IMMUNOMODULATORY EFFECT OF ABELMOSCHUS ESCULENTUS ON HUMORAL AND CELLULAR IMMUNE RESPONSES

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ABSTRACT
In the present investigation evaluated the phytochemical constitution of *Abelmoschus esculentus* (Bhendi) and its immunomodulatory efficiency. The immunomodulatory effect of the extract was analysed in vivo method using animal modelalbino mice. The immunomodulatory potential of the extract represents using antibody titration and estimating the B cell production. The result shows the potentiality of all kind of treated animals when compared to control. The increment in 'B' lymphocyte number was much pronounced in mice by the administration of *A. esculentus* in combination with immune enhansive drug than the lone effect of immunoenhansive drug.

Keywords: Immunomodulation, *Abelmoschus esculentus*, B- Cells and T- Cells.

INTRODUCTION
Traditional medicinal plants are the most important to the health of each and every individuals and societal communities. The medicinal importance of these plants containing chemical substances produce a definite physiological action on the human body (Edeoga *et al*.,2005). *Abelmoschus esculentus* L. is broadly taken as a raw vegetable in both temperate and tropical countries. The largest percentage of linoleic acid (42%) of *A. esculentus* seed oil and the available amino acid pattern of the protein renders with largest supplementation to the legume or cereal based diets (Jambhale and Nerkar, 1998).

The specific plants to be used and the methods of application for particular ailments are passed down through oral tradition. The information regards with the medicinal plants was reported in herbal phamacopoeias (Balunas, 2005). Plant derived medicines are widely used because they are relatively safe and cheaper (Iwu *et al*., 1999). Many plant species have been evaluated for their antimicrobial activity in the past 20 years (Castello *et al*.,2000 and Shajahan and Ramesh, 2004). Medicinal plants serve as therapeutic alternatives, safe choices, or some cases, as the only effective treatment. A large number of these plants and their isolated constituents have shown beneficial therapeutic values, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and immunomodulatory effects (Miller *et al*., 2004).

MATERIALS AND METHODS

Clean and disease-free fruit part of *A. esculentus* was collected from organic farm located at Thiruvallur with Latitude and Longitude of 13.1231° N, 79.9120° E. The collected samples were authenticated by Botanist using standard keys and descriptions and confirms with the help of herbarium. Then it was allowed to be dried at room temperature followed by grinding. Aliquots were extracted using soxhlet extractor at a temperature not reaching the boiling point of the solvent by using 200 mL of methanol (99%) for 6 to 8 h. The rotatory evaporator was used to concentrate the filtrate at 40 °C to viscous the extract until all the traces of solvent were removed. The crude extract was further lyophilized for preservation.

The immunomodulatory effect of *A. esculentus* fruit extract on the dynamics of SRBC antibody response was assessed in a mice (Swiss albino) model. Laboratory breed mice of either sex (2 months of age; weighing 25-30g) were used to evaluate the immunomodulatory activity of different organic extracts of the tender fruits of *A. esculentus*. Mouse were housed in polyvinyl cage littered with paddy husk under standard condition of temperature (27°C), 12h/12h light /dark cycles and fed with balanced pellet diet (Lipton, India Ltd) and tap water ad libitum.

A total of nineteen groups (each group containing six mice) of mice were experimented for immunological studies with an inclusion of control and immunised control. Drugs were administered to various groups of mice in the following manner. After treatment, the blood sample was drawn from each group of animals with a time interval of 7 days up to 21 days.

From the normal and antigens injected mice, serum sample was taken and the antibody levels were estimated (Dhasarathan et al., 2014). B and T cell E rosette assay: Blood was collected from treated and control mice as previous mentioned using a heparin pretreated vials. B and T cell count in the blood samples were carried out by the standard method.

RESULTS AND DISCUSSION

The collected fruit sample was evaluated as free from physical damage by visual observation. This sample is also free from disease. Since it was collected from organic garden there is absence of pesticides traces. The sample was regular in morphological structure with green and fresh. The length of the fruit was identified as 15.3 cm.

Quantification of antibody titre in the experimental groups of mice after the administration of plant drugs will reflect on the immunomodulatory effect of the plant drugs. The ethanol extract of *A. esculentus*showed highest antibody titre (7 $\log_2^2$) followed by hexane and chloroform extract (6 $\log_2^2$) and butanol and water extract (5.5 $\log_2^2$). Immunomodulatory efficiency of *A. esculentus*was compared with the standard immuno suppressive and immune enhansive drugs (Table 1). Wistar rats with acute gastric mucosal damage received an ethanolic extract of okra, which reduced inflammation (Soares et al., 2012).

