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Full Length Research Paper

Immunomodulatory activity of various extracts of *Adhatoda vasica* Linn. in experimental rats

G. Vinothapooshan[1]* and K. Sundar[2]

[1]Arulmigu Kalasalingam College of Pharmacy, Krishnankoil – 626 190, Tamil Nadu, India.
[2]Department of Biotechnology, Kalasalingam University, Krishnankoil – 626 190, Tamil Nadu, India.

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Methanolic, chloroform and diethyl ether extracts of leaves of Indian medicinal plant *Adhatoda vasica* Linn . were pharmacologically validated for its immunomodulatory properties in experimental animals. Oral administration of extracts at a dose of 400 mg/kg in adult male Wister rats significantly increased the percentage neutrophil adhesion to nylon fibers (P<0.001). It extracts were also found to induce Delayed Type Hypersensitivity reaction by sheep erythrocytes (P< 0.001). The observed results at different doses were significant when compared to control groups. These findings suggested that the extracts of this plant, *A. vasica Linn* positively modulates the immunity of the host.

Key words: *Adhatoda vasica* Linn., immunomodulatory, neutrophil.

INTRODUCTION

Medicinal plants are coming into prominence because of the conventional medicine such as antibiotics which have developed resistance to many of the infection organisms which are no longer responsive to conventional medicines. Herbal preparation can be more effective and safer than conventional medicines. Non-toxic could be administered for a long period.

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of non specific system, that is, granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors.

Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunelogical functioning. Hence both immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world. (Patwardhan et al., 1990).

Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system (Diasio and LoBuglio, 1996).

Traditional Indian systems of medicines like Siddha and Ayurveda have suggested means to increase the body's natural resistance to disease. A number of Indian medicinal plants and various 'rasayanas' have been claimed to possess immunomodulatory activity (Atal et al., 1986; Patwardhan et al., 1990; Puri et al., 1994; Balchandran and Panchanathan, 1998; Ziauddin et al., 1996).

Adhatoda vasica (acanthaceae) known as chue Mue, is a stout stragling prostrate shrubby plant with the compound leaves which gets sensitive on touching, spinous stipules and globose pinkish flower heads, grows as weed in almost all parts of the country (Ghani, 2003). Leaves and stems of the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and root contains tannins (Ghani, 2003). *A. vasica* is used for its anti–hyperglycemic (Uma maheswari, 2007), anti– diarrhoeal (Balakrishnan et al., 2006), anti–convulsant (Bum et al., 2004) and cytotoxic

*Corresponding author. E-mail: g_pooshan@yahoo.com.

Table 1. The chemical constituents and family name of A. *vasica.*

Plant name	Synonym	Family	Chemical constituents
Adhatoda vasica	*Adhatoda zeylancia*	Acanthaceae	Quinazoline vasicinone, essential oil.

Table 2. The percentage yields of *A. vasica* Extract.

Weight of drug	Extraction pattern	Solvent used	Weight obtaining) (g)	Percentage yield (%)
500 g *A. vasica* powder	Soxhlet apparatus	Methanol,	16	7
		Chloroform,	14	6.2
		Diethyl Ether	12	5.2

properties. The plant also contains turgorins, leaves and roots are used in treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing sinus. Plant is also used in treatment of sore gum and is used as a blood purifier (Ghani, 2003). In ayurvedic and Unani system of medicine, this plant has been used in diseases arising from corrupted blood and bile, bilious fever, piles, jaundice, leprosy, ulcers, small pox. The objective of present investigation was to study the immunomodulatory activity of the methanolic, chloform and diethyl ether extracts of the leaves of *A. vasica* in animal models. The chemical constituents of *A. vasica* were listed (Table 1).

MATERIALS AND METHODS

Plant material

The plant material was collected in Arulmigu Kalasalingam College of Pharmacy Medicinal Garden, Krishnankoil, Tamil Nadu. It was authenticated by Dr.Stephan, Deparmentt of Botany, The American College, Madurai, Tamil Nadu, India.

