 ml subcutaneously and once in the dorsal area behind the neck and after two weeks injected with 1 ml of protoscolices suspension, and the sixth group injected each animal with a mixture of 2 ml of hydatid cyst fluid and 1 ml of the ethanolic extract for propolis under the skin and only once in the dorsal behind the neck region and after two weeks

injection each animal with 1 ml of protoscolices suspension, and the seventh group I give each animal 1 ml of ethanolic extract for propolis by mouth every day for a week and then injected by 2 ml of hydatid cyst fluid subcutaneously and once in the dorsal area behind the neck and after two weeks injection each animal with 1 ml of protoscolices suspension , and the eighth group injected only with by 2 ml of hydatid cyst fluid subcutaneously but once in the dorsal area behind the neck and after a week each animal was given 1 ml of ethanolic extract for propolis by mouth every day for a week and after two weeks injection each animal with 1 ml of protoscolices suspension, and the ninth group injected only with 2 ml of hydatid cyst fluid subcutaneously and once in the dorsal region behind the neck and immediately give each animal 1 ml of ethanolic extract for propolis by mouth every day for a week injection each animal and after two weeks injection each animal with 1 ml of protoscolices suspension. Treatment with ethanolic extract and hydatid cyst fluid were repeated in all experimental groups after month and two months from the first treatment. Immunologicaltests were carried out afterthree months from the injection of protoscolices.The phagocytosis index was calculated according to the method of Weber *et al*. (1982), and the method by Mackeuzie (1988) was used in the account of T-rosette formation coefficient , as well as mitotic index was calculated according to the method of Allen *et al*. (1977), and the method Mackeuzie (1988) was used to estimate the concentration of immunoglobulinsIgA, IgG and IgM, by using single radial immuno diffusion assay (SRID).

Statistical Analysis

Resultswere analyzedstatistically byusingSPSSsoftware version16and use thet-testto find the significant differences between treatments (Morgan *et al*.2010).

RESULTS AND DISCUSSIONS

Results shown in (1) a significant increase in the level of probability of less than 0.05 in the percentage rate of phagocytosis index in the third , seventh, eighth and ninth groups, whilethe risewas not significantin the fourth, fifth and sixth groups. The highestpercentageof the phagocytosisindex in seventh group, which was (36.63%) compared to the positivecontrol(group II), which amounted to (25.4%). Phagocytic cells (macrophages) play an important role in getting rid of parasites within the host's body, wherein the process ofphagocytosisafter activated by gamma interferon (IFN-γ), which produces from the T-lymphocytes. The basic objective of the compounds with immunomodulation is macrophages, which play a key role in the formation of the immune response (Kaslow,1990), wherephagocytosislocatedat the front ofthe cellularimmunedefense mechanismsinnon-specificimmunity, where mediated primarily resistant parasites that enter into the host. Resultsindicateasdescribedin the current studyto thehigh percentage ofphagocytosisindexin allexperimentalgroupscompared tothe positivecontrol and can be explained to the efficiency of the active substances such as phenols and flavonoids in propolis that participate in the organization of many types of cytokines, including TNF-α, which mediates inflammation occurring in the body and then raise the immune system efficiency and operating immunomodulators and increase the viability of macrophages to attack the foreign objects (Zhang *et al*.2008), was also attributed caused to the extract to stimulate macrophage efficiency, which in turn lead to stimulate T cells to produce cellular mediators including the IFN- γ and IL-2 and TNF-B, as these mediators are working to attractthe macrophages to the site of injury leading to stimulate phagocytosis process and revitalization (Roitt *et al*.1998), or due to the activation of T-lymphocytes T-lymphocytes to the secretion of interferon gamma (IFN-γ), which in turn leads to increased phagocytic cells to stimulate the production of amounts of nitric oxide(NO), which is the process of the most efficient killing, and