Extract was found to increase the circulating antibody titre and antibody forming cells. In fact antibody forming cells were found to be stimulated much earlier 7th days then the maximum antibody titre obtained 14th day. However, an increased titre remained several days thereafter indicating that the immunological activity could be sustained for several days. In cyclophosphomide treated groups the antibody titre level was decreased into 80 %. In mice administered with *A. esculentus* the antibody titre levels were increased into 20, 10, 40, 20 and 10 percentages in hexane, butanol, ethanol, chloroform and water extracts respectively.
B cell production of control and treated animals were estimated by rosette forming assay and recorded in Table 2. The result shows that there is a changes in all type of treated animals when it was compared with control. The increase in B lymphocyte number was identified in mice by the administration of *A. esculentus* in combination with immune enhansive drug than the lone effect of immunoenhansive drug. B cell decrement was pronounced in mice treated with immunosuppressive drug while a moderate decrement was noticed due to *A. esculentus* in combination with immunosuppressive drug (Fig 1). The increment in 'B' cell count may be due to the impact of plant drug on the synthesis, proliferation and activation of 'B' cells in treated animals. Okra pods have been shown in earlier studies to be capable of ferric reduction and free radical scavenging. Ansari et al. (2005) reported that okra extract obtained through cold extraction and boiling the fruit in water demonstrated significant antioxidant activity.

T cell production of control and treated animals were estimated and recorded in Table 2. The result shows the identifiable changes in all kind of treated animals when it was compared with control. The increase in 'T' lymphocyte number was much pronounced in mice by the administration of *A. esculentus*in combination with immune enhansive drug than the effect of immune enhansive drug. T cell decrease was identified in mice treated with immunosuppressive drug while a moderate decrement was noticed due to *A. esculentus*in combination with immunosuppressive drug (Table 2). The increase in 'T' cell count may be due to the impact of plant drug on the synthesis, proliferation and activation of 'T' cells in treated animals.

SUMMARY AND CONCLUSION

Extract was identified to increase the circulating antibody titre and antibody forming cells. In fact antibody forming cells were found to be stimulated much earlier 7th days then the maximum antibody titre obtained 14th day. However, an increased titre remained several days thereafter indicating that the immunological activity could be sustained for several days. In cyclophosphomide treated groups the antibody titre level was decreased into 80 %. In mice administered with *A. esculentus* the antibody titre levels were increased into 20, 10, 40, 20 and 10 percentages in hexane, butanol, ethanol, chloroform and water extracts respectively. There was increase in the number of B lymphocytes. It was largely identified in mice by the administration of *A. esculentus* in combination with immune enhancive drug than the lone effect of immunoenhansive drug. In conclusion the compound phytol is responsible for the various property of *A. esculentus*. It is proved that it is potential immuno modulator.

Table 1. Estimation of antibody titre in normal and treated mice.

Group	Antibody titre (log_2^2)		
	I week	II week	III week
Group – 1	4	5	5
Group – II	5	6	7
Group – III	4	5	6
Group -IV	4	4	5.5
Group -V	5	6	7
Group – VI	4	5	6
Group – VII	4	5	5.5
Group – VIII	4	4	5
Group – IX	4	5	6
Group –X	4	5	6
Group – XI	5	4	5
Group - XII	4	4	4
Group – XIII	4	5	6
Group – XIV	4	5	6
Group – XV	5	7	9
Group – XVI	4	5	6
Group – XVII	4	5	5
Group –XVIII	4	3	1
Group - XIX	5	6	8

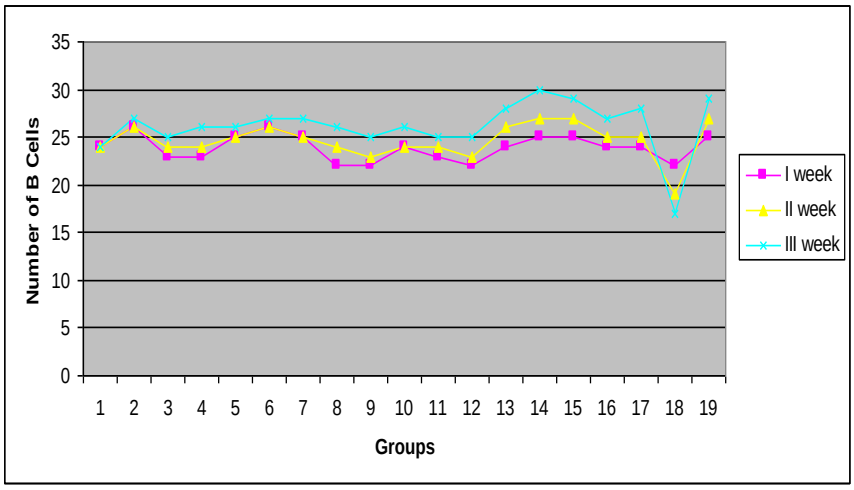

Figure 1. Enumeration of B cells using rosette-forming assay in treated mice.

Table 2. Enumeration of T cells in treated mice.

Group	**Number of T cells Rosette formed in 100 lymphocytes observed**		
	I week	**II week**	**III week**
Group – 1	57	59	51
Group – II	56	57	60
Group – III	48	43	47
Group -IV	48	48	47
Group -V	46	50	52
Group – VI	45	44	47
Group – VII	46	44	47
Group – VIII	43	44	45
Group – IX	43	45	45
Group –X	43	44	40
Group – XI	43	45	45
Group - XII	43	44	44
Group – XIII	43	44	49
Group – XIV	44	45	49
Group – XV	44	45	51
Group – XVI	43	45	49
Group – XVII	43	45	49
Group –XVIII	43	40	36
Group - XIX	45	47	52

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