International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 9 (11), pp. 001-005, November, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Immunomodulatory activity of fruits of *Randia dumetorum* Lamk

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Accepted 19 August, 2020

***Randia dumetorum* Lamk., a plant widely used in the traditional medicinal systems of India, has been reported to possess antiviral, antibacterial and anti-inflammatory activities. In present study, the attempt was made to screen immunomodulatory activity of methanol extract and its petroleum ether, chloroform, ethyl acetate and methanol fraction of fruits of *R. dumetorum*. The effects of *R. dumetorum* on cell mediated and humoral components of the immune system in mice were observed. Administration of chloroform fraction at dose 100 mg/kg produced statistically significant results as evidenced by increase in humoral antibody (HA) titre ($p < 0.05$), delayed type hypersensitivity (DTH) response ($p < 0.001$).This fraction also enhanced the total WBC level in cyclophosphamide induced mylosuppression model ($p < 0.001$) at dose 100 mg/kg. Petroleum ether fraction and methanol fraction affected only cell mediated immunity. Present study, therefore reveals that drug holds promise as immunomodulatory agent.**

Key words: *Randia dumetorum* Lamk, immunomodulatory, humoral immunity, delayed type hypersensitivity, myelosuppression.

INTRODUCTION

Traditional Indian system of medicines like Siddha and Ayurveda has 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. Diasio et al., 1996; Patwardhan et al., 1990).

Some plants are identified as rasayanas in Indian Ayurvedic system of medicine having various pharmarco-logical properties such as immunostimulant, tonic, neurostimulants, antiageing, antibacterial, antiviral, anti-rheumatic, anticancer, adaptogen etc (Agarwal and Singh, 1999). An entire section of Materia Medica of Ayurveda is devoted to drugs entitled as 'Rasayana'for enhancement of body resistance (Thatte and Dahanukar, 1997). 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. These attributes are similar to the modern concept of adaptogenic agents, which are known to afford protection of the human physiological system against diverse stressors (Bhattacharya et al., 2000).

A number of medicinal plants as rasayanas have been claimed to possess immunomodulatory activity. Some of the 'Rasayana' drugs known as immunomodulatory agents are *Withania somnifera, Tinospora cordifolia*, and *Mangifera indica* (Davis and Kuttan, 2000; Dahanukar and Thatte, 1997; Makare et al., 2001). A lot more are still to be explored and offer scope for further investigation.

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Randia dumetorum Lamk. (Rubiaceae) known as Madana (Sans), Mainphal (Hindi), Emetic nut (Eng) (Calis et al., 1997) is a small tree found in India in the tropical and subtropical region. Its fruits are considered to be tonic, alterative, demulcent, diuretic and restorative. The drug is claimed as a medical cure for piles, antidysentric agent, asthma, jaundice, diarrhea, emetic and gonorrhea (Kirtikar and Basu 1999). In the Ayurvedic texts, *R. dumetorum* is classified as a drug having properties similar to rasayanas (Agarwal and Singh, 1999).

An iridoid glycoside from leaves of *R. dumetorum* (Sati et al., 1986). Aucubin compound and napthaquinone are obtained from *R. dumetorum* (Kiramani et al., 1984). Saponins named as Dudumentoronin from fruit pulp of *R. dumetorum* Dumetoronin A, B, C,D, E and F etc. and Sugars produced on hydrolysis Dumetoronin A and B - Glucuronic acid, glucose, arabinose, xylose, rhamnose, unknown sugar. Dumetoronin C and D - Glucose, rabinose, xylose, rhamnose, unknown sugar. Dume-toronin E - Glucose, rhamnose, unknown sugar. Dume-toronin F - Glucose and unknown sugar (Varshney et al., 1978). A hemolytic triterpenoid saponins that is Randianin, from fruit of R. dumetorum (Subramaniam et al., 1989). Mannitol and leucoanthocyanidin from fruits, glycoside Randioside A, Beta- D-galactopyranosyl (1 - 3)-oleanolic acid (Rastogi and Mehrotra, 1980).

