Research Article

Immunomodulatory Activity of Aqueous Extract of Arachis Hypogaea Seeds (Fabaceae) in Rats

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Accepted on: 20-08-2015; **Finalized on:** 30-09-2015.

ABSTRACT

The objective of the study is to study the Immunomodulatory activity of aqueous extract of *Arachis hypogaea* seeds (Fabaceae) by using different methods. Effects of 100 and 400 mg/kg body weight of the aqueous extract of *Arachis hypogaea* seeds (Fabaceae) were studied on various immune paradigms like delayed type hypersensitivity reaction using SRBC as an antigen, determination of antibody titer, neutrophil adhesion test as an indicator for neutrophil index and carbon clearance assay as a measure of phagocytic activity. Aqueous extract of *Arachis hypogaea* substantially enhanced cellular immune response, humoral immune response, neutrophil index and phagocytic activity in doses of 100 and 400 mg/kg body weight. The Aqueous extract (400 mg/kg body weight) was efficient in improving immune response. The immunostimulatory effect produced by the extract may be due to cell mediated and humoral antibody mediated activation of T and B cells. It is therefore concluded that the *Arachis hypogaea* extract is a potent immunostimulator and its impact may be attributed to the flavanoids, proteins, vitamins and minerals present in the extract.

Keywords: *Arachis hypogaea,* immune response, humoral, immunostimulator.

INTRODUCTION

Immunomodulator is a substance that alters the immune response by augmenting the ability of the immune system to produce antibodies that recognize and react with the antigen that initiated their production. There are two types of agents, immunosuppressive agents which inhibits immune response, in organ transplantation and autoimmune diseases and immune stimulating agents which increase the immune response, useful in infections, immunodeficiency (AIDS and cancer). Herbal drugs are gaining interest nowadays because of their safety, low cost and easy availability.

There are many plants which are used traditionally to stimulate immune response.

Arachis hypogaea, is a species in the legume family Fabaceae. *Arachis* have seed containing pods, which mature underground. *Arachis hypogaea* is found all over world but mostly in Brazil, Bolivia, and America[1]. *Arachis hypogaea* have two varieties, one is wild and other is cultivated[2,3]. The seed is used mainly as a nutritive food.

It is reported to have hypoglycaemic, hypolipidemic, anti-oxidant, and effect on obesity parameters. Since, there are no reports of immunomodulatory activity of aqueous extract of *Arachis hypogea* seeds, the present work investigates the immumomodulatory activity of aqueous extract of *Arachis hypogea* seed (AEAH) by using different models.

MATERIALS AND METHODS

Plant material: Aqueous extract of seed *Arachis hypogaea* is obtained as gift sample from Green Chem. Herbal Extracts & Formulations, Bangalore, India.

Animals: Male albino Wistar rats (150-200 gm) were purchased from registered breeder and were maintained under standard animal house conditions in animal house of KLE University's College of Pharmacy, Bengaluru. Experimental protocol (IAEC/02/PA/2013-2014) was approved by Institutional animal ethics committee (IAEC) of KLE University's College of Pharmacy, Bengaluru.

Acute toxicity study:[4] Acute toxicity studies for aqueous extract were conducted as per OECD guidelines 425 to determine the safe dose using female albino Wistar rats weighing 150-200g.

Grouping of animals: Immunomodulatory activity was evaluated by Delayed hypersensitivity reaction, carbon clearance assay, humoral immune response, total leukocyte count and neutrophil adhesion test.

Albino Wistar rats (Male) weight (150-200g) were procured and randomly divided into IV groups of 6 animals each for the Delayed hypersensitivity reaction, carbon clearance assay, humoral immune response, total leukocyte count. Group I served as normal and it received 1ml water, Group II as positive control and Group III and IV treated with (100 mg/kg b.w. and 400 mg/kg b.w. p.o.) aqueous extract of *Arachis hypogaea* respectively and for neutrophil adhesion test model there is no positive control group.

Delayed hypersensitivity (DTH) reaction using SRBC as an antigen[5]

All rats were immunized on day 0 with 0.8 mL SRBC suspension in phosphate buffer containing 5×10^8 cells/mL, i.p. Wistar albino rats were treated with aqueous extract of seeds of *Arachis hypogaea* (AEAH) for 7 days. Edema was induced in the right hind paw of rats

by challenging with 0.1 mL SRBC suspension in the sub plantar region on day 7. The control lateral paw received equal volume of phosphate buffer and served as a control. Increase in the paw volume at 0, 24 and 48 h was assessed using a plethysmometer. The difference in the thickness of the right hind paw and left hind paw was used as a measure of delayed type hypersensitivity reaction.