attributed the importance of NO, which produces by phagocytic cells to its abilityto inhibitDNAbuildingof the organismcells(S'a- Nunes *et al*.2003). In study of Missima & Sforcin (2007) about the impact of propolison the effectiveness of macro phagesin the production of hydrogen peroxide(H_2O_2) and NO in mice exposed to stress, they found an increase in the production of H_2O_2 and a decrease in the production of NO by macrophages. Modern research has shown that some types of flavonoidsstimulates proliferation of peripheral white bloodcells.And it'ssignificantly increase theeffectiveness ofhelper Tcellsas well ascytokines and IL-2,IFN-γandmacrophagesandthisisuseful in treatingdiseasescausedweaknessin the immune system(Kawakita *et al*.2005). And reinforced these findings by Al-Jawadi (1999), which pointed to the high phagocytosis coefficient when using thyme extract asimmunomodulator, as well as Al-Humairi (2010) when studying the effectiveness of the extract of the seeds of *Datura stramonium*as immunomodulators on the growth and development of the hydatid cysts of *Echinococcus granulosus* in Balb / c mice, where thehigheffectivenessofphagocytosisinmicetreatedwith alkaloidsandphenols. That means the hydatid cyst components have an effective impact on the division of macrophages and so increase the phagocytosis process (Macintyre *et al*. 2000). The reason for the decrease of phagocytosisindex in the positive control may be duetothe parasite's role in the production oflymphokines by theinfectedhost, which inhibitthe process ofphagocytosisby killing macrophages(Jenkins *et al*. 1990).

Table1: Phagocytosisindexrate(%)in Albino Maleratsinfected Withsecondary Hydatid Cysts

Groups	Mean±Standard Deviation	T-Test	P- Value
First	19.83 ±1.98	-	-
Second	25.4 ±5.41	-	-
Third	29.36 ±1.89	1.1	0.44**
Fourth	26.8 ±3.07	0.38	0.71*
Fifth	27.9 ±4.76	0.60	0.58*
Sixth	27.23 ±5.2	0.42	0.69*
Seventh	36.63 ±0.89	3.5	0.024**
Eighth	31.9 ±7.4	1.2	0.040**
Ninth	36 ±3.5	2.8	0.033**

***=No significant Differences at Theprobability Valuemorethan 0.05. **=Significant Differences at The probability Valueless Than 0.05.**

Results indicated shown in (2) the existence of a significant rise in the level of probability of less than 0.01 and 0.05 in the percentage rateof T-rosette formation coefficient of bone in the third, sixth, seventh, eighth and ninth groups, while the rise was not significant in the fourth and fifth groups. The results showed in a (3) that there is a significant increase at the level of less than 0.01 and 0.05 in the percentage rate of T-rosette formation coefficient of the spleen in all experimental groups. According to results shown in the (4), the existence of significant increase at the probability of less than 0.01 and 0.05 in the percentage rate of T-rosette formation coefficient of lymph nodes in the third, seventh, eighth and ninth groups, whilethe risewas not significantin the fourth, fifth and sixth groups . The highestpercentageof the T-rosette formation coefficientof bone, spleen and lymph nodesin the seventh group, which was, 15.3%, 27.5%and 18.7% respectivelycompared to positivecontrol(group ll), which amounted to, 9.2%, 11.1%and 9% respectively**.**

The T-rosette formation coefficient one appropriate ways to measure of the cellular immune response level by T-lymphocytes of rats and the ability of these cells to bind antigen as a result of owning protein surface receptor (OX49) and this protein is as receptorwhich binds specifically with red blood cell antigens , which resemble the surface protein (CD2) located on the surface ofa human T cells being, who has the ability to overlap with the surface protein on the surface of sheep red blood cells (Hudson & Hay 1989). The reason forincreasing the percentage of T-rosette formation

coefficientmay be duethe ethanolicextractofpropoliscontaineffectivecompounds affectedinstimulate different cellularmediators thatits turn,stimulatethe proliferation ofT-lymphocyteproduction (Park *et al* .2004),or may be due to the presence of compounds in propolis affected in receptorson the surface ofT cells, which led to increasedformationof T-rosetteand these results are identical to the results of Al-Obeidi (2002), when studying the effect of cold and boiled aqueous extractof the roots of *Glycyrrhiza glabra* plant in albino mice, the results showedan increase inthe percentageof T-rosette formation. Also Yin *et al*. (2014) foundthatthe hydatid cyst fluid effects onTcells,by urgingthese cellsto differentiation and maturation toT-regular cellsfor organizationthe cellularimmuneresponse. These studyalsoshowed that thehydatid cyst fluid effects onspleencellsby growingthese cells*in vitro*, and this effectisthe production ofcytokines such asTransforming growth factor (TGF-β)andincrease the number of$CD4^+$ $CD25^+$ T- cells.

