# Study The Ability Of Using Sesame Oil As An Adjuvant

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## Abstract

The aim of the present study is to investigate the ability of sesame seed oil as an adjuvant by intramuscular injection of laboratory rabbits with 1ml of sesame oil conjugate with 1mg capsular polysaccharide of *Streptococcus pneumoniae* serotype 2. The serum (systemic) and trachea (mucosal) specific immunoglobulin titer of rabbits after 7 ,15 and 21 days was measured. The results demonstrated that there was significant increase (p= 0.0499) in specific serum immunoglobulin titer of rabbits (106.67) and in trachea of rabbits (46.66) post ٢١days from administration. Also the results proved that there was significant increase (p= 0.0481) in specific serum immunoglobulin titer of rabbits (213.333) post 21 days administration as compared to controls (106.76). The study recommended that the sesame oil can act as an adjuvant through inducing high level of specific serum immunoglobulin titer of rabbits.

## الخلاصة

هدف الدراسة الحالية هو التحقق عن إمكانية استخدام زيت بذور السمسم كمحفز مناعي عن طريق التجريع بالعضلة للأرانب المختبرية بمقدار ١مل من زيت بذور السمسم ممزوج مع ١ملغم من متعدد السكريات لبكتريا *Streptococcus pneumoniae* serotype 2 . تم قياس الكلوبيولين المناعي المتخصص في المصل(الجهازي) والقصبة الهوائية(المخاطي) بعد ٧,١٥و ٢١ يوما. أثبتت نتائج الدراسة وجود فرق معنوي (p= 0.0499) بين مستوى الكلوبيولين المناعي المتخصص في المصل (106.67) والقصبة الهوائية (46.66) للأرانب بعد ٢١ يوما من التجريع . كذلك أثبتت نتائج الدراسة وجود فرق معنوي (p= 0.0481) في مستوى الكلوبيولين المناعي المتخصص في المصل (213.333) بعد ٢١ يوما من التجريع بالمقارنة مع السيطرة (106.76) . توصي الدراسة بإمكانية استخدام زيت السمسم أن يعمل كمحفز مناعي من خلال تحفيزه لمستوى عالي من الكلوبيولين المناعي المتخصص في سيرم الأرانب.

## 1:Introduction:

Sesame oil result from the seeds of *sesamum indicum* , a plant relate to the family pedaliaceae. It is composed of various fatty acids and nonfat antioxidants, consist of tocopherol, sesamin, sesamolin and sesamol and its long been categorized as traditional health food in India and other east Asian countries (Saleem *et al*.,2011). Sesame oil has been found to contain considerable amounts of the sesame lignans :sesamin ,episesamin and sesamolin, the lignin presents in sesame oil are thought to be responsible for many of its unique chemical and physiological properties ,including its antioxidant and antihypertensive properties (Sankar *et al*.,2006). The immunological response to a number of antigens can be improved by the addition of certain extraneous materials like plant oil, the oil from the nutrient rich seed is popular in alternative medicine or traditional massages and treatments to modern day fads (Aljanaby ,2010).

The water-in-oil (w/o) emulsion gave good adjuvant effect on the antibody formation to thymus-dependent antigens without thymus-independent antigens, in hapten-carrier system the priming with carrier in the w/o emulsion enhanced more

effectively the carrier specific helper function than did the priming with carrier in free solution (Mosayebi,2007). The cells responsible for the helper function were radio resistant, in the adoptive cell transfer system, the w/o emulsion was shown to enhance T helper cell function (Aljanaby ,2010) . Therefore the aim of the present study was to investigate the ability of sesame oil to induce a systemic or mucosal specific immunoglobulin titer in rabbits post intramuscular administration of sesame oil conjugate with capsular polysaccharides of *Streptococcus pneumoniae* serotype 2.

## 2:Material and Methods:

**Laboratory Animals:** A total of 18 New Zeland white rabbits weighting (1.5-2) kg and 12 balbC albino mice weighting (30-35)g were used . Animals were left for two weeks for adaptation to the laboratory environmental conditions with adequate water and food before being used for experimentation.

