The effect of *Aloe vera* extraction on immunity
تأثير مستخلص الصبير على المناعة

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Abstract

***Aloe vera* aqueous extraction was examined for its immunomodulatory effect. Concentrated aqueous extraction was administrated orally 2ml/day for 30 days to the white mice, immunomodulatory effect was studied on total white blood cells (WBCs)count, phagocytosis, antibody titer and plaque forming cell (PFC). There is a significant increase in total count of WBC, reach 11300cell/ml^3 at the day 28 after administration. Increase in phagocytosis activity was observed clearly after three and four week compared with control. Remarkable increase in the antibody titer against SRBCs for primary and secondary immune response (10.4±022, 14.2±2) respectively, and finally apparent effect on plaque forming cell (PFC) and maximum value was found after 5 days after immunization with SRBCs. It was 1200 PFC/10^6 of spleen cell.**

Key words: *Aloe vera*, phagocytosis, immunomodulator, immunopotentiator, immune system.

المستخلص

لقد تم اختبار قابلية التغيير المناعي للمستخلص المائي لنبات الصبار.باستعمال الفئران المختبرية وبجرعة 2مل عن طريق الفم يوميا ولمدة شهر. تمت دراسة تاثير التغيير المناعي على العدد الكلي للخلايا البيض، عملية البلعمه، عيارية الاضداد واخيرا دراسة التاثير على الخلايا المكونه للويحات الخلايا البائية (الخلايا الفارزه للاضداد). وجد زياده ملحوظه في العدد الكلي لكريات الدم البيض بلغ عددها 11300 خليه/مل^3 في اليوم 28 بعد التغذيه بالمستخلص المائي مقارنة بالسيطره. كما وجد زياده في كفاءة عملية البلعمه عند الاسبوع الثالث والرابع مقارنة بلسيطره. كما وجد تاثير واضح في زيادة عياريةالاضداد ضد كريات الدم الحمر للخروف عند الاستجابة المناعية الاولى والثانية حيث بلغ عند الاستجابة المناعية الاولى 0.22 ± 10وعند الاستجابة المناعية الثانيه 2±4.2 واخيرا وجد ازدياد في عدد الخلايا المكونة للويحات(الخلايا الفارزه للاضداد) عند اليوم الخامس للتمنيع بكريات الدم الحمر للخروف وكان العدد 1500 لكل مليون خليه طحال.

الكلمات المفتاحية: مستخلص الصبار ، التحوير المناعي،المحفزات المناعية، البلعمة

Introduction

Natural products are important resources in traditional medicine and have been long used for prevention and treatment of many diseases. *Aloe vera* (Liliaceae) is one of the herbal medicines widely used in wound and burn healing [1], in treatment disorders such as arthritis, gout, dermatitis, peptic ulcer [2]. *Aloe vera*is a naturalsubstance containing enzymes, amino acids, and other active ingredients which have antimicrobial activity [3] and biological and pharmacological activities such as mitogenic activity for lymphocytes, complement activation, anti-inflammatory activity and immunomodulatory activity on immune response [4]. Both polysaccharides and glycoprotein are involved in such activities especially in connection with immune system [5].

Our study investigated the immunological effect of *aloe vera* extract when adminstrated orally in white mice.

Material and method

Plant: *Aloe vera* purchased from plant nursery in Baghdad, identified in Department of biology / University of Baghdad. Healthy mature leaves washed and cut in the middle to get inner sap material by scratching with knife. The inner pulp was cut into small pieces, weighted to get 400gr, homogenized with 400ml of phosphate buffer saline (PBS) pH-5 by blender in cold condition and kept overnight in refrigerator, then filtered through muslin clothes to get filtrate which centrifuged at 2000 rpm for 15min under cooled condition, discarded the green pellet and taken clear yellow supernatant concentrated by drying in sterile condition to 200ml and conserved in cold condition until used in experiment [6].