Extract preparation

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (500 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with methanol, chloroform and diethyl ether and the residual marc was collected. The extract was evaporated under reused pressure using a rotovac evaporator until all the solvent had been removed to give an extract sample with a yield of 16, 14 and 12% w/w in relation to the dried starting material. Preliminary phytochemical analysis was carried out to identify the % yields of extracts in *A. vasica* (Table 2).

Experimental animals

Adult male Wister rats of 150 to 200 g and Swiss albino mice of 25 to 30 g of either sex were used for the study. They were provided with a standard diet (Pranav Agro, India) and water *ad libitum* in animal house facility and maintained under standard laboratory conditions. The experimental protocol has been approved by institutional animal ethics committee, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. (Regd No.509/02/C/CPCSEA/2002.)

Qualitative chemical evaluation

All the extracts obtained were subjected to qualitative tests for various plant constituents and observed that presence of glycosides, phytosterol and alkaloids as major active constituents are confirmed by suitable chemical tests.

Neutrophil adhesion test in rats

Adult male Wister rats were weighing about 150 to 200 g were divided into 5 groups of 5 animals each. The dosages of drugs administered to the different groups were as follows:

Group-1: Control (normal saline 10 ml/kg)
Group-2: *Cedrus deodara* wood oil (100 mg/kg)
Group-3: Alcoholic extract of *A. vasica* (400 mg/kg)
Group-4: Chloroform extract of *A. vasica* (400 mg/kg)
Group-5: Diethyl ether extract of *A. vasica* (400 mg/kg)

The method of Wilkinson (Wilkinson, 1978) was used .The rats were treated orally with *C.deodara* wood oil and *Adhatoda* extracts at the doses of 100 and 400 mg /kg/day for 8 days .On the 8^{th} day blood samples were collected from the retro-orbital plexus in heparinized vials and analyzed for total leukocyte count (TLC) Using Erma PC -607 cell counter (Transasia Ltd., Mumbai, India). The differential leukocyte count (DLC) was performed by fixing the blood smear and staining with leucofine and percent neutrophils in each samples were determined. After the initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 10 minutes at 37°C .The incubated blood samples were again analyzed for TLC and DLC. Likewise the standard and various extracts were administered and analyzed. The product of TLC and percent neutrophils gave the neutrophil index of blood sample. Percent neutrophil adhesion was calculated from the following formula:

$$\text{Neutrophil adhesion (\%)} = \frac{\text{NIu-NIt}}{\text{NIu}}$$

Where, NIu = Neutrophil index of untreated blood sample. NIt=Neutrophil index of treated blood sample.

Table 3. Effect of Leaf extracts of *A.vasica* on neutrophil Adhesion test in rats.

Treatment	Dose (mg/kg)	TLC (cells/mm^3) (A)		Neutrophils (%) (B)		Neutrophil index(AXB)		Neutrophil Adhesion (%)	Inhibition (%)
		UB	NFTB	UB	NFTB	UB	NFTB		
Control (normal saline)	10	11.25±2.54	10.6±2.16	26.75±3.84	8.75±0.92	300.93±64.6	92.75±16.4	67.28±3.2	_
C. deodara wood oil (standard)	100	13.33±2.9	13.05±2.86	20.0±2.76	15.25±2.72	266.6±5.08	199.01±8.04	23.75±18.4	64.69***
Test extract -I	400	11.34±1.09	10.98±1.07	18.56±2.7	17.9±1.68	210.47±2.12	196.54±10.92	3.55±2.62	94.72***
Test extract -II	400	11.43±2.54	11.2±2.5	18.56±2.86	16.6±2.83	212.14±6.4	185.92±5.6	10.56±8.14	84.30**
Test extract –III	400	10.12±2.32	9.28±2.3	17.26±2.7	13.0±2.68	174.67±5.12	120.64±8.12	24.68±16.12	63.31*

Results are expressed as mean ± SEM from five observations as compared to control group by students "t" test. n=5,* P<0.01,**P<0.002, ***P<0.001.

Table 4. Effect of *A. vasica* extracts on delayed type hypersensitivity footpad thickness.