**Sesame oil and incomplete Freund adjuvant:** were provided from Hi media CO. , India, and conjugated by mixing well(1ml of sesame oil and 1ml of incomplete Freund adjuvant**)** under pressure by two sterile syringe (connected together by sterile tube).(Aljanaby ,2010).

***S.pneumoniae* isolation and identification:** *S. pneumoniae* isolation from patients with pulmonary infection and identified according to its culture characters , morphology staining and its biochemical reactions according to Macfaddin (2000).

**Serotype identification test was made by slide agglutination method:** Serotyping kit (Antisera) for *S.pneumoniae* identification was used according to the manufacturing company instructions (DENKA SEIKEN CO.,LTD) JAPAN.

**Mouse virulence test:** A volume of 0.3 ml of broth culture of *S.pneumoniae* serotype2 contain $1x10^8$cfu/ml was intraperitonealy injected into three mice. Animals were observed for pneumonia onset over 6 hours. The liver and kidney of dead mice were removed and emulsified with normal saline and streaked on gentimicine blood agar plate and incubated at $37C^0$ with (5-10) %CO2 for 24hours. All plates were checked for the presence of *S.pneumoniae* growth and the plates with presumptive *S.pneumoniae* were checked by morphology staining , culture characters and biochemical reactions. Three mice were injected with normal saline intraperitoneally as control (Sottile and Rytel, 2007).

**Polysaccharide isolation method:** This method was recommended by Kwapinsiki(1972) as follows:

1-*Streptococcus pneumoniae* cultured (by swab to obtain heavy growth)on Trypticase Soy Agar for 24hours at $37C^0$ with (5-10)% CO2.

2- The growth was harvested by 5ml normal saline.

3- One ml Hcl(0.5N)was added to the 5ml of suspension

4- The suspension was heated at 75C for 30min. in a water bath.

5- The suspension was cooled and centrifuged at 2500 rpm / 10 min .and the pellet was discarded.

6- The supernatant was neutralized to pH 7.0 by NaOH.(0.5N).

7- Three volumes of(1% ethyl sodium acetate)were added to the supernatant and kept at 4C for 24hours.
8- It was then centrifuged at 2500 rpm / 10 min. the pellet was collected and dissolved in one ml D.W.
9- Three volumes of (1% ethyl sodium acetate)was added and kept for 1hours at $4C^{o}$.
10- The preparation was centrifuged at 2500 rpm / 10 min. and the pellet was collected.

**Capsular polysaccharide detection methods:**

**1- Molisch test and Iodine test:** These methods were recommended by Hall(1980).

**2- Total protein concentration calculation:** The biuret method was used to estimate the total protein concentration in the capsular polysaccharide solution via Biolab reagent (kit) ,France, by spectrophotometric measurement (Gornall *et al* .,1949).

**3-Capsular polysaccharide pathogencity test:** This method was recommended by Tian *et al* (2007).An 0.3ml normal saline containing 0.1mg of capsular polysaccharide was intraperitoneally injected into mice( three replicates). Mice were observed over 6 hours after injection (three control mice were injected with normal saline).

**Immunization protocols:**

Eighteen rabbits were used for the immunization protocols (3 replicates for each protocol).(**Figure 1**).

**Protocol 1**: Intramuscular 1mg capsular polysaccharide of *S.pneumoniae* serotype2 conjugate with 1ml of incomplete Freund adjuvant for 7 , 15 and 21 days respectively (as control).

**Protocol 2**: Intramuscular 1mg capsular polysaccharide of *S. pneumoniae* serotype2 conjugate with 1ml sesame oil for 7 , 15 and 21 days respectively.

Intramuscular 1mg capsular polysaccharide of *S.pneumoniae* serotype2 conjugate with 1ml of incomplete Freund adjuvant and conjugate with 1ml sesame oil for 7 , 15 and 21 days respectively.

