Promoting effect of Echinacea extract co administrated with PPR vaccine in sheep as an immune stimulant.

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Abstract:

The medicinal plants like Echinacea (*Echinacea angustifolia*) may enhance immune function by increasing antigenic-specific immunoglobulin production. This study was conducted to evaluate the immunostimulatory effect of Echinacea extract when given PPR vaccine in sheep. For accomplishing this; four groups of sheep (five animals/ each) were vaccinated with PPR vaccine, one kept as a control(G1), the other three groups (G2, G3 and G4) during vaccination were orally treated with super critical carbon dioxide Echinacea extract ,60% ethanolic extract of Echinacea and 20% ethanolic extract of Echinacea respectively. Blood samples were collected from each animal at 7, 14,21,28,35 days for serum separation. Antibody titre was estimated by ELISA and SNT techniques. The highest titre of antibodies was observed in animals treated with 60% ethanolic extract of Echinacea followed by 20% ethanolic extract and super critical carbon dioxide extract respectively. In conclusion; giving 60% ethanolic extract of Echinacea improve the response of the immune system during vaccination with PPR vaccine in sheep.

Introduction:

Echinacea is one of the most widely used over the counter herbal preparations .That purport to improve immune system function especially when taken as short course of therapy (6-8 weeks) .(Bauer and Wagner ,1991 ; Diana *et al*. , 2003) .

The German commission approves the use of Echinacea purpurea herb as follows internally as supportive therapy for cold and chronic infections of respiratory tract and externally on poorly healing wounds and chronic ulcerations.(Blumenthal *et al*.,1998). Investigation towards identification of the principles responsible for the immune modulatory effects of Echinacea preparations have focused on five classes of Echinacea constituents namely, caffeic acid derivative ,alkyl amides polyacetylenes, glycoprotein's and polysaccharides. (Bauer, 1988 ; Pietta *et al*.,1998).

Several studies supported the role of Echinacea as immune stimulant. In vitro researches have demonstrated increased phagocytosis, NK cell activity and increased antibody – dependent cellular cytotoxicity mediated by TNF-a (Mel chart *et al*., 1995; See *et al*., 1997). The cellular evidence of the immunostimulatory activities of Echinacea extracts and its enhanced resistance to infectious diseases have proved to be through macrophage activation, phagocytic indices and macrophage derived cytokine concentration as well as activation of polymorph nuclear leukocytes, natural killer cells. Changes in the numbers and activities of T-and B-cell leukocytes have been reported also but are less certain. (Burger *et al*., 1997 ;Barrett, 2003)

Materials and methods:-

1-Echinacea

The Echinacea extract preparations for the present study were developed from a single lot of *Echinacea angustifolia* root. The root material was extracted with either by supercritical carbon dioxide, 60%ethanol or 20% ethanol to prepare three different forms of the extract according to method described by Ronald *et al*.(2005).

Phytochemical analysis of the extracts revealed that the supercritical carbon dioxide extract contain no polysaccharides but contain 78.8% alkamids. The 60% ethanol extract contained 41.9%polysaccarides and 2.9% alkamides, while the 20%ethanol extract contained 40.1% polysaccharides and only 0.2% alkamides. Repeated analysis of the study treatment ensured that the composition of the treatment remained constant.

2-peste des petites ruminant's vaccine

A Vero cells adapted peste des petites ruminant's vaccine prepared from the Egyptian strain (egypt-87) according to Khodeir and Mouaz (1998).This vaccine was used for animal inoculation. It had a titer of 5 log 10 TC ID \ ml and it stored at – $20C^0$ till used. Where the titration of the used vaccine was carried out using microtitre plate according to Ferreira (1976) using Vero cell.

3- Viral antigen:

The supernatant fluid of infected Vero cell culture with des petites ruminants was collected at full cytopathic effect (CPE).These supernatant was concentrated using polyethylene glycol and used as antigen in ELISA.

4-Vaccination and serum samples collected from experimental animals:

The serum samples of 20 sheep were screened using serum neutralizing test (SNT). They were free from neutralizing antibodies of peste des petites ruminants.

Twenty sheep were randomly divided into four groups. One group kept as control positive (G1), where it received the vaccine. The other three groups (G2, G3, G4) were treated 5 weeks with Echinacea extracts super critical carbon dioxide, 60% ethanol or 20%ethanol respectively as 1.5ml tincture containing the equivalent of 300mg of Echinacea root three times /day according to (Turner *et al*.,2005) directly after vaccination of sheep . During the experiment the serum samples were collected from each sheep on 7, , 14, 21 , 28,35 days.

