

Original Research Article

Immunomodulatory activity of methanol extract of *Adansonia digitata* L

Amrish Sharma¹ and Vinod Rangari^{2*}

¹Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur 342003, Rajasthan, ²Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur - 495 009 (CG), India

*For correspondence: **Email:** dr.rangarivinod@gmail.com; **Tel:** +9893939894

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Abstract

Purpose: To evaluate the immune-modulatory activities of various plant parts *Adansonia digitata* L. using delayed-type hypersensitivity rat model.

Methods: Defatted leaf, root bark and fruit pulp of *A. digitata* were extracted with methanol. Immunomodulatory activity of the methanol extracts (250 and 500 mg/kg) were evaluated in sheep RBC (SRBC)-induced delayed-type hypersensitivity model, cell mediated immune re-sponse and phagocytic activity using carbon clearance test.

Results: The extracts exhibited significant increase in delayed-type hypersensitivity reaction, indicating the ability of the extracts to stimulate T-cells. It also increased SRBC induced anti-body titer in immune-suppressed rats, and produced significant increase in phagocytic index by rapid removal of carbon particles from the blood stream.

Conclusion: These results indicate that methanol extracts of the leaf, root bark and fruit pulp of *A. digitata* hold promise as immunomodulatory agents.

Keywords: *Adansonia digitata* L.; Immuno modulation; Delayed type hypersensitivity; Humoral Antibody titre; Phagocytic index

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INTRODUCTION

Immunomodulation is a procedure that alters the immune system of an organism by interfering with its functions. Variety of plant extracts, pure phyto-constituents, natural adjuvants, synthetic agents, and antibody reagents are used as immune modulators which may work either as immunosuppressive and immune-stimulating agents. The use of these agents is often limited by factors such as increased risk of infection and generalized effects throughout the immune system [1,2]. Studies have shown that a number

of Indian medicinal plants may possess immune-modulatory activities [3-5].

Adansonia digitata L., family Bombaceae, is known by various names such as Baobab, Dead-rat tree, Bottle tree, Monkey-bread tree, and Lemonade tree. It is native to Africa. In India it grows naturally in Mandu region of Madhya Pradesh and is very common in Andhra Pradesh, Uttar Pradesh, Bihar, Tamil Nadu and Maharashtra [6]. Different parts of *A. digitata* are widely used as food, medicine, clothing and shelter. In folk medicine, it is used as antipyretic, febrifuge, astringent in diarrhoea and dysentery,

and as a substitute for cinchona in various systems of medicine [7]. The fruit pulp contains high levels of ascorbic acid, tartaric acid, and citric acid and is used in producing a refreshing drink, while the seeds are eaten fresh or dried. The leaves are said to be rich in vitamin C, sugars, potassium tartrate, and calcium. Various parts of *A. digitata* have shown anti-inflammatory, analgesic, antipyretic, antibacterial and antiviral activities [8]. A variety of phytochemical constituents such as terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates, and lipids have been reported from *A. digitata* [9]. The fruit pulp contains sterols, triterpenes, saponins, tannins, carbohydrates, and glycosides [8,10].

The purpose of this study was to evaluate possible immune-modulatory activities of the leaves, fruits and bark of *A. digitata*.

EXPERIMENTAL

Plant collection and authentication

Fresh leaves, fruits and bark of *A. digitata* L. were collected from Mandu, Madhya Pradesh, India. The plant was authenticated by Dr. Pramod Patil, Professor, Department of Botany, M.L.B. Girls Postgraduate College, Bhopal, India. A voucher specimen, number 00919 was deposited in their laboratory for future reference.

Extraction

The plant materials were washed thoroughly with water and air-dried under a shade. They were then pulverized to coarse powder and 500 g of each sample was extracted with petroleum ether (60-80 °C) for 24 h to remove fatty substances. The defatted, dried samples were further extracted with methanol and the extracts were evaporated to dryness under vacuum using a rotary evaporator at 40 °C.

Preliminary phytochemical screening

The methanolic extracts were subjected to preliminary phytochemical screening using standard chemical tests for alkaloids, amino acids, glycosides, steroids, triterpenoids, flavonoids and tannins [11]. Thin layer chromatographic analysis of the extracts was carried out on silica gel G adsorbent, using toluene: ethyl acetate: diethylamine (7:3:0.5 volume ratio) solvent system for the leaf and fruit pulp extracts while toluene: ethyl acetate (9:1) was solvent system for the root bark extract.

Vanillin-sulphuric acid was used as a visualizing agent.