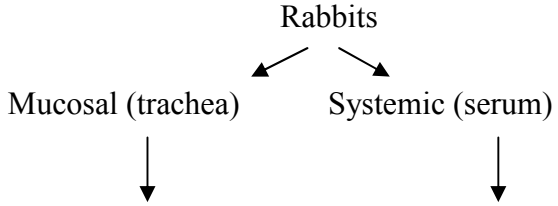


**Fig. 1.** Flow chart for immunization protocols for specific immunoglobulin titer detection in rabbits.

**Mucosal sample:** Mucosal samples were collected from treated rabbits after being sacrificed by chlorophorm , this method was described by Shnawa and Thwaini (2002).

**Separation of serum immunoglobulin:** The method used was that of Garvey *et al* (1977) as follows:

1- To 1ml of serum, 1ml of PEG 6% was added. The mixture was left in refrigerator for 30min at $4C^{o}$

2- It was centrifuged at 4000 rpm for 20min

3- The supernate was discarded and the precipitate was taken.

4- Five ml of formal saline & 5ml of PEG 6% was added and mixed well and left at room temperature for 15min.

5- It was centrifuged at 3500rpm for 20min.

6- The supernatant was discarded and the pellet was dissolved in 0.25ml formal saline.

**Separation of mucosal immunoglobulin:** This method was performed according to Shnawa and Thwaini (2002) as follows:

1-Equal volume of PEG was added to mucosal isolate and was left for 24hours at $4C^0$.

2-It was centrifuged at 4000 rpm for 20min. and the supernatant was discarded .

3- The pellet was dissolved in 1ml of formal saline and stored at 4C until used.

**Passive heamagglutination test (PHA):** This method was made according to Garvey *et al* (1977).

Passive haemgglutination test (PHA) was used to determine the antibody titer in systemic and mucosal secretion of immunized rabbits.

**Statistical analysis:** Statistical analysis was made using (graph pad prism version 4) computer software according to T test. The mean value and standard error (SE) for each value was determined. P value less than the 0.05 level of significance was considered statistically significant.

## 3:Results:

**Table 1.** Results of identification of *S.pneumoniae*.

| Gram stain | Catalase test | Capsule stain | Optochin test | Bile solubility test | Inulin fermentation test | Mice virulence test | Kit Identification |
|---|---|---|---|---|---|---|---|
| $G^+$ Diplococci | - | + | + | + | + | + | Serotype 2 |

**Table 2.** Capsular polysaccharide detection parameters.

| capsular polysaccharide | Culture on blood agar | Molisch test | Iodine test | Total protein concentration | pathogenicity test in mice |
|---|---|---|---|---|---|
| parameters | _ | + | + | 0.015 g/dl | _ |

The results indicate that no significant difference between systemic and mucosal of specific immunoglobulin titer in rabbits post intramuscular 1mg capsular polysaccharide of *S.pneumoniae* serotype 2 conjugate with 1ml of incomplete Freund adjuvant for 7 days and for 15 days respectively. On the other hand the present study demonstrated that there was significant increase (p= 0.0499) with 21 days (table 3).

**Table 3.** Comparison of specific immunoglobulin titer in serum and trachea of rabbits post intramuscular 1mg capsular polysaccharide of *S.pneumoniae* serotype 2 conjugate with 1ml of incomplete Freund adjuvant for 7,15 and 21 days. By PHA test .N=3.

| Specific immunoglobulin titer in rabbits | | Mean ± SE | *P value | Significance |
|---|---|---|---|---|
| Post 7 days | Systemic (blood) | 33.333±6.6667 | 0.0890 | Non significant |
| | Mucosal (trachea) | 16.6667±3.333 | | |
| Post 15 days | Systemic (blood) | 66.667±13.333 | 0.0890 | Non significant |
| | Mucosal (trachea) | 33.333±6.666 | | |
| Post 21 days | Systemic (blood) | 106.67±26.667 | 0.0499 | Significant P=<(0.05) |
| | Mucosal (trachea) | 46.667±17.638 | | |

SE= Standard error *= Measured according to T test.

