

Evaluation of Immunomodulatory activity of *Aesculus indica*

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Abstract: To investigate the immunomodulatory effect of *Aesculus indica* in rats. *Aesculus indica* was administered orally at doses of 50 and 100 mg/kg to healthy rats divided into five groups consisting of six animals each. The assessment of immunomodulatory activity was carried out by testing the humoral (antibody titre) and cellular (foot pad swelling) immune responses to the antigenic challenge by sheep RBCs and by neutrophil adhesion test. On oral administration of the extract, a significant increase in neutrophil adhesion and delayed type hypersensitivity response whereas the humoral response to sheep RBCs was unaffected. Thus *Aesculus indica* significantly potentiated the cellular immunity by facilitating the foot pad thickness responses to the sheep RBCs in sensitized rats. With a dose of 50 and 100 mg/kg the DTH response (mean \pm S.D. % increase in paw volume). The responses were statistically significant when they were compared with the control. The study stated that *Aesculus indica* shows a significant stimulation of the cell mediated immunity and no effects on the humoral immunity.

Keywords: *Aesculus indica*, immunomodulatory activity

Introduction :

Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases¹. Thus the present investigation was aimed at evaluating the immunomodulatory activity of *Aesculus indica* (Linn.) leaves on standard animal models.

Aesculus indica (Linn.) belonging to the family Sapindaceae is an herbaceous, soft perennial plant abundantly found in Northern Western Himalayas. Generally it is called as Bankhor. It is generally known for its medicinal properties. Seeds are used as astringent, nutritious, while the oil is used in the treatment of skin disease and rheumatism². Fruit are used in colic disorders³; roots are used in leucorrhoea treatment⁴. As a medicine, it is especially used for the complaints of the veins, such as phlebitis, haemorrhoids, varicose veins; in ulcers; to prevent thrombosis; in some cases of migraine, effusions of blood; for limb complaints and forst bite. The plant is reported to contain a mixture of saponins, one of which is described as aescine, which easily crystallizes. In addition it also contains flavonoid glycosides, aesculine, albumin and fatty oils. Aesculin is used medically and extracts from the seeds are used industrially. The hydroscoumarin glycoside aesculin from the bark of the branch absorbs ultra-violet rays and is an ingredient for suntan oil⁵.

Experimental:

Materials and Methods

Plant Material: Fresh leaves of the plant *Aesculus indica* (Linn.) were obtained and identified from authentic sources. A voucher specimen has been identified and deposited at the Department of Pharmacognosy, School of Pharmacy and Technology Management, Shirpur. The collected leaves were dried in shade, crushed to coarse powder and used for further studies.

Preparation of Extract: The dried plant material leaves (1 kg) were subjected to continuous hot extraction with petroleum ether and ethanol for 36 hours. After extraction with petroleum ether and ethanol the powder was again extracted with water by cold maceration for 72 hours. The extract was filtered, concentrated and the solvent was removed by rotary evaporator. The extract was dried over a dessicator. The residue (480 g) was used for this study⁶.

The extracts were subjected to preliminary qualitative tests to identify the various phytoconstituents present in leaves⁷. It was observed that petroleum ether extract contained steroids whereas alcoholic and aqueous extracts contained steroidal saponins, flavonoids, tannins, phenolic substances and carbohydrates.

Antigen: Sheep Red Blood Cells were collected in Alsever's solution, washed three times in large volumes (30 ml) of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5×10^9 cells/ml for immunization and challenge.

Animals: Healthy Wistar male rats (100-120 gm) were used for the study. All the animals were housed from

Table 1. Effect of *Aesculus indica* on neutrophil adhesion in rats

Groups	Neutrophil index		Neutrophil Adhesion (%)
	UB	FTB	
I (Untreated)	276.80 ± 70.21	227.19 ± 49.20	
II (50 mg/kg, p.o.)	300.64 ± 44.21	240.12 ± 55.10	21.99
III (100 mg/kg, p.o.)	314.44 ± 41.40	242.71 ± 45.12*	44.58

The values are mean ± S.D. of 6 rats in each group. One way ANOVA followed by Dunnett's test, *p, 0.001 Vs group I, UB= untreated blood; FTB= Fiber Treated Blood.

