

EFFECT OF *DODONAEA VISCOSA* ON SPECIFIC AND NONSPECIFIC IMMUNE SYSTEM MODELS

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Summary

Dodonaea viscosa L.F. a member of the family Sapindaceae popularly known as 'vilayati mehandi' in India. Plants were employed largely as analgesic, anti-inflammatory, antiviral, laxative and antimicrobial agent. The present study was performed to evaluate immunomodulatory activity of this plant in various experimental models. Administration of *D. viscosa* treated group at 200, 400 mg/kg defended the mice at 12 hours, 24 hours, 48 hours and up to 168 hours against death due to cecal ligation and puncture (CLP) induced abdominal peritonitis against control. Significant decrease in mean difference, in the foot paw thickness in delayed type of hypersensitivity (DTH) revealed its previous reported anti-inflammatory activity. In % neutrophil adhesion test increase in % neutrophil adhesion indicated improved natural defence system when animal administered with *D. viscosa*. Thus the results, obtained justify the traditional use of *D. viscosa*.

Keywords: *Dodonaea viscosa* – immunomodulators – delayed type of hypersensitivity

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Introduction

Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of 'Rasayana' in Ayurveda is based on related principles. Rasayana, listed as a class in the texts of traditional Indian medicine literature, consists of a number of plants reputed to promote physical and mental health, improve defense mechanisms of the body and enhance longevity (1). Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, especially when host defense mechanism has to be activated under the conditions of impaired immune response or when a selective immunosuppression is desired in situations like autoimmune disorders. In recent years there has been a renewed interest into the biological activity of traditional plant medicines and the role of natural products in drug discovery (2).

Plant drugs which so far have been used in traditional medicine for their antibacterial, antiviral, antifungal, or antitumoral activities are good candidates for screening immunostimulant potency. Also there are great numbers of compound like alkaloids, terpenoids, saponins, simple phenolic compounds, essential fatty acids, vitamins, polysaccharides having potential immunostimulating activity (3). Number of medicinal plants as rasayana have been claimed to possess immunomodulatory activity. Some of the rasayana drugs known as immunomodulatory agents are *Withania somnifera*, *Tinospora cordifolia*, *Asparagus racemosus* and *Mangifera indica* (4, 5, 6, 7). A lot more are still to be explored and offer scope for further investigation.

Dodonaea viscosa L.F. (Sapindaceae) popularly known as 'vilayati mehendi' in India. It is an evergreen shrub or small tree abundantly available in Western Ghats of Tamilnadu and distributed throughout India. The leaves were reported to possess local anesthetic, smooth muscle relaxant (8), antibacterial (9, 10) and antifungal (11, 12). 95% of ethanolic extract of *D.Viscosa* leaves has shown anti-

ascariasis, anthelmintic, cardiac depressant, hypotensive, uterine relaxation and vasoconstrictor activity in different experimental models (13).

The structural similarity of the *Dodonaea* saponins to antiexudative saponin esters from *Aesculus hippocastnum*, *Thea sinensis*, *Sanicula eutopaea*, *Eryngium planum*, *Hydrocotyle vulgaris* and *Polemium caruleum* leads to subject them to the Viscarin Carrageenin oedema test on a rat paw showed inhibition of oedema up to 33 %, while according to the investigation of (14). *Dodonaea* saponins were also tested in two *in vitro* granulocyte systems, challenged with yeast and zymosan, respectively. In the granulocyte test a dose dependant enhancement of phagocytosis up to 25% was observed (15), whereas a phagocytosis-independent increase of luminescence up to 65 %, was found in the chemiluminescence's test (16).

Thus the most important area in which it has not been possible to have any breakthrough is the development of adjuvant to be used in vaccination programs or immunosuppressant which can be safely used in organ transplant cases. These basic areas of immunomodulators are currently receiving in adequate attention. A number of plant products are being investigated for immune response modifying activity (17). Present study was attempts immunomodulatory activity of *Dodonaea viscosa* in mice for their possible applications in immunotherapeutic and immunochemical industry.