Experimental animal

Healthy white mice 15-20g of both sexes where selected for the study, the animal were feed on commercial water and food pellet. They where acclimated to laboratory hygienic condition for 10 days.

Experimental design

Animals were randomly divided into two groups, group A, control not received *aloe vera* extraction, 2ml of PBSwas added to the water. Group B, animal received 2ml of *Aloe vera* concentrated extraction added to the water. Tested extract applied daily to the water.

1-Effect of *aloe vera* extract on total number of white blood cells (WBCs)

Total number of white blood cells(WBCs) count were determined before treatment and at day 7, 14, 21, 28day.of treatment. Blood was collected from tail vein, WBCs total count determined by using hemocytometer.

2-Effect of *Aloe vera* extraction (AVE) on phagocytosis

Phagocytic activity was assessed by the modified method of [7], in summary:25µl of blood from tail vein was collected at day 0, 7, 14, 21, 28, of experimental animal and controlseparately, mixed with 0.25 µl of 1×10^4*Staphylococcus aureus* overnight culture in heparinated plastic tube, incubated for 30 min. in $37C^0$, smear of blood stained with Leichman'sstain for 2min. washing with water and left drying for 8 min. microscopic examination at 100x to find phagocytic index of neutrophilswas carried out :

$$\mathbf{PI} = \frac{\mathbf{phagocytic(engulfed)}}{\mathbf{total\ number\ of\ phagocyte}} \times \mathbf{100}$$

(3mice of each group were taken and compared with control).

3- Effect of *Aloe vera* extraction on plaque- forming cell

Three mice from group A and 3 from group B was immunized with 0.5ml of 1×10% SRBcintraperitoneal, after 5 days of immunization., all animals were killed,spleens were processed and the number of plaque forming cell (Ab forming cell) were determined by the method of [8].

4- Effect of *aloe vera* extraction on humoral response (Ab production)

Three mice from group A and three from group B, injected with 0.5 ml of 10% SRBcintraperitoneal.Ab titer was measured according to [9]. Blood samples were collected from caudal vein .Ab titer was determined by the heamaglotination test. The blood sample were centrifuged to collect the seraand equal volume of individual serum sample of each group was pooled. To serial two fold dilution of pooled serum sample made in 25µl in normal saline microtitration plates was added 25µl of 1% SRBCs suspension in saline, aftermixing, the plates were incubated at $37C^0$for one hour and examined for heamglutination titration.

Statistical Analysis: All the results were expressed as ± standard error. Data were analyzed using one way analysis of variance (ANOVA). P-value≤0.05 were considered as statistically significant.

Results and discussion

Aloe vera is one of the herbal medicines widely used in natural treatment and alternative therapy for various type of diseases .Previous scientific studies on *Aloe vera* extract showed ability to modulate immune response specific and non specific immunity when it was injected intraperitoneally [10] and improve wound healing when it was administrated orally and topically [11]. In our study aqueous extraction of *aloe vera* showed remarkable effect on immune responses when it was administrated orally. There is significant increase in WBC total count compared with control. Highest value was seen at the day 28 after treatment it was 11300cell/ml. Fig (1),