The fruits, seeds and bark possess anthelmentic, insecticidal, antidysentric and diaphoretic properties (Chopra and Nayar, 1992). The ethno medicinal use of the plant *R. dumetorum* shows having antibacterial, anti-fungal (Kumar and Chuahan, 1992). Antifertility (Rajani and Saxsena, 1995), analgesic, anti- inflammatory activity (Ghosh and Thejomoorthy, 1995), antitumor (Yakugaku, 1980). However, there is paucity of data available on the effect of *R. dumetorum* on humoral and cellular immunity on animals. Therefore, the present work aimed at studying the immunomodulatory effect of the methanol extract and its fractions of fruit of *R. dumetorum* on mice.

MATERIALS AND METHODS

Animals

Swiss albino mice of sex, weighing 20 - 25 gm, household in standard conditions of temperature, humidity and light were used. They were fed with standard rodent diet and water *ad libitum.*

Plant material and extract preparation

Dried fruits of *R. dumetorum* were purchased from local market, Pune, India. The fruits were identified and authenticated from Botanical Survey of India, Pune, India and assigned voucher specimen no BSI/WC/Tech./2006/622). Maceration of air-dried powdered fruits of *R. dumetorum* afforded (17% w/w) methanol extract (w/w). Methanol extract so obtained was then fractioned by maceration into different polarity solvents like petroleum ether, chloroform, ethyl acetate and methanol. All respective fractions were concentrated under vacuum.

Phytochemical screening

Phytochemical screening was done for methanol extract and its fraction (Khandelwal, 2003). Methanol extract gave positive tests for alkaloids, phenolics, steroids, terpenoids, saponins, flavonoids and carbohydrates. Petroleum ether and chloroform fraction gave positive tests for terpenoids and steroids. Ethyl acetate fraction gave positive test for flavonoids, terpenoids, steroids and tannins whereas methanol fraction gave positive tests for saponins, alkaloids, phenolics, flavonoids and carbohydrates on phyto-chemical screening.

Test samples

Weighed quantities of test extracts were suspended in 1% sodium carboxy methylcellulose to prepare suitable dosage form. The control animals were given an equivalent volume of sodium carboxy methylcellulose vehicle.

Drugs

Cyclophosphamide was used as a standard immunosuppressant agent. Antigen: Fresh blood was collected from sheep's sacrificed in the local slaughterhouse. Sheep red blood cells (SRBCs) were washed three times in normal saline and adjusted to a concentration of 20% for immunization and 1% for challenge as antigen.

Humoral antibody (HA) and delayed type hypersensitivity (DTH) response

The method described by (Bafna and Mishra, 2004) Animals were divided into eleven groups of six animals each. Group I (control group) received the vehicle (1% SCMC, 0.25 ml each orally) for a period of 7 days. Groups II–III given the methanol extract (50 and 100 mg/kg, p.o.). The animals of groups IV - XI were given the fractions (petroleum ether, chloroform, ethyl acetate and residual methanol fraction) of methanol extract (50 - 100 mg/kg, p.o.) daily for 7 days. The animals were immunized by injecting 0.1 ml of 20% of fresh sheep red blood cells suspension intraperitonially on day 0. Blood samples were collected in microcentrifuge tubes from individual animal by retro-orbital plexus on the 7^{th} day and serum was separated. Antibody levels were determined by haemagglutination technique. Briefly, equal volumes of individual serum samples of each group were pooled. Two fold dilutions of pooled serum samples were made in 25 µl volumes of normal saline in microtitration plate and to that were added 25 µl of 1% suspension of sheep red blood cells in saline. After mixing, the plates were incubated at room temperature for 1 h and examined for haemagglutination under microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titre.