Materials and Methods

Plant materials

The leaves of *Dodonea viscosa* were collected from and authenticated from botanical Survey of India, Pune (Voucher Specimen No: VSJAA1). Weighted quantity of powdered *D. viscosa* (1kg) defatted in soxhlet apparatus with petroleum ether (40-60 °C) about 35-40 complete cycles. The defatted material was dried to remove petroleum ether and subjected

to extraction using 01 liter of ethanol (95%) in a soxhlet apparatus for 24 hours. The solvent was evaporated under vacuum.

The extract was kept in air tight containers for further studies. Phytochemical investigation of ethanolic extract of *Dodonaea viscosa* showed the presence of steroids, triterpenoids, saponins, flavonoids, tannins and proteins.

Drugs

Accurately weighed quantities of the ethanolic extract of *D.viscosa* were prepared into water as vehicle using Tween 80 as a suspending agent. Cyclosporine was used as standard immunosuppressant. A Sheep red blood cell (SRBC's) was collected from local slaughter house in Alsevers solution. SRBC were used as an antigen.

Animals

Male albino rats (Wistar strain, 125– 150 g) / mice (Swiss albino, 25-30g) were obtained from Yash farms, Pune used for the study. The animals were maintained at 25 ± 2 °C in the institute's animal house with food (Amrut feeds, Chakan Oil Mills, Pune, India) and water *ad libitum*. The study was approved by Institute's animal ethical committee and confirmed to national guidelines on the care and use of laboratory animals (CPCSEA/IAEC/PC10/07-2K8).

Acute toxicity study

Healthy adult albino mice (18- 22g) were subjected to acute toxicity studies as per guidelines (OECD 425) suggested by the OECD. The mice were observed for 02 hours for behavioral and neurological profiles & after 24, 72 hours for any lethality. (18).

All mice were free of any toxicity up to the dose of 2 gm/kg. From this data, two different doses of *Dodonaea viscosa* 200, 400 mg/kg were selected for further study.

Statistical analysis

Data were expressed as mean \pm S.E.M. and statistical analysis was carried out using unpaired Student's 't' test. *p<0.05 was regarded as statistically significant.

Methods

Cecal ligation and puncture (CLP) induced abdominal peritonitis

A number of experimental models have been applied in the study of the physiopathology of peritonitis and abdominal sepsis. One of the earliest reports on experimental peritonitis described was adopted (19). Animals were divided into three sets, each set have four groups. Animal in group I (sham laprotomy) and in group II (sham CLP) received orally 10 ml/kg distilled water, at 18 hours, 02 hours and 02 hours before laprotomy in group I and CLP in group II. Animal in group III and group IV were administered *D.viscosa* at doses of 200 and 400 mg/kg, p.o. at 18 hours, 02 hours and 02 hours before CLP. Mice were anaesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and immobilized with adhesive tape onto a cork board. 02 mm midline incision was performed at the mesogastric region; the cecum was identified and ligated with 3-0 silk surgical thread 1cm from the tip. Care was taken not to cause bowel obstruction. With the help of 20-gauge needle single puncture was performed on cecal wall and squeezed lightly to express a small amount of stool from the puncture site in order to assure full thickness of perforation. The cecum was returned to the abdominal cavity and the incision was closed with 3-0 silk thread. In group I only midline laprotomy performed, cecum was exteriorized, return to abdomen and the wound was closed with 3-0 silk thread. Measurement of mortality was carried out for 7 days after CLP.

Delayed Type Hypersensitivity (DTH)

The method described by Joharpurkar et al., (20) was adopted. The mice were divided into 4 groups, each containing six animals. Normal control Group I was given distilled water orally for 21 days. Negative control Group II received cyclosporine 100 µg/mouse, on 14th day of study i.p. Animals in group III, IV were administered *D viscosa* at a dose of 200 and 400 mg/kg/day, orally for 21 days. Immunized mice with 0.1 ml of 20% SRBC's in normal saline i.p. on 14th day of study. On day 21st, animal from all

group get challenged with 0.03 ml of 1% SRBC's in subplantar region of right hind paw. Footpad reaction was assessed after 24 hours i.e. on 22nd day. Increase in foot paw edema was measured with the help of Digital Plethysmometer – LE7500 (Panlab, USA).