Carbon clearance assay

After treatment for 7 days, rats were injected with 0.1 mL of carbon suspension (Indian ink) intravenously through tail vein on the 8th day. Blood samples were collected from retro-orbital plexus at 10 and 20 min time intervals[6]. Blood samples were lyzed with 2 mL of 0.1% acetic acid and absorbance of samples was recorded at 675 nm using a spectrophotometer. The graph for absorbance versus time was plotted for each animal in respective test groups and phagocytic index was calculated using the formula:

$$K = \frac{OD_1 - OD_2}{t_2 - t_1}$$

Where, OD_1 and OD_2 are the optical densities at times t_1 and t_2 respectively and K represents the slope of regression line.

Neutrophil adhesion test[7]

All the groups received the treatment for 14 days by oral route. After 1 h of the last dose on the 14th day, blood samples were collected in heparinised vials by retro-orbital puncture and subjected to total as well as differential leukocyte count by fixing blood smears and staining with Leishman's stain. After initial counts, the blood samples were incubated with 80mg/mL of nylon fibers at 37 °C for 15 min. The incubated samples were analyzed for total and differential leukocyte count. The product of total leukocyte count and % neutrophils known as neutrophil index was determined for each of the respective groups[7]. The % neutrophil adhesion for each of the test groups will be determined as follows:

$$Neutrophil\ adhesion\ (\%) = \frac{Nl_U - Nl_t}{Nl_U}$$

Where NI_U = neutrophil count is untreated blood

Where NIt =neutrophil count in fiber-treated blood

Humoral immune response[8]: All rats were immunized on day 0 with 0.8 mL SRBC suspension containing 5×10^8 cells/mL (i.p.). Cyclophosphamide (50mg/kg) was administered orally on 5th day of the experiment to animals of all the groups except group-I. On 7th day, 2 h after the last dose blood was withdrawn from the retro-orbital plexus under light anaesthesia. Serum (25µL) was serially diluted with 25µL of phosphate buffered saline. SRBC suspension (0.1 mL) was added to each of these dilutions and incubated at 37°C for 1 h. The value of highest serum dilution carrying visible haemagglutination will be taken as the antibody titre expressed in terms of wells.[5]

Statistics

The interpretation of results was done after subjecting the data obtained from various studies to statistical analysis which included t-test and one way ANOVA followed by post- test Dunnet's multiple comparison test.

RESULTS

Acute toxicity studies

Aqueous extract of *Arachis hypogaea* were conducted as per OECD guidelines 425 using female albino Wistar rats weighing 150-200g. There was no change in normal behavioural pattern of animals. No sign and symptoms of toxicity were observed during the observations which was done continuously for the first 4 h and then observed up to 24 h for mortality and continued after each dose. The derivatives were safe up to a dose of 2000 mg/kg b.w. The in-vivo studies were carried out for aqueous extract of *Arachis hypogaea* at doses of 100,400 mg/kg b.w.

Delayed Hypersensitivity Type (DTH) Reaction using SRBC as an antigen

The impact of 100 and 400 mg/kg of AEAH on T-cell mediated DTH reaction is depicted in **Table 1.**

Group treated with AEAH at dose 100 mg/kg showed significant increase ($p<0.05$) in DTH response whilst group treated with AEAH at dose 400 mg/kg showed significant increase ($p<0.01$) DTH response in terms of Paw volume compared against control.

Carbon clearance assay

Administration of AEAH (100 and 400 mg/kg p.o) increased the clearance of carbon particles from blood as indicated by a significant increase in phagocytic index (***P<0.001) when compared with control group.

This indicates that AEAH extract enhanced the phagocytic activity by stimulating the reticuloendothelial system in a dose dependent manner as shown in **Table 2**

Neutrophil adhesion test

Incubation of neutrophils with nylon fibers (NF) produced a decrease in the neutrophil counts due to adhesion of neutrophils to the fibers and it is an indicative of the marginalization of phagocytic cells in the blood vessels, i.e. an indication of immunostimulation.

A dose dependent increase in percentage of neutrophil adhesion was observed with AEAH 400 mg/kg which was significant compared to control (**P<0.01). The results are shown in **Table 3.**

Humoral Immune Response

AEAH (100 and 400 mg/kg) increased humoral antibody titre compared with the control.

The antibody titre level was significant in animals treated with higher dose of AEAH (400 mg/kg) compared to the control (*P<0.05). The results are shown in **Table 4.**

Table 1: Effect of aqueous extract of *Arachis hypogaea* on delayed type hypersensitivity reaction. (% increase in paw volume)

Groups	Treatment	0 hour	24 hour	48 hour
I	Normal	0.35 ±	0.39 ±	0.31 ±
II	Positive	0.75 ± **	0.76 ± **	0.61 ± **
III	100 mg/kg	0.70 ± **	0.63 ± **	0.55 ± **
IV	400 mg/kg	0.63 ± *	0.60 ± *	0.41 ± *

n=6, values are expressed as Mean± SEM, In Each Group; Statistical Analysis by One Way ANOVA Followed by Dunnett's Multiple Comparison Test Using Graph pad Prism Software. **P<0.01 and P<0.05 compared to control.