Specific immunoglobulin titer in serum and trachea of rabbits post intramuscular 1mg capsular polysaccharide conjugate with 1ml of sesame oil for deferent days.

The results reveal no significant increase in specific immunoglobulin titer in serum and trachea of rabbits post intramuscular 1mg capsular polysaccharide of *S. pneumoniae* serotype 2 conjugate with 1ml sesame oil for 7 days . But The results indicate that there was significant difference in specific immunoglobulin titer for 15 days and for 21 days p= 0.0259, p=0.0137 respectively.(**Table 4**).

**Table 4.** Comparison of specific immunoglobulin titer in serum and trachea of rabbits post intramuscular 1mg capsular polysaccharide of *Streptococcus pneumoniae* serotype 2 conjugate with 1ml of sesame oil for 7,15 and 21 days. By PHA test .N=3.

| Specific immunoglobulin titer in rabbits | | Mean ± SE | *P value | Significance |
|---|---|---|---|---|
| post 7 days | Systemic (blood) | 53.333±13.333 | 0.1481 | Non significant |
| | Mucosal (trachea) | 26.667±6.6667 | | |
| post 15 days | Systemic(blood) | 106.26 ±26.667 | 0.0259 | Significant P=<(0.05) |
| | Mucosal (trachea) | 40.033±6.666 | | |
| post 21 days | Systemic(blood) | 213.333±53.333 | 0.0137 | Significant P=<(0.05) |
| | Mucosal (trachea) | 53.333±13.333 | | |

SE= Standard error *= Measured according to T test.

**Specific immunoglobulin titer in serum and trachea of rabbits for deferent days.**

The intramuscular 1mg capsular polysaccharide of *S.pneumoniae* serotype 2 conjugate with 1ml sesame oil gave the highest level in specific immunoglobulin in serum of rabbits then conjugate with 1ml of incomplete Freund adjuvant with significant increase p=0.04508 post 15 days and with significant increase p= 0.0481 post 21 days (**Table 5**). while the result reveal no significant difference in specific immunoglobulin titer in trachea of rabbits post intramuscular 1mg capsular polysaccharide of *S.pneumoniae* serotype 2 conjugate with 1ml sesame oil and post conjugate with 1ml of incomplete Freund adjuvant after 7 , 15 and 21 days (**Table 6**).

**Table 5.**Comparison between specific immunoglobulin titer in serum of rabbits .

| **Specific immunoglobulin titer in serum of rabbits.** | **Mean ± SE** | ***P value** | **Significance** |
|---|---|---|---|
| Post 7 days with incomplete Freund adjuvant. | 33.333±6.6667 | p=0.0562 | Non significant |
| Post 7 days with sesame oil. | 53.333±13.333 | | |
| Post 15 days with incomplete Freund adjuvant. | 66.667±13.333 | p=0.0450 | Significant P=<(0.05) |
| Post 15 days with sesame oil. | 106.26 ±26.667 | | |
| Post 21 days with incomplete Freund adjuvant. | 106.67±26.667 | p= 0.0481 | Significant P=<(0.05) |
| Post 21 days with sesame oil. | 213.333±53.333 | | |

SE= Standard error *= Measured according to T test.

**Table 6.** Comparison between specific immunoglobulin titer in trachea of rabbits.

| **Specific immunoglobulin titer in trachea of rabbits.** | **Mean ± SE** | ***P value** | **Significance** |
|---|---|---|---|
| Post 7 days with incomplete Freund adjuvant. | 16.6667±3.333 | p=0.0832 | Non significant |
| Post 7 days with sesame oil. | 26.667±6.6667 | | |
| Post 15 days with incomplete Freund adjuvant. | 66.667±13.333 | p=0.0562 | Non significant |
| Post 15 days with sesame oil. | 40.033±6.666 | | |
| Post 21 days with incomplete Freund adjuvant. | 46.667±17.638 | p=0.1262 | Non significant |
| Post 21 days with sesame oil. | 53.333±13.333 | | |

SE= Standard error *= Measured according to T test.