Neutrophil adhesion test

The effect of extract on % neutrophil adhesion was evaluated in rats (21). Animals were divided into three groups each of six rats. Group I was kept as a control and received vehicle only. Group II and group III were administered orally with *D.viscosa* at doses of 200, 400 mg/kg p.o. daily for 14 days.

After 14 days of treatment of all three groups, blood samples were collected by retro-orbital puncture, anticoagulated and subjected to total (TLC) as well as differential leukocyte count (DLC). After initial counts the blood sample were incubated with 80 mg/ml of nylon fibers at 37 °C for 15 minutes. The incubated samples were again analyzed for DLC and TLC. The product of TLC and % neutrophil known as neutrophil index was determined for each of the respective group (22). % neutrophil adhesion =

(Difference of neutrophil count of untreated and fiber treated group / Neutrophil count of untreated blood) × 100

Results

Cecal ligation and puncture (CLP) induced abdominal peritonitis

In mice mortality due to CLP induced abdominal peritonitis was observed and results were expressed as percentage survival (Fig. 1.). *D. viscosa* treated group significantly protected the mice against death due to CLP induced abdominal peritonitis at 12 hours 24 hours, 48 hours and up to 168 hours as compared to control group. After 12, 24, 72 hours of CLP the percentage survival in group III (200 mg/kg,) p.o. was found to be non significant against control. Wherein after 48 (44.44 ± 5.55%) and up to 168 hours (26.26 %) it was found to be significant (p<0.05) against control (22.21 ± 5.55 %). In group IV (400 mg/kg) p.o. the percent survival at 12 hours (83.33 ± 0.00 %) was found to be significant (p<0.05) against control (61.10 ±5.55 %). After

24 hours (77.77 ± 5.55 %) and 48 hours (66.66 ± 0.00 %) of CLP there was significant increase ($p < 0.01$) in percentage survival wherein at 72 hours (61.10 ± 5.55) and up to 168 hours (44.44 ± 5.55) it was highly significant ($p < 0.001$) against control. In the laparotomy control group I, percentage survival was found to be 100 % up to 168 hrs.

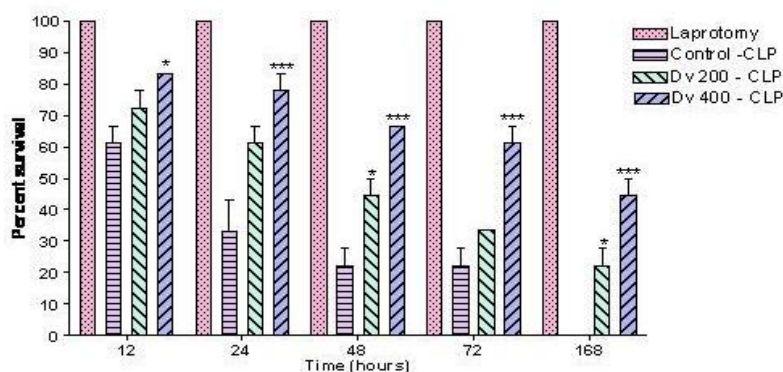


Fig. 1. Effect of Ethanolic Extract of *D. viscosa* on % Survival in Mice Using CLP Induced Abdominal Peritonitis. Results are expressed as mean \pm SEM. (n = 6). Data were analysed by one way analysis of variance (ANOVA) followed by *Dunnnett's* test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. CLP = Cecal Ligation and Puncture, DV 200-CLP = Ethanolic extract of *D. viscosa* 200 mg/kg with CLP; DV 400-CLP = Ethanolic extract of *D. viscosa* 400 mg/kg with CLP.

Delayed Type Hypersensitivity (DTH)

The result obtained in Fig. 2 indicates that there was significant decrease in mean difference, in the foot paw thickness at doses of 200, 400 mg/kg in ethanolic extract of *D. viscosa* administered group when compared against normal control. Negative control group having treatment cyclosporine (100 μ g/mouse) showed significant decrease, ($p < 0.01$) in the mean difference, in the foot paw thickness as compared to control group. Group treated with *D. viscosa* at dose 200 mg/kg showed significant decrease ($p < 0.05$) in DTH response whilst group treated with *D. viscosa* at dose 400 mg/kg showed significant decreases ($p < 0.01$) DTH

response in terms of mean difference, in the foot paw thickness, when compared against control. The drug influences cell mediated immune response in dose dependent manner.

