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The role of bird thymus extract on some immunological parameters in rabbits vaccinated with killed *Salmonella typhi*

دور مستخلص توثة الطير على بعض المعايير المناعية في الارانب المحقونة بالسالمونيلا المقتولة

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Abstract

Three groups of rabbits were used to study the effect of bird thymus extract with protein concentration of 1.5 mg /ml .First group was injected with thymus extract ,second group was injected with thymus extract and killed *Salmonella* antigen and third group was injected *wiN n th* killed *Salmonella* antigen .After the period of immunization schedule was completed ,Blood samples , intestinal and appendix were collected to study some immunological parameters (direct agglutination with Salmonella antigen ,IgM ,IgG concentrations ,LIF ,IFN-γ ,phagocytic activity and delayed type hypersensitivity).Thymus extract was effect on humoral immunity by increasing the mean of specific titers against *Salmonella* in second group(533.33 ,53.3, 53.3)while third group were (266.6 ,26.6 ,26.6),the concentrations of immunoglobulin (IgM, IgG)also increased in second group compared with other groups .while in cellular immunity the results were differences between groups .

In this study we confirm, The immunomodulatory effect on previously mention parameters

الخلاصة :

لمعرفة تاثير مستخلص توثة الطير بتركيز بروتيني1.5 ملغم /مل تم استخدام ثلاثة مجاميع من الارانب وحقنت المجموعة الاولى بمستخلص التوثة وحقنت المجموعة الثانية بمستخلص التوثة مع مستضد السالمونيلا اما المجموعة الثالثة فحقنت بالسالمونيلا المقتولة وبعد اكمال فترة التمنيع .جمعت عينات الدم وعينات الزائدة الدودية والامعاء الدقيقة لدراسة بعض المعايير المناعية والمتضمنة (التلازن المباشر مع مستضدات السالمونيلا المقتولة وتركيز IgG, IgM وتركيز الانترفيرون كاما وعملية البلعمة وعامل تثبيط هجرة الخلايا البيض وواختبار فرط الحساسية المتاخر .فأظهرت النتائج ان المستخلص له تاثير على المناعة الخلطية حيث زادت معدل عيارات الضد المتخصص للسالمونيلا في المجموعة الثانية (533.3,53.3,53.3)مقارنة بالمجموعة الثالثة (26.6, 26.6, 266.6)وكذلك ازداد تركيز الIgG, IgM في المجموعة الثانية مقارنة مع المجاميع الاخرى زاما المناعة الخلوية فكانت النتائج متغايرة بين المجاميع نستنتج من هذه الدراسة ان لمستخلص توثة الطير تاثير محور على المعايير المناعية للارانب الممنعة بمستضد السالمونيلا المقتولة .

Introduction.

The thymus is an important organ which play a crucial role in the maturation of lymphocyte structures in the parenatal and early post natal period of life and orchestrating the lymphoid system thought the life (1).various studies with thymus extract have got remarkable immunomodulatory effects .The thymic extract restored the depressed functions of humoral and cellular immune systems in alloxan diabetic mice (2) .Thymus extract has been found to enhance the antibody titers in bird vaccinated against infectious bronchitis virus (3) ,another studies found that calf thymic extract increase antibody titers against New castle virus in chicken (4).It was being shown that administration of thymic extract has positive immunomodulatory effect on T cell functions as T cell functions as T cells obtained from thymic extract such as administered Dalton s lymphoma bearing mice show an increased INF-γ, production and improved antigen specific proliferative ability (5) .The aim of this study to know the effect of thymus extract on some immunological parameter in the present of antigen .

Materials and Methods

1- crude thymus extract :

sterile Petri dishes .TP put in . The thymic tissues (avian thymic tissues) were collected in sterile phosphate buffer saline (PBS) PH 7.2 and washed thoroughly with this saline in Petri plate then minced and homogenized . The tissue homogenate solution was centrifuged at 3500 r.p.m. for 30 minutes .Supernates represent the crude thymic extract (CTE). Equal volumes of CTE and polyethylene glycol PEG 6000 6% were mixed and left at room temperature for 30 minutes and centrifuged at 3500 r.p.m. for 30 minutes .Pellet were the thymic protein precipitate was dissolved in 1 ml of normal saline(4) .LTP concentrations were estimated by Biurate method (5) and adjusted to 1.5 g/L. .