Table 2: Effect of aqueous extract of *Arachis hypogaea* on phagocytic index.

Groups	Treatment	Phagocytic index
I	Normal	0.002 ± 0.001
II	Positive control	0.07 ± 0.019*
III	100 mg/kg	0.11 ± 0.018***
IV	400 mg/kg	0.27 ± 0.019***

n=6,values are expressed as Mean± SEM, In Each Group; Statistical Analysis by One Way ANOVA Followed by Dunnett's Multiple Comparison Test, Using Graph pad Prism Software. ***P<0.001 and *P<0.05 compared to control.

Table 3: Effect of aqueous extract of *Arachis hypogaea* on Neutrophil adhesion test.

Groups	Treatment	Total Leukocyte count		Total Neutrophil count		
		Untreated blood	Nylon fibre treated blood	Untreated blood	Nylon fibre treated blood	Neutrophil adhesion (%)
I	Normal	8,215 ±	5,515	7,092 ±	5,580	21.31
II	100	8,947 ±	6,365	8,083 ±	5,945	26.45
III	400	10,870±	7,308	10,058	6,395	36.41**

n=6,values are expressed as Mean± SEM, In Each Group; Statistical Analysis by One Way ANOVA Followed by Dunnett's Multiple Comparison Test Using Graph pad Prism Software. ***P<0.001, **P<0.01 and *P<0.05 compared to control.

Table 4: Effect of aqueous extract of *Arachis hypogaea* on humoral immune response.

Groups	Treatment	Haemagglutination antibody
I	Normal	3.167 ± 0.60
II	Positive control	1.167 ± 0.47*
III	100 mg/kg	3.500 ± 0.42
IV	400 mg/kg	5.333 ± 0.42*

n=6,values are expressed as Mean± SEM, In Each Group; Statistical Analysis by One Way ANOVA Followed by Dunnett's Multiple Comparison Test Using Graph pad Prism Software. ***P<0.001 and *P<0.05 compared to control.

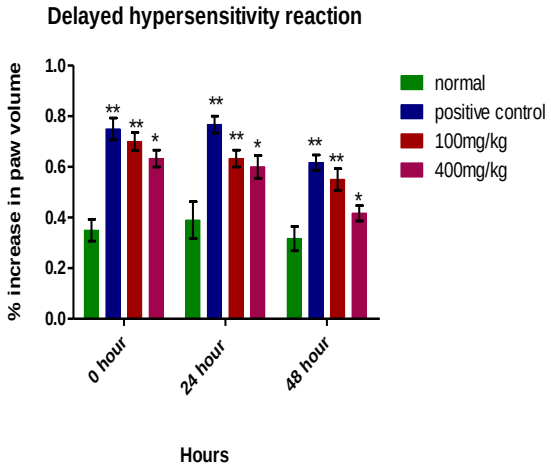

Figure 1: Effect of aqueous extract of *Arachis hypogaea* on delayed type hypersensitivity reaction. (% increase in paw volume)

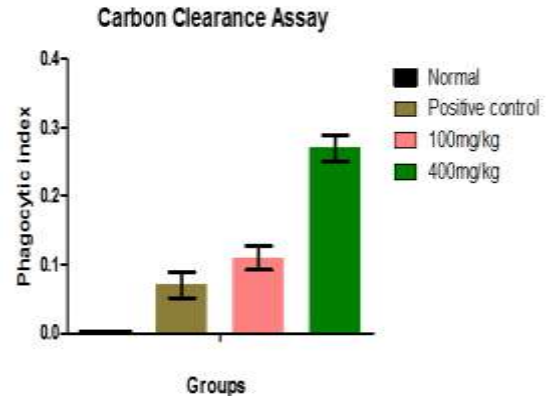

Figure 2: Effect of aqueous extract of *Arachis hypogaea* on phagocytic index

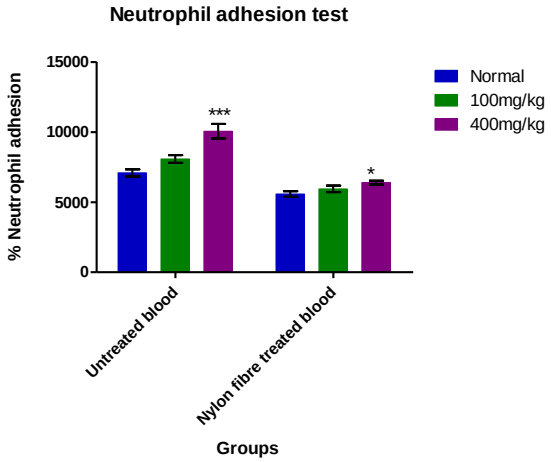

Figure 3: Effect of aqueous extract of *Arachis hypogaea* on Neutrophil adhesion test.