The thickness of the right hind footpad was measured using digital Vernier caliper on the 7^{th} day. The mice were then challenged by injecting 20 µl of 1% SRBCs in right hind footpad and after 24 and 48 h of this challenge the foot thickness was measured again. The pre- and post -challenge difference in the thickness of footpad was expressed in mm and taken as a measure of DTH.

Cyclophosphamide-induced myelosuppression

Cyclophosphamide induced myelosuppression was studied according to the method described by (Manjarekar et al., 2000).Animals were divided into twelve groups of six animals each. Group I (control group) and Groups II (cyclophosphamide group) received

Table 1. Effect of *R. dumetorum* on footpad thickness and antibody titre.

S/N	Groups (n = 6)	Dose/day	Footpad thickness (mm) after 24 h Mean ± S.E.M.	Footpad thickness (mm) after 48 h Mean ± S.E.M.	Antibody titre Mean ± S.E.M.
1	Control	D.W.	0.54 ± 0.028	0.76 ± 0.031	358 ± 24.06
2	ME	50 mg/kg	0.63 ± 0.034	0.80 ± 0.031	381± 21.09
3	ME	100 mg/kg	0.85 ± 0.048	0.95 ± 0.038	395 ± 13.73
4	PE F	50 mg/kg	0.68 ± 0.031	0.88 + 0.030	370 ± 124.80
5	PE F	100 mg/kg	0.77 ± 0.024 **	1.25 + 0.050***	434 ± 19.43
6	CH F	50 mg/kg	0.98 ± 0.038***	1.08 + 0.041***	381.47 ± 17.06
7	CH F	100 mg/kg	1.15 ± 0.056***	1.19 + 0.040***	939 ± 42.10*
8	EA F	50 mg/kg	0.58 ± 0.037	0.81 + 0.052	360 ± 24.19
9	EA F	100 mg/kg	0.65 ± 0.042	0.95 + 0.031	355 ± 23.85
10	ME F	50 mg/kg	0.72 ± 0.022*	1.26 + 0.063***	365 ± 24.52
11	ME F treated	100 mg/kg	0.78 ± 0.078**	1.32 + 0.050***	375 ± 125.20

ME: Methanol extract, PE F: petroleum ether fraction, CH F: Chloroform fraction, EA F: ethyl acetate fraction, ME F: methanol fraction. Values are expressed as Mean ± S.E.M. * = $p < 0.05$,** = $p < 0.01$ and *** = $p < 0.001$.Test drug treated groups were compared with control Group. (Statistically analysed by one way ANOVA followed by Tukey-Krammar multiple comparisons test.)

the vehicle (1% SCMC, 0.25 ml each orally) for a period of 13 days. Groups III - IV administered methanol extract (50 - 100 mg/kg, p.o.) and groups V - XII fractions (petroleum ether, chloroform, ethyl acetate and residual methanol fraction) of the methanol extract (50 - 100 mg/kg, p.o.) daily for 13 days. The animals of groups II - XII were injected with cyclophosphamide (30 mg/kg, i.p.) on the 11th, 12th and 13th day, 1 h after the administration of the respective treatment. Blood samples were collected on the 14th day of the experiment and the total white blood cell (WBC).

Statistical analysis

Data were expressed as mean ± S.E.M. and differences between the groups was statistically determined by analysis of variance followed by Tukey-Kramer Multiple Comparions test, with level of significance set at $p < 0.05$.

RESULTS

Humoral antibody (HA) titre and delayed type hypersensitivity (DTH) response

The HA titre was used to assess humoral immune response. The augmentation of the humoral immune response to SRBCs by methanol extract and its fractions was evidenced by increase in the antibody titre (Table 1). The humoral antibody titre value in control group was found to be 358.4 ± 94.06. Administration of chloroform fraction produced dose dependent increase in humoral antibody titre however statistically significant result ($p < 0.05$) was at dose 100 mg/kg. Methanol extract showed slight increase in HA titre but it was not statistically significant. Other fractions of methanol extract showed statistically insignificant effect on humoral immune function.