Animals

This study was done as per the Standard Operating Procedures for Institutional Animal Ethics Committee of the CPCSEA [12] and ethical approval was obtained from the Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA), Chennai, India (1410/c/11/CPCSEA, IAEC approval no. DL/AMSR/2013/V/101). Wistar rats (150 - 200 g) maintained under standard environmental conditions (temperature $25 \pm 1^\circ\text{C}$, relative humidity $55 \pm 10\%$ and 12 h light/12 h dark cycle) were fed on standard laboratory feed and water *ad libitum* and allowed to acclimatize to the laboratory conditions. Groups of six animals each were used in all sets of the experiments. Animals in Group I (control) did not receive any drug or extract but those in group II (standard) receive dexamethasone (0.2 mg/kg). The extracts of the leaves, root bark and fruit pulp were given in two oral doses (250 mg/kg and 500 mg/kg) to two groups of animals namely Groups III and IV, groups V I and VI and groups VII and VIII, respectively. The drug and extracts were administered orally for 5 days.

SRBC-induced delayed type hypersensitivity

The method of Lagrange *et al* [13] was used to analyze the effect of Delayed Type Hypersensitivity (DTH) responses in rats treated with Sheep Red Blood Cells (SRBC). Wistar rats were immunized with 0.1 ml 10 % SRBC subcutaneously, and administered test samples at 250 mg/kg or 500 mg per kg body weight. The control group received 25 μL of normal saline while dexamethasone (0.2 mg/kg) was used as standard immune-suppressive agent. Standard drug and all the extracts and were administered by oral route for 5 days. On the 4th day after immunization, the rats were administered 0.1 ml 1 % SRBC subcutaneously, and on the 5th day rats in all groups were administered 0.1 ml of 1 % SRBC in the paw. Increases in foot paw volume were measured after 24h using a digital vernier caliper (Mitutoyo JAPAN). Difference between the thickness of paw just before and 24 h after SRBC challenge (in mm) was taken as a measure of DTH [14].

SRBC-induced haemoglobin (HA) titre assay

The method described by Atal *et al* [3] was utilized for the SRBC-induced haemoglobin (HA) titer assay. As in SRBC-induced delayed type hypersensitivity, Wistar rats were immunized with

0.1 ml 10 % SRBC subcutaneously, and administered extract samples at 250 mg/kg or 500 mg per kg orally while animals in control group received 25 μ L of normal saline. Dexamethasone (0.2 mg/kg) was used as standard immune-suppressant. On the 4th day after immunization, all the animals (groups I-VIII) were administered 0.1 ml 1 % SRBC subcutaneously. On the 5th day blood was withdrawn and serum was separated, diluted two-fold and incubated with 1 % SRBC in a 96 well plate. Antibody levels were determined by the method described by Shinde *et al* [15]. Serial two-fold dilutions of the serum were made using 25 μ L of normal saline each time of transfer across the plate. To the 25 μ L of diluted serum in each well was then added 25 μ L of 1 % v/v SRBC suspension in normal saline. The microtiter plate was maintained at room temperature for 1 h and the well contents examined for hemagglutination. Each experiment was repeated twice (n=3).

Carbon clearance test

The method of Biozzi *et al* [16] was used to analyze phagocytic activity of the white blood cells in the rats. Carbon ink suspension was injected via the tail vein into the rats of each group 48 h after the five-day treatment. Blood samples (25 μ L) were then withdrawn from the retro-orbital plexus under mild ether anesthesia at 5 and 15 minutes after injection of colloidal carbon ink. The blood samples were lysed in 0.1 % sodium carbonate solution (3 ml), and optical

density was measured at 660nm in a spectrophotometer.

Statistical analysis

Data were obtained in triplicate and expressed as mean \pm SD (n \geq 3). Comparison of means was performed by Dunnett-test to determine difference between groups. $P < 0.05$ was considered statistically significant.

RESULTS

The yield of methanol extracts of *A. digitata* leaves, bark and fruit pulp were 3, 2.3 and 1.4 %w/w, respectively. Preliminary phytochemical screening of all the extracts revealed the presence of flavonoids, steroids, tannins, glycosides and amino acids. Thin layer chromatographic study of the methanolic extract of the leaves revealed nine spots in the solvent system (toluene: ethyl acetate:diethyl-amine, 7:3:0.5) while the fruit pulp methanolic extract in the same solvent system showed five spots when sprayed with vanillin-sulphuric acid. However, the methanolic extract of the bark in the solvent system (toluene:ethyl acetate, 9:1) revealed one major and nine minor spots.

The results of delayed type hypersensitivity (DTH) response to SRBC, haemagglutinating anti-body titer and carbon clearance test are given in Table 1.