S/N	Group (Treatment)	Dose (mg/kg p.o)	Paw volume at 24 h (%)
1.	Control PBS (pH 7.4)	10	30.44 ± 5.24
2.	Alcoholic extract	400	11.56 ± 4.37* (62.02)
3.	Chloroform extract	400	15.60 ± 4.30* (48.75)
4.	Diethyl ether extract	400	20.10 ± 4.22* (33.96)

Values are expressed as mean ± S.D, n = 5 per group. p<0.05 (compared to respective control). Student's t-test. In brackets, inhibition percentage is reported.

Statistical analysis

Data were expressed as the mean ± the correspondent standard deviation (S.D), n=5 and statistical analysis was carried out by employing Student's t-test.

Delayed type hypersensitivity (DTH)

Hypersensitivity reaction to SRBC was induced in mice following the method reported by Ray et al. (1996). Sheep's erythrocytes (SRBCs) collected in elsever's solution, were washed three times with pyrogen-free sterile normal saline and adjusted to a concentration of 1×10^8 cells/ml for sensitization and challenge. Animals were sensitized with 10% SRBC (1×10^8 cells) at Days 0 and 7 subcutaneously (s.c). After administration of various test extracts of *A.* vasica (400 mg/kg /day). Whereas control group was administered with equal volume of PBS (pH 7.4).On Day 9, both groups were challenged with 1×10^8 SRBC cells, intradermally into the left footpad of each mouse, while PBS (pH 7.4) was injected into right hind paw. The increase in footpad thickness (FPT) was measured 24 h after SRBC challenge by volume differential meter. The degree of DTH reaction was expressed as the percentage increase in FPT over the control values.

RESULTS

Various extracts of *A. vasica* was administered at a dose of 400 mg/kg/oral for 8 days, which significantly inhibited the adhesion of neutrophils to nylon fibres which simulates the process of margination of cells in the blood vessels, this indicates that at the site of inflammation alcoholic extracts of *A. vasica* reduces the number of neutrophils, thus decreasing their phagocytosis action and the release of various enzymes and mediators that make inflammation.

Percentages of neutrophil adhesion were observed on the 8th day at a dose of 400 mg/kg/oral, respectively. The percentage increase in neutrophil adhesion showed a dose dependent activity (P<0.001) (Table 1 and 3). The alcoholic extract at a dose of 400 mg/kg showed highly significant activity (P<0.001) (Figure 1).

Various extracts of *A. vasica* at a dose of 400 mg/kg was found to suppress delayed type hypersensitivity reactions induced by SRBC in mice, respectively (Table 4). Decrease in delayed type hypersensitivity reactions revealed the inhibitory effect of various extracts of *A. vasica* on T lymphocytes and accessory cell types required for expression of the reaction (Figure 2).

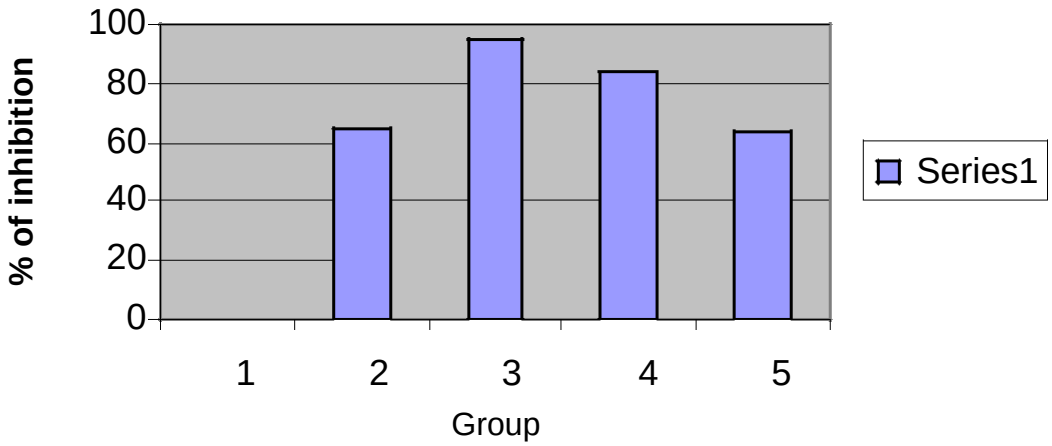

Figure 1. Effect of leaf extracts of *A.vasica* on neutrophil adhesion test in rats. Group-2: C.deodara wood oil (standard), Group-3: Alcoholic extract of *A. vasica*, Group-4: Chloroform extract of *A. vasica*, Group-5: Diethyl ether extract of *A. vasica.*