Table 2: T- Rosette Formation Coefficientratein the Bone (%) in Albino Maleratsinfected with Secondary Hydatid Cysts

Groups	Mean±standard deviation	t-test	p- value
First	0.55±7	-	-
Second	0.65±9.2	-	-
Third	0.95± 12.63	5.1	0.007***
Fourth	0.23± 9.53	0.83	0.45*
Fifth	0.23± 10.26	2.6	0.057*
Sixth	0.80± 11.46	3.7	0.019**
Seventh	4.30± 15.3	2.4	0.003 ***
Eighth	4.88± 14.13	1.7	0.005 ***
Ninth	4.8± 15.16	2.1	0.0041 ***

***= No significant Differences at the probability Value more than 0.05. **=Significant Differences at the probability Valueless Than 0.05. ***=Highly significant Differences at the high probability Value lessthan 0.01.**

Table 3: T- Rosette Formation Coefficient rate in the Spleen (%) in Albino Male rats infected with Secondary Hydatid Cysts

Groups	Mean ± Standard Deviation	T-Test	P- Value
First	2.25±9.9	-	-
Second	3.87 ±11.1	-	-
Third	7.8 ± 17.7	1.3	0.003 ***
Fourth	4.1 ± 13.6	0.77	0.028**
Fifth	1.24 ± 13.9	1.1	0.026**
Sixth	2.3 ± 17.3	2.3	0.0041 ***
Seventh	2.00 ± 27.5	6.5	0.001 ***
Eighth	2.17 ± 19.76	3.3	0.0022 ***
Ninth	2.07± 20.9	3.8	0.001 ***

****=Significant Differences at The probability Valueless than 0.05. ***=Highly significant Differences at The high probability Value lessthan 0.01**

Table 4: T- Rosette Formation Coefficient rate in the Lymph Nodes (%) in Albino Male rats infected with Secondary Hydatid Cysts

Groups	Mean ± Standard Deviation	T-Test	P- Value
First	2.02±7.2	-	-
Second	3.01 ±9	-	-
Third	5.0± 13.3	1.2	0.026 **
Fourth	3.2± 10.4	0.54	0.61*

Fifth	2.04± 10.56	0.74	0.49*
Sixth	1.05± 11.5	1.3	0.24*
Seventh	3.5± 18.7	3.6	0.0024***
Eighth	1.85± 14.4	2.6	0.022 **
Ninth	3.7± 18.3	3.3	0.0035***

***= No Significant Differences at the probability Value more than 0.05. **=Significant Differences at the Probability Value lessthan 0.05. ***=Highly Significant Differences at the High Probability Value lessthan 0.01**

Results shown in the (5) the existence of a significant rise in the level of probability of less than 0.01 and 0.05 in the percentage of mitotic index ratein the bone in the third, fifth, sixth, seventh, eighth and ninth groups, while not a significant rise in the fourth group. The results showed in s (6) and (7) that there is a significant increase at the probability of less than 0.01 and 0.05 in the percentage of mitotic index ratein the spleen and lymph nodes in the third, seventh, eighth and ninth groups, while was not significant rise in the fourth, fifth and sixth groups . The highest percentage of mitotic index in the bone, spleen and lymph nodes in animals of seventh group, which was, 28.93%, 32.8% and 15.9% respectively, compared to positive control (group II), which amounted to, 18.06%, 20.1% and 9.46% respectively.

The account of mitotic index can give a clear picture of the humoral and cellular immune response, where noted a rise in the percentage of mitotic indexin the bone, spleen and lymph nodes in the experimental groups treated with propolis, compared to positive control,in order to contain the Artepillin C compound that stimulates cell division, where increases of T-lymphocytes and B- lymphocytes rate and found that this compound stimulates the lymphocytes to apoptosis optionally in the event of any defect in the process of DNA building (Kimoto *et al*. 1998),Also found that the most important compounds that are found in Albropouls is Caffeic acid phenethyl ester (CAPE),which has importance in the inhibition of tumor cell growth by stopping cell growth in the first growth phase and stimulate apoptosis (Kuo *et al*. 2005). These results are consistent with the results of Fahmi *et al*. (2011) in the study of the influence of anti-mutagenic Saudi propolis.The study showed that propolis acts as anti-mutagenesis and anti-toxins also stimulates mitotic coefficient, where increases the activation and proliferation of lymphocytes in the bone marrow in albino mice.As well as Al-Obeidi (2002) study results compatible with the results of the current study about the effect of boiled and cold aqueous extract of the roots of licorice plant Glycyrrhiza glabra in albino mice, where the results showed an increase in cell division in the cells of the bone marrow, spleen, lymph nodes and thymus gland . In another study by Macintyre *et al*. (2000), about the impact of hydatid cyst fluid onthe division of T-lymphocytes *in vitro*, found that the fluid components increase of T cellsDNA about five-fold.