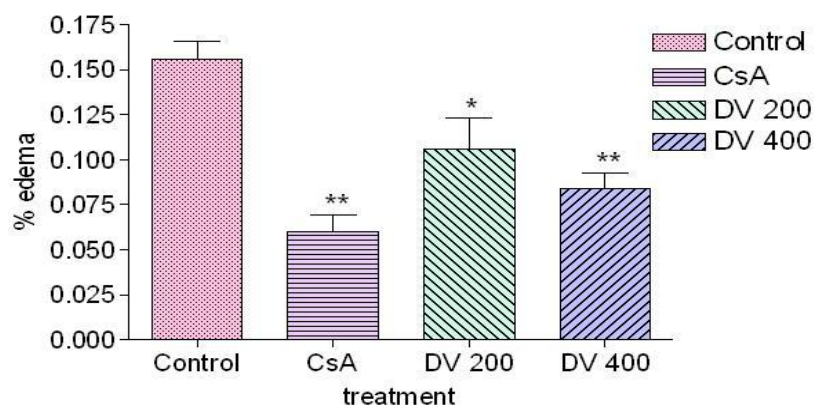


Fig. 2. Effect of *D. viscosa* on Mean Difference, in Foot Paw Thickness in Mice as Assessed by Delayed Type of Hypersensitivity.

Results are expressed as mean \pm SEM. (n = 6). Data were analysed by one way analysis of variance (ANOVA) followed by *Dunnett's* test. * $p < 0.05$ and ** $p < 0.01$. DV 200: Ethanolic extract of *Dodonaea viscosa* 200 mg/kg, DV 400: Ethanolic extract of *Dodonaea viscosa* 400 mg/kg, CsA: Cyclosporine.

Neutrophil adhesion test

Fig. 3 depicts that % neutrophil adhesion in control group was found to be 10.096 ± 0.4414 ; in ethanolic extract *D. viscosa* treated group II (200 mg/kg) it was 11.531 ± 2.265 whilst in group III (400mg/kg) it was found to be 16.31 ± 1.763 . As an evident from the result obtained in % neutrophil adhesion test, nearly 1.5% increase in % neutrophil adhesion in group II found to be insignificant, whilst in group III it was found to be 6 % ($p < 0.05$).

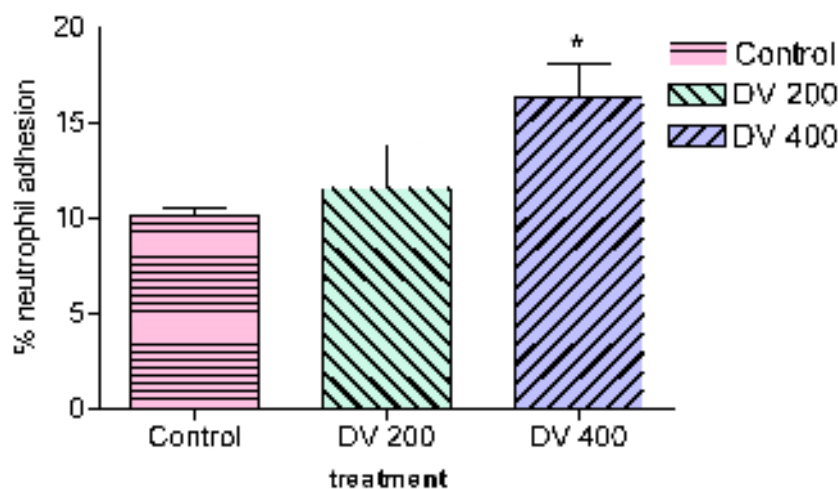


Fig.3. Effect of Ethanolic Extract of DV on Neutrophil in Rat Using % Neutrophil Adhesion Test

Results are expressed as mean \pm SEM. (n = 6). Data were analysed by one way analysis of variance (ANOVA) followed by *Dunnnett's* test. * $p < 0.05$ and ** $p < 0.01$. DV 200: Ethanolic extract of *Dodonaea viscosa* 200 mg/kg, DV 400: Ethanolic extract of *Dodonaea viscosa* 400 mg/kg, CsA: Cyclosporine.