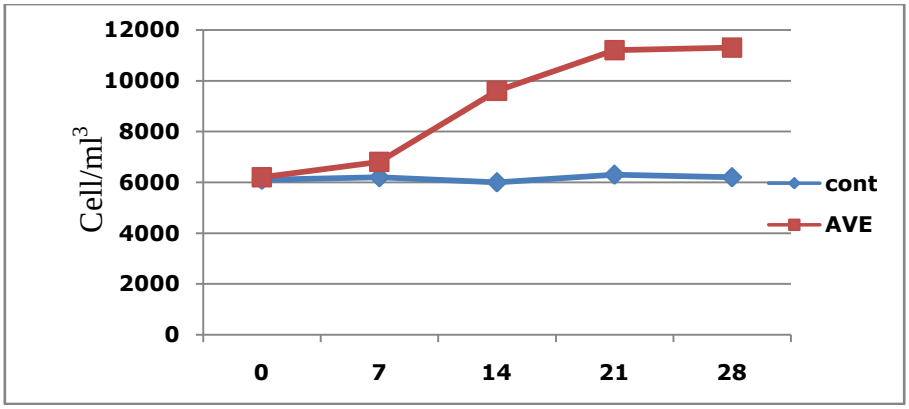

Fig. 1: Effect of AVE on Total WBC count

similar result was obtained by [12] when AVG was administrated intraperitoniallyand the total count of WBCs reached 14000cell/ml.

There is remarkable increase in phagocytic index (PI).Table (1), this increase is proportional with days after administration. Highest value of PI was 39±1 .Study, by [13]observed that AVE produce increase in the phagocytic activity and microbicidal activity of the peritoneal macrophages and increase in the size, number of psedopods and number of vacuoles of these cells.

Table (1): Effect of *Aloe vera* extract on phagocytic index (PI)

Days after Treatment	PI
0	26 ± 1
7	28 ± 1.4
14	35 ± 3.2*
21	38 ± 2*
28	39 ± 1*

***Significant increase P≤0.05compared with control**

*Aloe vera*extraction enhances and increases plaque forming cell (antibody secreting cell) and this in turn influenced antibody titer .This results in Fig2 and Table (2) show the effectAVE on number of PFC and a marked increase in antibody titer against SRBCs.

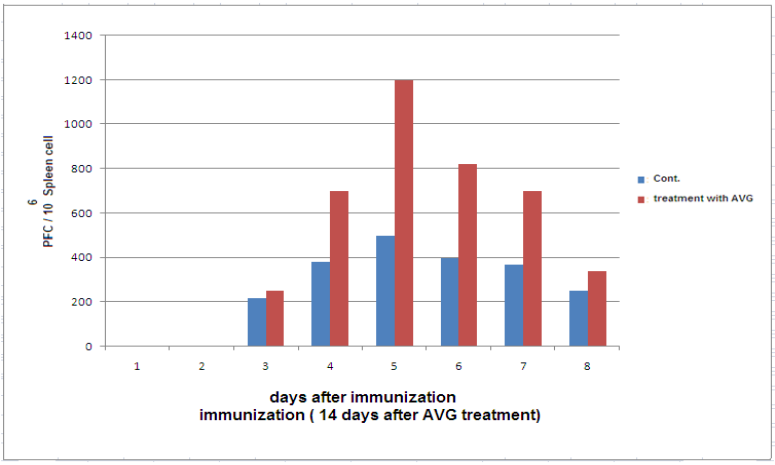

Fig.2: Effect of AVE on plaque forming cell (PCF)

Table(2): II Effect of AVE on antibody titer

Ab titer	primary immune response response	secondary immune
Control	7.5 ±03	11.3±2.1
Orally administration(AVE)	*10.4±2.1	*14.2±0.8

n=3mean ±standard error *significant increase p≤0.05compared with control

Many chemical components as polysaccharides and glycoprotein are involved in modulation of immune responses[14],Acemannan, the major fraction of aloe polysaccharides, hasbeen extensively studied for immunomodulatory effect. Reports showed that these β (1, 4)-linked acetylated mannans are able to increases phagocytic activity [15]. An acemannan stimulate leucocytes and lymphocyte in dose dependant manner as well as triggered the release of IL-1, IL-6 and TNF-α. The study found that

AVE modulateboth specific and non specific immunity andAVE effective components not affected or diminished by digestive enzyme.

Present study showed theimmunomodulatory effect of *Aloe vera* extract on both cellular and humeral immunity when it administrated orally. Hence it can be concluded that *Aloe vera* extract may be a potential candidate in several immune suppressed clinical condition. Further studies are needed in animal models and human to clarify the modulatory effect of *A.vera* on immune function. Extraction and purification of *A.vera*compound also needed to find out effective molecules.

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