Table 1: Effect of methanol extracts of *A. digitata* leaves, root bark and fruit pulp on foot pad oedema, haemagglutinating antibody titre and phagocytic index

Treatment	Animal group	Treatment (mg/kg, p.o.)	Foot pad thickness at 24 h (mm)	Haemagglutinating antibody (HA) titre	Carbon Clearance Abs at 5 min.	Carbon Clearance Abs at 15 min	Phagocytic Index (K, mean \pm SD)
Untreated (control)	I	-	9.78 \pm 0.11	3.66 \pm 0.51	0.084 \pm 0.003	0.038 \pm 0.005	0.035 \pm 0.005
Dexamethasone (standard)	II	0.2	7.20 \pm 0.08	2.00 \pm 0.00	0.067 \pm 0.005	0.065 \pm 0.004	0.001 \pm 0.005
Leaves Extract	III	250	9.55 \pm 0.16 ^{ns}	3.33 \pm 0.51 ^b	0.077 \pm 0.006	0.023 \pm 0.007	0.041 \pm 0.045 ^{ns}
	IV	500	10.93 \pm 0.70 ^a	5.33 \pm 1.03 ^a	0.132 \pm 0.003	0.032 \pm 0.007	0.063 \pm 0.011 ^{ns}
Root bark extract	V	250	9.58 \pm 0.18 ^{ns}	3.16 \pm 0.40 ^b	0.087 \pm 0.005	0.030 \pm 0.004	0.047 \pm 0.007 ^{ns}
	VI	500	11.56 \pm 0.95 ^a	4.33 \pm 0.51 ^a	0.098 \pm 0.005	0.027 \pm 0.004	0.055 \pm 0.014 ^{ns}
Fruit pulp extract	VII	250	9.83 \pm 0.10 ^{ns}	4.66 \pm 0.81 ^a	0.087 \pm 0.003	0.029 \pm 0.003	0.049 \pm 0.005 ^{ns}
	VIII	500	12.23 \pm 0.29 ^a	5.66 \pm 0.51 ^a	0.087 \pm 0.008	0.018 \pm 0.001	0.069 \pm 0.006 ^b

Values are mean \pm S.D. ^a $p < 0.0001$, highly significant; ^b $p < 0.05$, significant; ^{ns} $p > 0.05$, non-significant (compared to respective control); Dunnett's t-test (n = 6)

Compared to the control, the extracts of the leaves, fruit pulp and root bark produced significant high levels of oedema, HA titre and phagocytic index at the high doses (500 mg/kg) of the extract. At the low doses of the extracts (250 mg/kg), other than HA titre values where significantly higher values than the controls were obtained, variable responses were obtained (Table 1).

DISCUSSION

DTH is antigen-specific and causes erythema and induction at the site of antigen infection in immunized animals; T-cells are required to initiate the reaction. Active phagocytosis is the major defense mechanism against infection. The clearance rate of granular foreign bodies from circulation reflects the phagocytic function of mononuclear macrophages. In this study, only animals treated with high dose (500 mg/kg) of test extracts showed increase in paw edema (Table 1). This reveals stimulatory effects of the methanolic extracts on T-lymphocytes.

Based on the HA titre values, the interaction of B cells with antigen and their subsequent proliferation and differentiation into antibody secreting cells occurred at higher dose of test extracts. Even at a lower dose of fruit pulp extract (250 mg/kg), there was highly significant haemagglutination ($p < 0.0001$). The antibodies produced by the stimulatory effect of methanolic extracts on humoral immune response bind to antigens and neutralize them or facilitate their elimination by cross linking to form latex that is more readily ingested by the phagocytic cells. The immune stimulant activity might be attributed to the presence of flavonoids and vitamin C in the plants.

The effect of the extracts on reticulo-endothelial system (RES) was measured by the rate of removal of carbon particles from the blood circulation. This is a diffuse system comprising phagocytic cells, fixed tissue macrophages and mobile macrophages. The phagocytic cells in this system comprise mononuclear phagocytic system (MPS), and the macrophage is the major differentiating cell in the MPS. Cells of RES and MPS are known to be important in the clearance of particles from the blood stream. When colloidal ink containing carbon particles are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation. Specifically, a faster removal of particles is correlated with an enhanced phagocytic activity of RES cellular components [17]. In this study, the immunosuppressant

dexamethasone completely inhibited the carbon clearance while administration of leaf, bark and fruit pulp extracts significantly increased the rate of carbon clearance in a dose-dependent manner. More significant rate of carbon clearance in fruit pulp extract may be attributed to the presence of ascorbic acid in the fruits.

CONCLUSION

A. digitata L is widely used as food, beverage and traditional medicine in parts of Africa and India. The methanolic extracts of its leaves, bark and fruit pulp produce significant increases in delayed type hypersensitivity (DTH), humoral antibody (HA) titre and phagocytic index thereby indicating significant immune stimulant activities in the experimental animals. This present study provides scientific basis for the ethnomedical uses of *A. digitata*. Further studies might be required to determine detailed mechanisms and active phytochemicals responsible for immunomodulatory activity.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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