Table 2. Effect of *Aesculus indica* on HA titre and DTH response to antigenic challenge by sheep RBCs in rats

Groups	HA titre	DTH response (% increase in paw volume)
I (Untreated)	4.84 ± 0.99	6.02 ± 1.84
II (50 mg/kg, p.o.)	5.34 ± 0.71	10.53 ± 3.11*
III (100 mg/kg, p.o.)	5.89 ± 0.39	14.51 ± 2.39*

The values are mean ± S.D. of 6 rats in each group. One way ANOVA followed by Dunnett's test, *p, 0.001 Vs group I,

animal house which were under standard conditions of temperature (23± 2°C), 12 h light/dark cycles and fed with standard pellet diet (D.S. Trading Company, Mumbai) and water *ad libitum*. Fresh sheep red blood cells (SRBC) in Alsever's solution was obtained from authentic sources. The animals were divided in to three groups consisting of six animals each. A group of six untreated rats were taken as control (Group I). The extract was fed orally for 14 days at a dose of 50 mg/kg/day (Group II) and 100 mg/kg/day (Group III) for assessment of immunomodulatory effect.

Neutrophil Adhesion Test⁸: On the 14th day drug treatment, blood samples were collected (before challenge) by puncturing the retro orbital plexus into heparinized vials and analyzed for total leucocyte count (TLC) and differential leucocyte count (DLC) by fixing blood smears and staining with Field stain I and II-Leishman's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37 °C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below

$$\text{Neutrophil adhesion(\%)} = \frac{\text{NI}_t - \text{NI}_u}{\text{NI}_t} \times 100$$

NI_u

Where NI_u= Neutrophil index of untreated blood samples
NI_t= Neutrophil index of treated blood sample.

Haemagglutinating Antibody (ha) Titre⁹: Rats of group II and III were pretreated with AI for 14 days and each rat was immunized with 0.5X10⁹ SRBC/rat by i.p. route, including control rats. The day of immunization was referred to as day 0. The animals were treated with AI for 14 more days and blood samples were collected from each rat on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBC (0.025X10⁹ cells). The micro titre plates were incubated at room temperature for 2 hours and examined visually for agglutination. The highest number of dilution of serum showing haem agglutination has been expressed as HA titre.

Delayed Type Hypersensitivity (dth) Response⁹: Six animals per group (Control and treated) were immunized on day 0 by i.p. administration of 0.5X10⁹ SRBC/rat and challenged by subcutaneous administration of 0.025X10⁹ SRBC/ml in to right hind foot pad on day +14. The extract of AI was administered orally from day-14 until day + 13. DTH responses were measured at 24 h after SRBC challenged on day +14 and expressed as mean percent increase in paw volume (plethysmometrically).

Statistical Analysis: The data was analyzed using one way analysis of variance (ANOVA) followed by Dunnett's Test. P values <0.001 were considered as significant.

Results :

AI evoked as a significant increase in neutrophil adhesion (P, 0.001, significance value) at a dose of 100 mg/kg.day in rats. The results of neutrophil adhesion test are shown in Table 1. HA titre did not show any significant increase when AI was orally administered in different dose. The DTH response to SRBC which corresponds to cell mediated immunity showed a dose dependent increase due to treatment with AI. The differences in DTH response were statistically significant which are shown in Table 2. Thus it can be said that AI induced a remarkable enhancement in DTH Response to SRBC in animals.

Discussion:

Immunomodulatory agents obtained from plant and animal origin generally enhances the immune responsiveness of an organism against a pathogen by activating the system. In the present investigation AI when administered orally, significant increased in the adhesion of neutrophils to nylon fibers which interrelates to the [process of margination of cells in blood vessels. It was found to be highly significant when compared with control. The HA titre did not show any increase with AI administration. The DTH response directly correlated the cell mediated immunity and was found significant. Thus in this process the T-lymphocytes gets sensitized when they are challenged by any antigen which there by gets converted in to lymphoblasts and secrets lymphokines, and attracts the scavenger cells to the site of reaction. The increase in the response indicated that AI has a

stimulating effect on the lymphocytes. Thus it can be concluded that methanolic extract was found to be highly stimulating agent for both cell mediated immune responses.

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