## 4:Discussion:

The results demonstrate that intramuscular 1mg capsular polysaccharide of *streptococcus pneumonia* serotype 2 conjugate with 1ml of sesame oil can induce high level of specific immunoglobulin in serum and trachea of rabbits as compared to control.

The mechanisms by which oil promote increased immune response are slowly becoming more defined as the molecular aspects of antigen recognition and immune response become understood. Adjuvant may have up to five of the following mechanisms of action: the “depot” effect, an antigen presentation effect, an antigen distribution or targeting effect, an immune activation/ modulation effect, and cytotoxic lymphocyte induction effect (Cox and Coulter,1997).

One classic mechanism of oil action is the “depot” effect, in which the adjuvant protects the antigen from both dilution and rapid degradation and elimination by the host. By localizing and slowly releasing intact antigen, the adjuvant permits a slow, prolonged exposure of the immune system cells to a low level of antigen. This prolonged exposure results in continued stimulation of antibody producing cells, resulting in the production of high levels of antibody by the host (Harold and Stills, 2005 ; Aljanaby,2010).

The importance of continued low-level antigen stimulation for the production of high-affinity antibody is best explained by the antigen selection hypothesis proposed by Siskind and Benacerraf (1969) they proposed that low-dose antigen exposure resulted in the stimulation of only B cells with high-affinity receptors, whereas at higher doses, B cells with medium- and low-affinity receptors would be stimulated (Harold and Stills ,2005).

The conjugation of oil to capsular polysaccharide to allow polysaccharides -specific responses that elicit T-cell help (Lee *et al* ., 2001) .Stimulation of the B cell (with consequent production of capsular polysaccharide -specific antibody) and activation of the peptide-recognizing $CD4^+$ T cell result in T-cell help,Th2 helper which promotes immunoglobulin class switching to IgG and memory responses. Immunoglobulin class switching and B-cell memory depend on co-stimulation of the B cell through CD80 and/or CD86 interacting with CD28, through CD40 interacting with CD40L and perhaps through other interactions between co-stimulatory molecules. Bacterial capsular polysaccharide are classic antigens for B cells and are recognized by a B-cell receptor (BCR) that has the correct specificity, interaction between the capsular polysaccharide and the BCR is sufficient to induce the signals that are required to stimulate clonal expansion of B cells and antibody production (Aljanaby,2010). However, this pathway by itself does not result in immunological memory. Capsular polysaccharide – oil interact with a BCR in a similar manner to pure polysaccharides; however, in addition, they elicit T-cell help, through antigen presentation of the protein component to $CD4^+$ T cells, which provide the necessary co-stimulation to induce memory B cells and memory T cells. Therefore, antibody production is achieved, and the consequent immunological memory results in antigen-specific immunity to the capsular polysaccharide . This strategy has been exploited to produce pathogen-specific vaccines that target bacterial capsular polysaccharides (Sarkis and Dennis,2006).

The mechanism of the enhanced response to a bacterial antigen when combined with the adjuvants here in described is obviously complex. Under the ordinary conditions of inoculation with watery suspensions of antigen the antigenic stimulus is apparently short-lived, for the antigen is absorbed by the body and probably destroyed within a short period of time, this is reflected in the characteristic antibody response in which the highest titers are reached within 2 weeks after inoculation and are followed by a progressive decrease to low antibody levels thereafter (Kutuna,2005).The antibody response to inoculation of the antigen in the form of an emulsion containing bacterial capsule and oil, on the other hand, continues to increase up to 1 to 3 weeks after inoculation, reaching extraordinarily high titers ,the function of the oil is probably to set up a reactive tissue wall about the inoculums and thus localize and maintain the antigenic material at the inoculation site and the

adsorption base (Falba) undoubtedly contributes to the tissue reaction as well as combining the watery and the oily components into a stable water-in-oil emulsion (Aljanaby,2010).

## 5:Conclusion:

The results of the present study indicate that sesame oil can act as an adjuvant because it induce high level of specific immunoglobulin titer in serum of rabbits after it conjugate with capsular polysaccharide of *S.pneumoniae* serotype2.

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