Table 5: Mitotic Index rate in Bone (%) in Albino Male rats infected with Secondary Hydatid Cysts

Groups	Mean±Standard Deviation	T-Test	P- Value
First	1.95±16.7	-	-
Second	0.46±18.06	-	-
Third	1.9± 25.5	6.5	0.003***
Fourth	1.63± 20.5	2.4	0.068*
Fifth	1.63± 21.19	3.9	0.017**
Sixth	1.85± 22.36	3.8	0.018**
Seventh	2.0± 28.93	9.1	0.001***
Eighth	2.13± 26.66	6.8	0.002***
Ninth	1.74± 27.76	9.2	0.001***

= No Significant Differences at the probability Value more than 0.05. **=Significant Differences at The probability Value lessthan 0.05.=Highly significant Differences at The High probability Value lessthan 0.01**

Table 6: Mitotic Indexrate in spleen (%) in Albino Maleratsinfected with Secondary Hydatid Cysts

Groups	Mean±Standard Deviation	T-Test	P- Value
First	2.13±15.93	-	-
Second	1.44±20.1	-	-
Third	1.37± 25.76	4.9	0.008***
Fourth	1.45± 20.56	0.39	0.714*
Fifth	2.05± 21.26	0.80	0.466*
Sixth	3.0± 21.76	0.85	0.442*
Seventh	2.40± 32.8	7.8	0.001***
Eighth	1.65± 28.6	6.7	0.003***
Ninth	2.12± 29.5	6.3	0.003***

***= No Significant Differences at the Probability Value morethan 0.05. ***=Highly Significant Differences at the High probability Value lessthan 0.01**

Table 7: Mitotic Index rate in Lymph Nodes (%)in Albino Male ratsinfected with Secondary Hydatid Cysts

Groups	Mean±Standard Deviation	T-Test	P- Value
First	1.49±7.23	-	-
Second	2.05±9.46	-	-
Third	1.37± 14.23	3.3	0.029**
Fourth	1.31± 11.7	1.5	0.187*
Fifth	2.51± 11.8	1.2	0.281*
Sixth	1.7± 12.4	1.9	0.126*
Seventh	2.6± 15.9	3.3	0.027**
Eighth	1.15± 14.7	3.8	0.018**
Ninth	1.30± 15.8	4.5	0.011**

***= No Significant Differences at the Probability Value Morethan 0.05. **=Significant Difference sat the Probability Valueless Than 0.05**

Results of single radial immune diffusion assay described in the s (8) , (9) and (10) that there is a significant increase in the level of probability of lessthan 0.01and 0.05in the concentration rate of immunoglobulinsIgA ,Ig Gand IgMinallthe study groups compared to the positive control(group II) and was the highest concentration in the seventh group, which reached to (941.7,3040.4and450.6) g /dl, respectively, compared to the positive control, which stood at(412.77,1266.7and183.43)) g /dl, respectively.

The single radial immune diffusion assay of the most common immune methods of measuring the amount of antibody by measuring the circle diameter which is complex consists of deposition of antibody and antigen that surrounds the sample, which is intended to measure the amount of antibody in terms of spreading the complex in the center of agar media containing the specific antibodies to that antigen, which is increasing in diameter with the passage of time and this precipitate insoluble (Goldsby *et al*. 2007). It has been observed by the results of the current study, the high concentration of antibodies IgA, IgG and IgM in animals groups that have been treated with ethanolic extract of propolis through the mouth and then treated with hydatid cyst fluidsubcutaneously (seventh group) or treatment with hydatid cyst fluid subcutaneously and then ethanolic extract of propolis orally (ninth group), this is due to the ability of propolis for modulating and synthesis of antibodies (Sforcin *et al*. 2005) or may be due to efficiency of the ethanolic extract for propolis in stimulate the activity of phagocytic cells which in turn leads to stimulate T cells to increase production of cellular mediators such as IL-1, IL2 and IL-4, which in turn stimulate B cells to differentiate into plasma cells producing