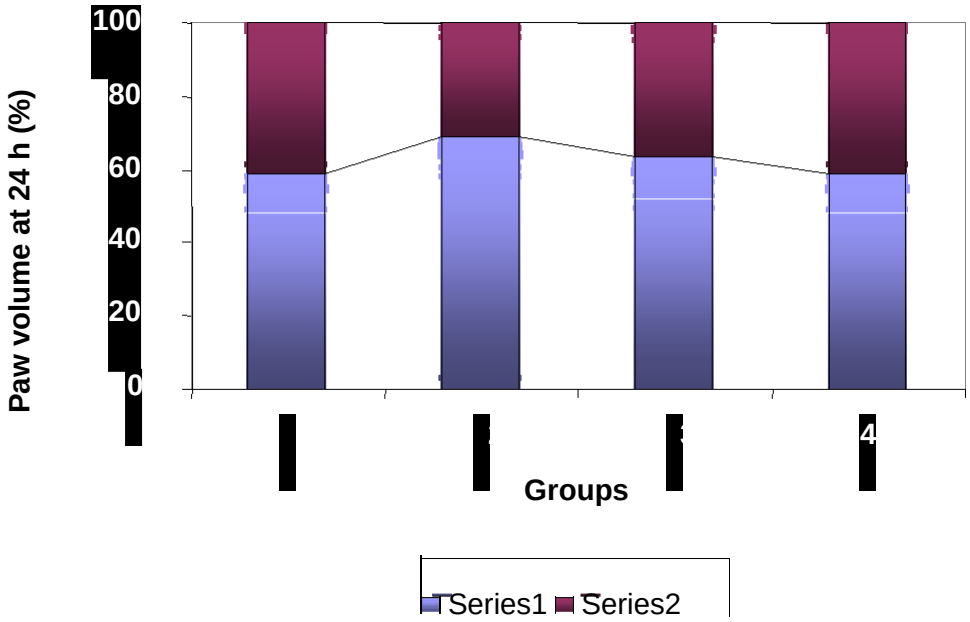

Figure 2. Effect of *A. vasica* extracts on delayed type hypersensitivity footpad thickness. Group 1: Control PBS (pH 7.4), Group 2: Alcoholic extract, Group 3: Chloroform extract, Group 4: Diethyl ether extract.

DISCUSSION

Intensive course of chemotherapy can have the opposing effects of tumor elimination and immunosuppression; it is incumbent upon clinicians and basic scientists to continue to explore the maximization of tumor killing balanced with the reconstitution of immunity. Control of disease by immunological means has two aspects, namely the development and improvement of protective immunity and the avoidance of undesired immunological side reactions. Modulation of the immune system by cytostatic agents is emerging as a major area in pharmacology, especially in cases where undesired immunosuppression is the result of therapy (Bach, 1976). The modulation of immune response by using medicinal plant products as a possible therapeutic measure has become a subject of active scientific investigations. The main objective of the present study was to evaluate the immunomodulatory activity of plant *A. vasica* Linn.

A. vasica administered orally showed significant increase in adhesion of neutrophils to nylon fibers which correlates to the process of margination of cells in blood vessels. The neutrophil adhesion was significantly increased with the dose of 400 mg/kg/day when compared

to untreated control. Neutrophils circulate in the vasculature in a passive state and become more adhesive upon stimulation at sites of inflammation, while it was marginated to the vessel wall, subsequently by transmigration and phagocytosis.

In the present study, we found various extracts of *A. vasica to* inhibit DTH reactiveness, but the maximum inhibition was observed using the treatment of alcoholic extract of *A. vasica*. The interaction of sensitized T-cells with presented antigen is known to be associated with the release of mediators, such as histamine. Products of arachidonic acid metabolism (Griswold et al., 1982) and interferon-gamma eventually lead to DTH. Therefore, the inhibitory action could be due to an influence of fraction on the biological mediators (Desai et al., 1966).