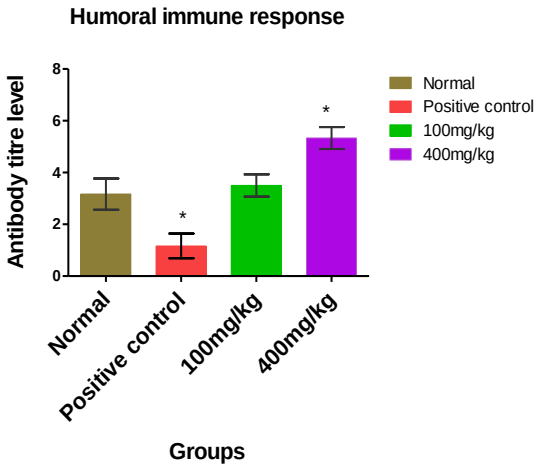

Figure 4: Effect of aqueous extract of *Arachis hypogaea* on Humoral immune response

DISCUSSION

The task of the immune system is to boost immunity and protect the physiological system from infection. Modulation of immune response through stimulation or suppression may help in maintaining a disease free state.

The DTH response, which is a direct correlate of cell mediated immunity (CMI), was found to be significantly increased at a dose of 100 and 400 mg/kg/day for aqueous extract. During CMI responses, sensitized T-lymphocytes, when challenged by the antigen, are converted to lymphoblast's and secrete lymphokines, attracting more scavenger cells to the site of reaction. The infiltrating cells are thus immobilized to promote defensive (inflammatory) reaction. In our studies, paw volume was enhanced after aqueous treatment suggesting cell mediated immune enhancement[5].

AEAH showed significant immunostimulant activity in carbon clearance test by increasing phagocytic index in a dose dependent manner. The reticuloendothelial system is clearing particulate substances, such as bacteria, and altered endogenous materials, such as fibrin aggregates. Phagocytosis is the mechanism by which microorganisms and foreign bodies, dead or injured cells are removed. Measurement of the activity of the reticuloendothelial system depends upon estimation of the rate of clearance from the blood of foreign materials, such as colloidal carbon.[9]

When blood samples were incubated with nylon fibers, a reduction in neutrophil percentage due to the adhesion of neutrophils to the nylon fibers was observed. The percentage reduction in neutrophil count in nylon fibre treated blood samples from the treatment with *Arachis hypogaea* extract was significantly more than the control group. The adhesion of neutrophil to nylon fibers indicates the migration of cells in the blood vessels and the number of neutrophils reaching the site of inflammation.[10] AEAH extract at selected doses in the albino rats have showed a significant increase in the neutrophil adhesion to the nylon fibers. This may be due to up regulation of the â2 integrins that are present on the surface of the neutrophils through which they adhere firmly to the nylon fibers.

Leukocytes play a vital role in the production of antibodies. An elevation in total leukocytes was observed following treatment with AEAH.

B lymphocytes responsible for humoral immunity produce immunoglobulin's which recognize and eliminate extra cellular antigens.

Antigenic exposure could facilitate the proliferation and differentiation of B cells resulting in enhanced antibody titre.

Cyclophosphamide facilitates senescence of immune cells resulting in a decline in antibody titre. Challenge with SRBC produces rise in the haemagglutination antibody titre owing to sensitization of macrophages, T and B lymphocytes. A significant elevation in antibody titre was seen with 400 mg/kg of AEAH in immunosuppressed animals.[5]

The aqueous extract of *Arachis hypogaea* seeds produced significant immunostimulatory effect may be due to cell mediated and humoral antibody mediated activation of T and B cells. It can therefore be concluded that the aqueous extract of *Arachis hypogaea* seeds is a potent immunstimulator and can be tried for clinical trials for the use in therapeutics.

CONCLUSION

The immunostimulatory effect produced by the *Arachis hypogaeae* extract may be due to cell mediated and humoral antibody mediated activation of T and B cells. It is therefore concluded that the *Arachis hypogaeae* extract is a potent immunostimulant and this may be attributed to the flavanoids, proteins, vitamins and minerals present in the extract.

Acknowledgement: Authors are thankful to Green Chem. Herbal Extracts and Formulations, Bangalore, for providing *Arachis hypogaeae* extract as a gift sample.

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Source of Support: Nil, **Conflict of Interest:** None.