antibodies (Park *et al*. 2004). Previous studies reported that the ethanolic extract of propolis efficient in the production of antibodies (Orsolic & Basic, 2003 ; Ziaran *et al*. 2005).Also the study of Çetin et al. (2010) on the impact of different concentrations of propolis in chicken and was the highest rise in the immunoglobulin concentration level, IgG and IgM when using 3 g / kg during the 12-week period. Also found that one of the active ingredients in propolids which is Caffeic acid phenethyl ester (CAPE) increase the proliferation of lymphocytes and also increase the secretion of IL-1 and IL-2 by the spleencells (Park *et al*. 2004)). Also Chu (2006) who mentioned that the propolis important in the activation of T and B cells. In another context, a number of studies have reported the effectiveness of the immune stimulation of propolis increase the production of immunoglobulins especially IgG (Zeedan *et al*. 2014). The impact of hydatid cyst fluid in increase in the level of antibodies, because it contains antigens where Hashemi Tabar & Ramzi (2009) said that the immunization of the lamb animal with hydatid cyst fluid led to increased production of the amount of antibodies. Also the study by Mamuti *et al.* (2002) on antigen isolated from the hydatid cyst fluid labeled Antigen B in urging the humoral immune response to produce large quantities of specific antibodies of class IgG. As some studies have indicated that the humoral immunity,active in the stage of the cyst growth,which is characterized by rising the level of antibodies IgA, IgG andIgM (Wen & Craig, 1994).The results obtained by Youssefi *et al*. (2010) agreed with the results of the current study where it was found that immunization of mice with the hydatid cyst fluid of the*E.granulosus* parasite led to increasing the level of antibodies after 4, 8 and 12 weeks of immunization when used ELISA technique.The conclusion from this study that the ethanolic extract of propolis very effective in stimulating cellular and humoral immunity in albino male rats infected with protoscolices of *Echinococcus granulosus* parasite.

Table 8: The Concentration Rate of Iga(G / Dl) in the Serum of Albino Maleratsinfected with Secondary Hydatid Cysts

Groups	Mean±Standard Deviation	T-Test	P- Value
First	12.15±337.8	-	-
Second	11.72±412.77	-	-
Third	15.3± 524.8	10.4	0.001***
Fourth	24.9± 467.8	5.2	0.006***
Fifth	18.8± 615	17.9	0.001***
Sixth	17.1± 762	29.1	0.001***
Seventh	15.5± 941.7	41.2	0.001***
Eighth	13.85± 684.4	17.0	0.001***
Ninth	14.30± 830.2	37.4	0.001***

*****=Highly Significant Differences at the high Probability value lessthan 0.01.**

Table 9: The Concentration Rate Og (G / Dl) in the Serum of Albino Male rats infected with Secondary Hydatid Cysts

Groups	Mean±Standard Deviation	T-Test	P- Value
First	54.0 ±859.9	-	-
Second	41.1±1266.7	-	-
Third	47.1± 1751.8	13.4	0.001***
Fourth	44.3± 1523.1	7.3	0.002***
Fifth	49.8± 1994.3	19.5	0.001***
Sixth	55.3± 2520.2	31.5	0.001***
Seventh	60.3± 3040.4	42.0	0.001***
Eighth	52.6± 2250.4	25.5	0.001***
Ninth	58.1± 2803.7	37.4	0.001***

*****=Highly Significant Differences at The high Probability Value lessthan 0.01**

Table 10: The Concentration Rate of Igm(G / Dl) in the Serum of Albino Male ratsinfected with Secondary Hydatid Cysts

Groups	Mean±standard deviation	t-test	p- value
First	5.75±155.73	-	-
Second	9.30±183.43	-	-
Third	6.75±230.4	7.0	0.002***
Fourth	6.35± 204.2	3.1	0.033**
Fifth	7.15± 265.0	12.0	0.001***
Sixth	7.85± 332.5	21.2	0.001***
Seventh	9.0± 450.6	35.6	0.001***
Eighth	7.4± 294.2	16.0	0.001***
Ninth	8.2± 372.8	26.3	0.001***

****=Significant Differences at the Probability Value lessthan 0.05.***=Highly Significant Differences at the High Probability Value lessthan 0.01**

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