DTH response was checked by increased footpad thickness using digital vernier caliper. Administration of methanol extract and its all fraction produced dose dependent increase in thickness of footpad of mice as a measure of DTH response (Table 1).

After 24 h chloroform fraction showed significant increase in the paw thickness at dose 50 mg/kg ($p < 0.001$) and 100 mg/kg ($p < 0.001$).Methanol fraction shows significant difference at dose 50 mg/kg ($p < 0.05$) and 100 mg/kg ($p < 0.01$). Petroleum ether fraction showed significant increase in paw thickness at dose of 100 mg/kg ($p < 0.01$) but the methanol extract and ethyl acetate fraction were insignificant at dose of 50 and 100 mg/kg as compared to control.

After 48 h chloroform and methanol fraction showed significant increase in the paw thickness at dose 50 mg/kg ($p < 0.001$) and 100 mg /kg ($p < 0.001$). Petroleum ether fraction showed significant increase at dose of 100 mg/kg ($p < 0.001$). Methanol extract and ethyl acetate fraction were insignificant at dose of 50 and 100 mg/kg as compared to control.

Cyclophosphamide induced myelosuppression

Cyclophosphamide at the dose of 30 mg/kg, i.p. caused a significant reduction in total WBC count (Table 2) as compared to control group.

Chloroform fraction showed significant increase in total WBC count at dose 50 mg/kg ($p < 0.01$) and 100 mg/kg ($p < 0.001$). Petroleum ether fraction at dose 100 mg/kg showed significant rise ($p < 0.01$) in the mean WBC count while methanol extract and ethyl acetate, methanol fractions showed increase in WBC count but results were statistically insignificant when compared with

Table 2. Effect of *R. dumetorum* on Cyclophosphamide induced Myelosuppression.

S/N	Groups (n = 6)	Dose/ day	WBC (thousand/cu.mm) Mean ± S.E.M
1	Control	D.W.	8.86 ± 0.38
2	CYP	30 mg/kg	5.67 ± 0.56 ***a
3	ME	50 mg/kg	5.74 ± 0.74
4	ME	100 mg/kg	6.60 ± 0.69
5	PE F	50 mg/kg	7.24 ± 0.42
6	PE F	100 mg/kg	10.45 ± 0.74**b
7	CH F	50 mg/kg	9.45 ± 0.62**b
8	CH F	100 mg/kg	11.23 ± 0.72***b
9	EA F	50 mg/kg	6.12 ± 0.29
10	EA F	100 mg/kg	6.97 ± 0.43
11	ME F	50 mg/kg	8.34 ± 0.53
12	ME F	100 mg/kg	8.56 ± 0.73

ME: Methanol Extract, PE F: Petroleum ether fraction, CH F: Chloroform fraction, EA F: Ethyl acetate fraction, ME F: Methanol fraction. CYP: Cyclo-phosphamide. Values are expressed as 'a' when compared with control and expressed as 'b' when compared with CYP. (Statistically analyzed by one way ANOVA followed by Tuky-Kramar multiple comparisons test.)Values are expressed as Mean ± S.E.M.* = P < 0.05, ** = p < 0.01 and *** = p < 0.001.Test drug treated group compared with cyclophosphamide.

Cyclo-phosphophamide group.

DISCUSSION

Modulation of the immune responses through stimulation or suppression may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy (Wagner et al., 1984).

Immunomodulatory activity of *R. dumetorum* was explored by evaluating its effect on antibody titre, DTH response, cyclophosphamide induced myelosuppression in mice. Administration of methanol extract and its fractions showed immunostimulatory activity. Significant stimulation with respect to these parameters was obtained with chloroform fraction, petroleum ether and methanol fraction while methanol extract and ethyl acetate fraction showed insignificant changes.