The all serum samples were investigated to detect the formed antibodies using ELISA and serum neutralizing test (SNT).

5- The indirect enzyme linked immunosorbent assay (ELISA):

It was performed according to the method previously described by Anderson *et al*.(1982). The antigen was prepared from the infected Vero cell cultures with PPR virus as previously described and diluted with bicarbonate buffer (9.6) using ten fold serial dilution. From each dilution 100 ul were dispensed \ well horizontally and the plates were covered and incubated at $37C^0$ for one hour (coating). The plates were washed three times with the washing solution and 100 ul of the blocking buffer were added \well.

After that, the plates were incubated at $37C^0$ for 30 minutes. Then washed three times again with the washing solution. Two fold dilutions of the tested sera were prepared and 100 ul of each diluted serum were added to each well vertically. Positive control sera were included in each plate. The plates were incubated at $37C^O$ for one hour, then washed two times with the washing buffer and 100 ul of the diluted conjugate (1\10000) were dispensed in each well the plates were incubated at 37C for one hour then washed three

times with washing buffer and 100 ul of the substrate were add \ well and the plates were incubated at room temperature in a dark place for 15 minutes. The reaction was stopped by adding 25 ul \well of the stopping solution (1.25M).

6- Serum neutralization test (SNT):

Both screening and quantitative SNT were performed by the micro technique as described by Ferreira (1976) in flat bottom tissue culture micro titter plates containing Vero cells. The SNT was applied on sheep sera before and one weekly interval after inoculation with PPR vaccine. The end point neutralizing antibody titers were expressed as the reciprocal of the final dilution of serum inhibiting the CPE of 100-200 TCID50 (Singh *et al*., 1967).

Result:

The results showed that oral treatment of sheep by Echinacea extract during vaccination by peste des petites ruminants' vaccine increased the titers of antibodies as measured by indirect ELISA and quantitative SNT .

The control positive sheep (G1) responded to the vaccination where the average antibodies titer was 0.188 ± 0.52 in the first weak of experiment by ELISA. While antibodies titer by SNT was ≤2. This titer increased and reached the peak at the fourth weak .where the average antibodies titer was 1.294 ± 0.091 (64 by SNT) as shown in table (1,2) and figure (1,2).

The other three groups (G2, G3, and G4) which were treated by Echinacea extract during vaccination revealed difference in the average antibodies titer rising levels.

The immune response of (G2) which treated by Echinacea extracts supercritical carbon dioxide was 0.232± 0.063 by ELISA and ≤2 by SNT in the first week and reached peak at the fourth and fifth week. Where the average antibodies titer was 1.659± 0.054 by ELISA and 32 by SNT in the last two weeks as presented in figure (1, 2) and table (1,2).

Concerning, G3 and G4 responded to the immunostimulating effect of ethanol – Echinacea extract where the average antibodies titers of both groups were 0.581± 0.051 and 0.558±0.63 by ELISA and 4 and ≤ 4 by SNT respectively in the first week.

The later antibodies titers of G3 and G4 increased gradually and reached the peak 1.957± 0.053 and 1.892± 0.045 by ELISA and 128 and 64 by SNT respectively at the fourth and fifth week (table 1,2 and figure 1, 2).

Discussion:

The results of the present study indicated the effect of Echinacea extract as an immunostimulant through monitoring antibodies levels in sera of sheep after exposure to PPR vaccine.

Weekly regular collection of blood samples along 5 weeks of the experimental period revealed the highest titer of antibodies (1.951± 0.051 by ELISA and 128 by SNT) was in group three (which was treated by 60% ethanol extract) as comparing with the control and other treated groups followed by group four which was treated by 20% ethanol extract, as the titer was 1.885 ±0.050 by ELISA and 64 by SNT. While the lowest titer observed was in group two which treated by super critical carbon dioxide extract as it was near or slightly higher than the control titer. Our result comes in harmony with those mentioned by (Kathyn ,1999 ; Rehman *et al*., 1999 ; Bergeron , 2000 ; Barrett , 2003 and Bruce, 2006)

Those results showed that the treatment with 60% alcohol extract was the beneficial in yielding high antibodies titer than treatment with 20% alcohol extract and supercritical carbon dioxide extract respectively .this agreed with Bergeron (2000) who mentioned in their study on the optimal conditions for Echinacea extraction by different alcohol concentrations ,that 70% alcohol extract was found to be optimal in yielding soluble solids such as important phytochemicals (polysaccharides and alkyl amides) more than the lower concentrations(50%) and higher concentrations (85% - 90%)