Discussion

Phagocytosis by macrophages is important against the smaller parasites and its effectiveness is markedly enhanced by opsonisation of the parasite with the antibodies and complement activation leading to more rapid clearance of parasites from blood (23). Intra-abdominal infection may be a source for sepsis. Peritoneal Inflammatory response generated to polymicrobial organisms from these infection to be generated from the gastrointestinal tract. Clinical peritonitis may originate from a defect in an abdominal

viscus, such as acute intestinal perforation, that progress to sepsis resulting in high morbidity and mortality in both experimental animal and patient. Patient with sepsis may exhibit features of immunosuppression, including an inability to clear infection and predisposition to infection (24). Secondary peritonitis almost always involves a mixed aerobic and anaerobic flora, especially when the contaminating source is colonic. This infection can result primarily from chemical irritation (e.g., a ruptured gastric ulcer) or from bacterial contamination (e.g., a ruptured appendix). The cecal ligation and puncture (CLP) is the most commonly employed animal model for the induction of experimental peritonitis since it presents a marked similarity with the physiopathology of secondary peritonitis observed in humans, in which peritoneal irritation, necrosis, persistent discharge of faecal material within the abdomen and increase in the concentration of cytokines are observed. CLP is characterized by two distinct phases i.e. hyperdynamic phases and hyporesponsive phase. It is this hyporesponsive phase that may dictate the outcome of sepsis. It is proposed that in critically ill patient/animal, dysregulated lymphocyte apoptosis in the thymus and spleen may lead to immune suppression, leaving the patient vulnerable to subsequent infections or unable to fight existing sepsis, result in multi organ failure (25).

The result obtained in the present study in CLP model indicates that the drug *D. viscosa* might have enhanced the capacity of the non specific immunosystem i.e. monocyte macrophage system and also posses antiapoptotic ability that prevents the dysregulated apoptotic immune as well as non immune cell death.

DTH reaction is antigen specific and causes erythema and induration at the site of antigen injection in immunized animals when encountered with activated Th1 cells by certain antigens, viz SRBC's. DTH comprises of two phases, an initial sensitisation phase and effector phase. In initial sensitization phase Th1 cells are activated and clonally expanded by APC with class II MHC molecule. In effector

phase subsequent exposure to the SRBC's antigen induces DTH response, where Th1 cells secrete a variety of cytokines and other non specific inflammatory mediators (26, 27).

The drug *D. viscosa* might be capable of influencing the role of immune cells resulting in activation of suppressor T cell causing decrease in delayed type of hypersensitivity against SRBC's antigens. Moreover, decrease in DTH response supports the reported anti-inflammatory activity of ethanolic extract of *D. viscosa* (28).

Neutrophil adhesion test is an indication of the marginalization of phagocytic cells in the blood vessels, i.e. an indication of immunostimulation. Increase in % neutrophil adhesion is attributed to marginalization of phagocytic cells which reflects improvement in defensive response under normal circumstances.

The *Dodonaea viscosa* reported to contain flavonoids, terpenoids and saponins like aliarin, dodonic acid, viscosol (29) stigmasterol, isorhamnetin (30) penduletin, quercetin, doviscogenin (31) dodonosides A and B (32) have been isolated in *D. viscosa*. The triterpenoidal saponins, flavonoids are well documented for their immunomodulator potential in *Euphorbia neriifolia* alcoholic (33) extract and *Celastrus paniculatus* (34).

Hence in conclusion, the present investigation reports the immunomodulator activity of ethanolic extract of *Dodonaea viscosa* L.F. leaves, which can be represented by capacity to influence role of phagocytosis, cell mediated immunity model. The observed potential can be attributed to the presence of saponins and flavonoids present in plant. Further detail study is required to pinpoint the exact mechanism and active principle involved in it.

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