Antibody molecules, a product of B lymphocytes and plasma cells, are central to humoral immune responses, IgG and IgM are the major immunoglobulin which are involved in the complement activation, opsonization, neutralization of toxins, etc (Roitt et al.,1993).The augmentation of the humoral immune response to SRBCs by chloroform fraction, as evidenced by increase in the antibody titre in mice indicated the enhanced responsiveness of T and B lymphocyte subsets, involved in the antibody synthesis (Benacerraf et al., 1978). *Eclipta alba* (Jayathirtha and Mishra, 2004) is known for its effect on humoral immunity.

DTH is antigen specific and causes erythema and induction at the site of antigen infection in immunized animals. The histology of DTH can be different for different species, but the general characteristics are an influx of immune cells at the site of injection, macrophages and basophils in mice and induction becomes apparent within 24 - 72 h. T-cells are required to initiate the reaction (Waksman, 1979; Poulter et al., 1982). Increase in the DTH response indicates that drug has a stimulatory effect on lymphocytes and necessary cell types required for the expression reaction. DTH response, which is direct co-related to cell-mediated immunity, was significantly increased with chloroform, methanol and petroleum ether fraction as compared to untreated control. There are some plants like *Aesculus indica* (Chakraborthy, 2009), *Argyreia speciosa* (Gokhale et al., 2003) which are reported for acting on delayed type of hypersensitivity.

Statistically significant rise in HA titer, DTH response of chloroform, petroleum ether and methanol fraction suggest that active principles of fruits which are responsible for stimulation of antibody response, cell mediated response can be extracted with both polar and non polar solvents.

A high degree of cell proliferation renders the bone marrow a sensitive target particularly to cytotoxic drugs. In fact, bone marrow is the organ most affected during any immunosuppression therapy with this class of drugs. Loss of stem cells and inability of the bone marrow to regenerate new blood cells results in thrombocytopenia and leucopenia (Bafna and Mishra, 2006). Administration of the chloroform and pet ether fraction was found to increase the total WBC count, which was lowered by cyclophosphamide, a cytotoxic drug. The result indicates that these fractions can stimulate the bone marrow activity. But, methanol extract and ethyl acetate, methanol fractions fail to restore effect of cyclophosphamide induced myelosuppression.

Saponin are either triterpenoid or steroidal glycosides proven as important phytoconstituent with different pharmacological activities such as antiallergic, antiphlogostic, cytotoxic antitumour, antiviral, immunomodulating, antihepatotoxic, molluscicidal and antifungal activity. Recently three diosgenyl saponins isolated from *Paris polyphylla* reported for immuno-stimulating (Xiu-Feng Zhang et al., 2007) . Role of soponins in the immunomodulating effect of the plant, lymphocyte stimulation tests were performed on eight cycloartane-type saponins isolated from *Astragalus melanophrurius* (Calis, 1997).The higher concentrations of the tested compounds have been shown to possess inhibitory effect. Cycloartane and oleanane-type triterpenes from these species possess prominent IL-2 inducing activity (Erdem et al.,

2005).

The previous reports show that oleanolic acid (Ghosh et al., 1983) was isolated from seed and fruit of *R. dumetorum.* Oleanolic acid is well known for their hepatoprotective effect for both acute chemically induced liver injury and chronic liver fibrosis and cirrhosis (Liu, 2005). Immunomodulatory activity of terpenoid compounds glycyrrhizinic acid, ursolic acid, oleanolic acid and nomilin was reported (Raphael and Kuttan, 2003) .The activity of chloroform fraction may attributed to the presence of oleanolic acid and saponins which are reported in *R. dumetorum*.

The present study reveals that, *R. dumetorum* has immunostimulant activity and chloroform fraction which strongly affected immune system seems to be bioactive fraction of this plant. However, the mechanism of action could be unfolded only after detailed investigations.

ACKNOWLEDGEMENT

Authors wish to thank Dr. A. D. Deshpande, Director of Pharmacy, Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, India, for providing all the necessary facilities required to carry out the research.

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