The later two compounds are of high value as immune stimulants , this comes in accordance with Juliet(2003) who reported that alkamides from Echinacea root have stimulatory effect on the immune system. And polysaccharides from Echinacea being of two types, a heteroxlan and arabinogalacton, both act on the immune system to boost its effect. The immunostimulatory effect of Echinacea extract could be attributed to the ability of both polysaccharides and alkamides to stimulate proliferation of cells from bone marrow, activation of phagocytosis by macrophages. That hypothesis is agreed with many authors as Bauer *et al*.(1988) in his in vivo study by giving mice ethanolic Echinacea extract, it led to stimulating the proliferation of bone marrow cells and increase phagocytosis by macrophage and neutrophils . On the other hand, on his in vitro study by incubating mouse serum with polysaccharides from Echinacea promoted phagocytosis by macrophage. Also, Bukovsky(1993) and Barrett (2003) reported that Echinacea extract do indeed demonstrated significant immunomodulattory activities through macrophage activation, phagocytosis enhancement as well as T and B –cells activation. Moreover, Echinacea extract stimulated the ability of macrophage to engulf and destroy particles (Bauer and Wagner, 1991) and it induced significant augmentation of rat's primary secondary IgG response to antigen when injected to rats for six weeks (Rehman *et al*.,1999).In this study use ELISA as a simple and rapid test for screening of antibodies followed by SNT as a confirmatory test for the results .its well known that ELISA is one such test used for screening of antibodies to various morbilli virus(Saliki *et al*.,1993 ; Libeau *et al*.,1995) and does not require cell culture and strict sterility of sera samples (Taylor *et al*., 1990).

The results of this study revealed a great effect of Echinacea extract as an immunostimulatory and beneficial income will be obtained when administrating 60% alcoholic extract of Echinacea along the vaccination process. As it was found that Echinacea extract enhance the effect of vaccine through stimulation of immune system of sheep.

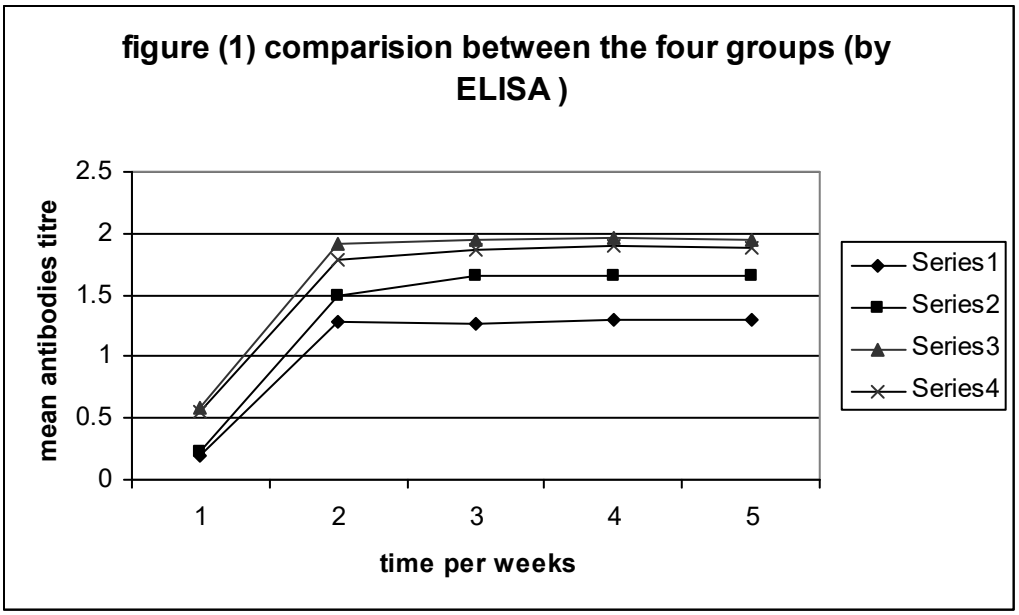
Table (1) presenting the immune response of sheep during the three treatments using ELIZA technique:

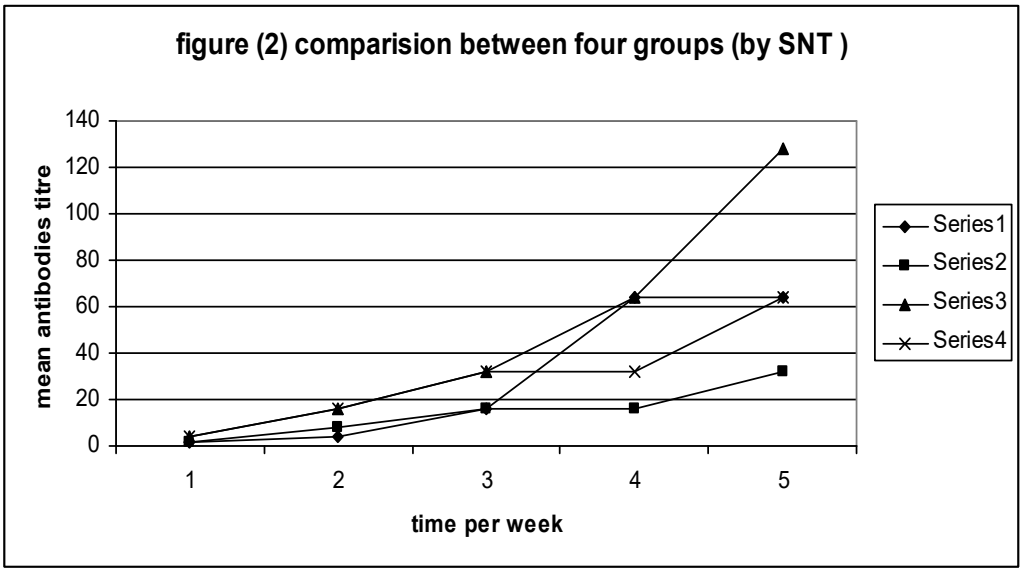
	7 days.	14 days.	21 days.	28 days.	35 days.
G1 (control).	0.188±0.052	1.280±0.045	1.271±0.0141	1.294±0.091	1.291±0.089
G2 (S. C. D.)	0.232±0.063	1.489±0.063	1.653±0.057	1.659±0.054	1.654±0.049
G3 (60% ethanol extract).	0.581±0.051	1.912±0.081	1.948±0.063	1.957±0.053	1.951±0.051
G4 (20% ethanol extract).	0.558±0.063	1.792±0.068	1.865±0.051	1.892±0.045	1.885±0.050

Table (2) presenting the immune response of sheep during the three treatments using SNT technique:

	7 days.	14 days.	21 days.	28 days.	35 days.
G1 (control).	< 2	4	16	64	64
G2 (S. C. D.)	< 2	8	16	16	32
G3 (60% ethanol extract).	4	≤ 16	32	64	128
G4 (20% ethanol extract).	≤ 4	16	32	32	64

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figure (1) comparision between the four groups (by ELISA)
mean antibodies titre
2.5
2
1.5
1
0.5
0
1
2
3
4
5
time per weeks
Series1
Series2
Series3
Series4


figure (2) comparision between four groups (by SNT)
mean antibodies titre
140
120
100
80
60
40
20
0
1
2
3
4
5
time per week
Series1
Series2
Series3
Series4

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التأثير المناعي لمستخلص نبات الاكينسيا أثناء التحصين بلقاح طاعون المجترات الصغيرة في الخراف

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المستخلص

النباتات الطبية مثل نبات الاكينسيا قد تحفز الأداء الوظيفي للجهاز المناعي من خلال زيادة أنتاج الأجسام المضادة المتخصصة . تناولت هذه الدراسة تقييم الأداء المناعي لمستخلص نبات الاكينسيا على الخراف تم تحصينها بلقاح طاعون المجترات الصغيرة.

تم تقسيم الخراف إلى أربع مجموعات (كل مجموعة تحتوي على خمسة خراف) ، المجموعة الأولى تم الاحتفاظ بها كمجموعة ضابطة بينما باقي المجموعات (2، 3، 4) تم معالجتها بمستخلص نبات الاكينسيا المستخلص بالطرق آلاتية Super critical carbondioxide والكحول الايثانولي 20 % الكحول الايثانولي 60% على التوالي وذلك أثناء التحصين . جمعت عينات الدم من كل مجموعة بعد (7 و 14 و 21 و28 و 35) يوم بغرض فصل السيرم (المصل). استخدم اختبار الاليزا واختبار المصل المتعادل لتقييم مستوى الأجسام المناعية في عينات الدم المجموعة.

أظهرت نتائج الاختبارين ان أعلى مستوى للأجسام المناعية كان في المجموعة التي تم علاجها بواسطة مستخلص الاكينسيا الكحولي الايثانولي 60 % تليها المجموعة المعالجة بواسطة متخلص ا الاكينسيا الكحولي الايثانولي 20% ثم تليها المجموعة المعالجة بواسطة مستخلص Super critical carbondioxide.