2- Killed bacterial antigen

*Salmonella typh*i was obtained from microbiology laboratory in biology department ,College of science ,Babylon university , was identified by Ep 20 and killed antigen was prepared as(6).

3- Rabbits :

Nine Rabbits(male 1-1.5 Kg) brought from local marked nine were proved to be free of ecto and endo parasite and bacterial .These were kept under ad labium conditions of food and drinks then adapts to housing conditions . These nine rabbits were sub grouped into three groups

4- Immunization protocol:

immunization protocol that used as the following table 1 . After third weeks of immunization ,fourth week left and in five week the blood and autopsy(appendix and intestinal) were **c**ollected .In this study positive control (G1 and G3)

Table (1) Immunization protocol

Groups	Time	Dose	Site of administration
G1	First week Second week Third week	1ml (0.5 TP+0.5 oil) 1ml TP 1ml TP	0.5 in subcutaneous in different site ,and 0.5 intramuscularly
G2	First week Second week Third week	1ml (0.5 TP+0.5 killed bacteria) 1ml = 1ml =	
G3	First week Second week Third week	1ml (killed bacteria) 1ml (killed bacteria) 1ml (killed bacteria)	

5- Mucosal immunology :

The mucosal immunoglobulins were separated by the methods of (7)

6- Serology

1- Tube agglutination test was prepared as (8) Killed bacteria was used as antigen and serum of animal and mucosal immunoglobulin as antibodies .

7- Radial immunodiffusion plates

IgG and IgM concentration , were determined by using radial immunodiffusion plates (LTA company) according to(9)

INF-γ concentrations were determined by ElISA kit ,the method was occurred according to manufacture company (R&D).

8-cellular immunity

Phagocytic activity was assessed by neutrobluetetrazolium reduction according (10). leucocyte migration inhibition factor was determined for both systemic (blood) and mucosal (appendix) according (11)and delayed type hypersensitivity according (12)

Results :

Specific antibody titers were higher in second group of animals (533.3, 53.3)compared with third group(266.6,26.6)and first group was appeared low antibody titer .The immunoglobulins (IgG& IgM) concentration was determined by using single radial immunediffusion ,in (table 3) the immunoglobulin concentrations were higher(366.63,3248.46) in second.INF-γ concentration were determined in the serum of rabbits ,the result appeared higher concentration (13.7) the rabbit that immunizes with Killed antigen and low in both first and second group (8.46,8.6)respectively .Phagocytic activity percentage was studied and the results was appeared higher activity with second group (0.77) while in first and third were (0.60, 0.72).leucocyte migration inhibitory factor percentage were determined in three groups both in systemic and mucosal ,LIF percentage were significant(the significant mean the percentage between 30-70) both in second and third groups as (table 6)delayed type hypersensitivity were higher in second group of rabbits both with thymus extract and *Salmonella* antigen (table 7).

Table (2)Specific antibody titers against killed *Salmonella* antigen

Groups	**Specific antibody titers**		
	Systemic	**intestinal**	**Appendix**
First group: Three rabbits injected with thymus extract	20 20 10	2 2 1	2 2 1
M ± SD	16.66±5.7	1.66±	1.66±0.5
Second group: Three rabbits injected with mixture of thymus extract and killed bacteria	320 640 640	32 64 64	32 64 64
M ± SD	533.33±184.7	53.3±**18.4**	53.3±**18.4**
Third group: Three rabbits injected with killed bacteria	160 320 320	16 32 32	16 32 32
M ± SD	266.6±**92.37**	26.6±9.2	26.6±9.2

Table 3 : IgG and IgM concentrations

Group	**IgG (mg/ dl)**	**IgM (mg/ dl)**
First group: Three rabbits injected with thymus extract	111.7 117.1 122.6	1662.2 1712.5 2137.7
M ± SD	**117.1 ± 5.450**	**1837.46± 261.2**
Second group: Three rabbits injected with mixture of thymus extract and killed bacteria	296.4 312.9 490.6	3513.4 3444.3 2787.7
M ± SD	**366.63± 107.67**	**3248.46± 400.52**
Third group: Three rabbits injected with killed bacteria	236.5 229.4 201.9	2193.7 2250.3 2307.5
M ± SD	**222.6±18.274**	**2250.5±56.900**