Delayed type hypersensitivity reaction is characterized by large influxes of non-specific inflammatory cells, in which the macrophage is a major participant. It is a Type IV hypersensitivity reaction that develops when antigen activates sensitized T_{DTH} cells (Dunnet, 1964). These cells generally appear to be a T_H1 subpopulation although sometimes TC cells are also involved. Activation of T_{DTH} cells by antigen presented through appropriate antigen presenting cells results in the secretion of various cytokines including Interleukin-2, Interferon- , macrophage migration inhibition factor and tumor necrosis factor- (Askenase and Van Loveren, 1983). The overall effects of these cytokines are to recruits macrophages into the area and activate them, promoting increased phagocytic activity vis- à-vis increased concentration of lytic enzymes for more effective killing. Several lines of evidence suggest that DTH reaction is important in host defense against parasites and bacteria that can live and proliferate intracellularly. Treatment of alcoholic extract of A*. vasica* enhanced DTH reaction, which is reflected from the increased footpad thickness compared to control group suggesting heightened infiltration of macrophages to the inflammatory site (Ecobichon, 1999). This study may be supporting a possible role of alcoholic extract of *A. vasica* in assisting cell-mediated immune response. T sesquiterpene lactones, which are isolated from *Tridax*, have been identified and are known to cause delayed type hypersensitivity (Picman, 1986). So it might be possible that these sesquiterpene lactones may be present in the alcoholic extract of *A. vasica.*

Conclusion

The present study has shown the immunostimulatory activity of the plant *A. vasica* by potentiating humoral as well as cellular immunity (Galighor, 1976). These findings also suggested that *A. vasica* does not suppress the immune system while treating cancer like other chemotherapeutic agents (Harbone, 1984). Promising approaches to immune reconstitution using natural products regimens continue to be developed and it appears that the future of cancer chemotherapy continues to be bright (Gennaro, 1995). Further detailed studies are required which might establish a possible mechanism of immunomodulation effects of this plant.

REFERENCES

Atal CK, Sharma ML, Kaul A, Khajuria A (1986). Immunodulating agents of plant origin. I: Preliminary screening. J. Ethnopharmacol., 18: 133-144.

Bach JF (1976). The Pharmacological and immunological basis for the use of immunosuppressive drugs. Drugs, 11: 1-3.

Balchandran P, Panchanathan S (1998). Immunomodulatory activity of semecarpus anacardium Linn. Nut milk jextract in aflatoxin B1-induced hepatocellular carcinoma in rats. Pharmacy Pharmacol. Comm., 4: 507-510.

Desai PD, Ganguly AK, Govindachari TR, Joshi Bs, Kamat VN, Manmade AH, Mohamed PA, Nagle SK, Nayak RH, Saksena AK, Sathe SS, Vishwanathan N (1966). Chemical investigation of some Indian Plants: Part II. Indian J. Chem., 4: 457-459.

Dunnet CW (1964). New tables for multiple comparisons with a control biometrics, 20: 482-491.

Ecobichon DJ (1999): The basis of toxicology testing 2nd edn, Newyork, CRCpress, 43-46.

Galighor AE, Kozloff EN (1976). Essentials of practical microtechnique 2nd edn, Lea and Febiger, NewYork.

Gennaro AR (1995). The science and practice of pharmacy,9th edn,vol 1,Mac publishing company,Easton PA.Glossary of Indian medicinal plants by .N. CHOPRA.

Harbone JB (1984). Phytochemical methods-A guide to modern technique of plant analysis,2nd edn, chapman and hall, Newyork, 85. Medicinal plants by S.G.JOSHI.

Patwardhan B, Kalbag D, Patki PS, Nagsampagi BA (1990). Search of immunomodulatory agents: a review. India drugs 28(2): 348-358.

Ray A, Banerjee BD, Sen P (1996). Modulation of humoral and cell-mediated immune responses by Azadirachta indica in mice: Indian J. Exp. Biol., 34: 698–701.

Wilkinson PC (1978). In: Vane JK, Ferreria SH, editors. Handbook of experimental pharmacology, Berlin: Springer-Verlag, p. 109.