Table(4) Interferon gamma INF- γ concentrations

Group	**INF-γ concentrations**
First group: Three rabbits injected with thymus extract	8.2 8.8 8.4
M ± SD	8.46±0.3
Second group: Three rabbits injected with mixture of thymus extract and killed bacteria	8.9 8.8 8.2
M ± SD	8.6±**0.3**
Third group: Three rabbits injected with killed bacteria	16.5 14.4 10 .2
M ± SD	13.7±**3.2**

Table (5) phagocytic activity

Group	**Phagocytic %**
First group: Three rabbits injected with thymus extract	0.48 0.60 0.72
M ± SD	**0.60±0.12**
Second group: Three rabbits injected with mixture of thymus extract and killed bacteria	0.8 0.8 0.72
M ± SD	**0.77±0.04**
Third group: Three rabbits injected with killed bacteria	0.8 0.66 0.7
M ± SD	**0.72±0.07**

Table (6)leucocyte migration inhibitory factor

Groups	**Leucocyte migration inhibitory factor%**		
	Systemic	**intestinal**	**Appendix**
First group: Three rabbits injected with thymus extract	0.76 0.8 0.75	0.85 0.77 0.7	0.8 0.8 0.75
M ± SD	0.77±**0.02**	0.77±**0.07**	0.78±**0.02**
Second group: Three rabbits injected with mixture of thymus extract and killed bacteria	0.33 0.6 0.55	0.35 0.4 0.5	0.4 0.6 0.5
M ± SD	0.49±**0.14**	0.41±**0.07**	0.5±**0.1**
Third group: Three rabbits injected with killed bacteria	0.66 0.6 0.63	0.58 0.54 0.56	0.4 0.5 0.43
M ± SD	0.63±**0.03**	0.56±**0.02**	0.44±**0.05**

Table (7) Skin test

Group	Injection by thymus extract		Injection by killed bacteria	
	Induration mm	**Erythema**	**Induration mm**	**Erythema**
First group: Three rabbits injected with thymus extract	10 9.5 9	+ + +	2 3 3	- - -
M ± SD	9.5±**0.3**		2.66±0.5	**M ± SD**
Second group: Three rabbits injected with mixture of thymus extract and killed bacteria	11 12 12	+ + +	10 8 8	+ + +
M ± SD	11.6±**0.5**		8.66±1.1	
Third group: Three rabbits injected with killed bacteria	2 2 2	- - -	7 8 7	+ + +
M ± SD	2±**0**		7.33±0.5	

Discussion

The specific antibody was appeared with *Salmonella* killed antigen , but the titers in second groups were higher than third group .Administration of bird thymus extract markedly increased the ab titers against *Salmonella* ag .,similar results appeared in chicken another study found calf thymus extract increased ab against New castle diseases virus in (4).Thymus extract effect on the concentration of IgG ,IgM in second groups .concentrations were increasing in second groups this may be due to the effect of thymus on humoral immune response .Interferon concentrations were increased in third group because the natural killer cells and macrophages were stimulated by killed antigen and also produced from Th1 due to clonal expansion of antigen specific T cell and provide a rapid source of INF- γ to activate macrophage (1) thymus extract had modulatory effect on production of IFN- γ in second group (5).Cellular immunity also studied by phagocytic activity .Nitrobluetetrazolium assay as a measure of nonspecific immunity (14)stated that intensity of NBT reduction correlated with bacteriacidal activity .the result obtained for the phagocytic activity in third group while modulated in second group ,this increasing could be due to the ability of thymus extract to act as an immunomodulator by excerating control on cytokine by blood mononuclear cells (15 ,4) .

LIF and delayed type hypersensitivity also effected by thymus extract this due to that thymus extract enhance T cell (16) .The present study found that thymus extract effect on humoral and cellullar immunity in rabbit that immunized with killed *Salmonella typhi* .

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