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Research Article

EVALUATION OF *IN VIVO* ANTICANCER AND IMMUNOSTIMULATORY ACTIVITY OF FLOWERS OF *MIMOSA PUDICA* LINN. (FABACEAE)

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ABSTRACT

Objective: To investigate the *in vivo* anticancer and immunostimulatory activity of dichloromethane (DCM) extract of flowers of *Mimosa pudica* and its isolated compound 11β hydroxy-3 methoxy 1,2 dehydro crinane.

Methods: The anticancer activity was performed on Ehrlich ascites carcinoma (EAC) cell line in Swiss albino mice. The activity was assessed by evaluating tumor volume, viable and nonviable tumor cell count, tumor weight, hematological, and histopathological parameters of EAC-bearing animals. The immunostimulatory activity was performed through carbon clearance, delayed type hypersensitivity (DTH), neutrophil adhesion and humoral antibody (HA) titer methods.

Results: At the dose of 500 and 1000 mg/kg/day p.o for the extracts and 2.5 mg/kg/day p.o. for the isolated compound, significantly decrease the tumor volume (3.46±0.135 ml, 2.25±0.153 ml, and 1.84±0.012), increased the life span (59.32%, 76.39%, and 82.43%) and significantly ($p<0.05$) decreased tumor weight as compared with control. Hematological profiles were found to be nearly normal level in extract treated mice compared with tumor bearing control mice. The immunostimulatory activity was also found to be effective in the above dosage regimen. The results revealed that animals treated with above doses show a significant increase in the rate of carbon clearance from blood, increase in HA titer value, increase in neutrophil adhesion and significant ($p<0.05$) increase in mean paw edema in DTH reactions in dose-dependent manner.

Conclusion: The results demonstrated that the extract is possessing dose-dependent anticancer activity and immunostimulatory activity attributed to the presence of crinane alkaloid.

Keywords: Humoral antibody titer, Ehrlich ascites carcinoma cell line, LD_{50}, Delayed type hypersensitivity, Dichloromethane, *Mimosa pudica.*

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INTRODUCTION

Cancer is the prominent cause of mortality worldwide, and most of the chemotherapeutic agents have been reported to exhibit severe toxicity to normal tissues, accompanied by undesirable huge side effects [1]. Moreover, most of these drugs are very expensive, mutagenic, and carcinogenic. Therefore, novel pharmaceutical agents that provide a more specific therapeutic regimen or increase the efficacy of conventional chemotherapy, without increasing toxicity toward normal cells, would clearly be of great clinical benefit. Increasing evidence suggests that immune responses are involved in the control of cancer and that the immune system can be manipulated in different ways to recognize and treat cancers [2]. Progress in immune-based strategies has introduced a new therapeutic avenue using a number of techniques destined to eliminate malignant cells [3]. Recently, cancer chemoprevention with ideas using foods and medicinal herbs has been regarded as one of the most visible fields for cancer control [4]. Most of the anti-tumor drugs currently used in chemotherapy are toxic to normal cells and cause toxicity for immune cells. Therefore, the identification of new anticancer drug with low side effects on the immune system or which boosts it has become an essential goal in many studies of immunopharmacology.

The *Mimosa pudica* Linn. (Fabaceae) invites the attention of the researchers worldwide for its traditional use in various parts of the world. In Assam part of India, it is known by the name "Lajukilata" and traditionally used as antitumor, stimulant, antioxidant and for wound healing activity. Phytoconstituents such as alkaloids, sterols, and tannins together with other compounds such as mimosine, mimosinic acid, and gallic acid were already reported from this plant [5]. Although some studies are reported on anticancer activity of leaves and aerial plant parts extracts of *M. pudica* but no studies were conducted with the flowering parts of the plants. This study was undertaken to explore the anticancer and immunostimulatory property of flowers of *M. pudica* Linn using some *in vivo* models.

EXPERIMENTAL

Plants material

Flower parts of *M. pudica* were collected from local areas of Guwahati, Assam, India in the month of January 2012. The plant material was authenticated by Dr. GC Sharma, Curator, Department of Botany, Gauhati University, Guwahati (voucher specimen no. 17777).

Preparation of the extracts

The collected plant parts were washed with water; shade dried in open air and pulverized using an electric grinder. About 200 g of *M. pudica* powder was packed into Soxhlet apparatus and subjected to successive extraction using petroleum ether, ethyl acetate, dichloromethane (DCM), and butanol as the solvent. The preliminary phytochemical and pharmacological screening shows a good result for DCM extract, therefore it is selected for further studies. The extract was filtered through Whatman filter paper No. 40, evaporated using vacuum rotary evaporator (Buchi) and heated on a water bath at 45±5°C and stored in vacuum desiccators for further use [6,7].

Isolation of compound

M. pudica flower DCM extracts were fractioned by column chromatography using silica gel 60-120 as stationary phase and

methanol–chloroform as mobile phase. The isolated compound was purified using preparative thin layer chromatography (TLC) techniques. The spot of the compound was scraped, collected, and then diluted with chloroform:methanol (7:3). The compound was obtained in the form of a crystal after filtration and evaporation. It was identified by ultraviolet, Fourier-transform infrared, nuclear magnetic resonance, and mass spectroscopic methods. The compound is identified as 11β hydroxy-3 methoxy 1,2 dehydro crinane.

Drugs and chemicals

Extra immune tablets (Charak pharmaceuticals) and cyclophosphamide in the form of cycloxan-50 tablet (Biochem Pharmaceuticals) were locally purchased from the medicine store. Trypan blue was purchased from Hi media Laboratories Pvt. Ltd, Mumbai. Nylon fiber purchased from local market Guwahati, Assam, India. Methotrexate was obtained from IPCA Laboratories, Mumbai.

Animals

The study was conducted using Swiss albino mice weighing 20±5 g. They were obtained from the animal house of GIPS. The mice were grouped, housed in polycrylic cages and maintained under standard laboratory conditions (temperature 25±2°C) with light/dark cycle (12/12 hr). They were allowed free access to a standard dry pellet diet and water *ad-libitum* [8].

Acute toxicity study

The acute toxicity study was conducted as per the OECD guidelines 425. Initially, the DCM extract was administered orally at a limit dose of 2000 mg/kg for the extract and 10 mg/kg for the isolated compound to single mice. The mice were observed closely for the first 4 hr and then periodically up to 24 hr for any toxic symptoms and mortality. After 24 hr same dose was administered to four more female rats. This study was approved by the Animal Ethics Committee of Girijananda Chowdhury Institute of Pharmaceutical Sciences, Guwahati, Assam (CPCSEA Regn. No.1372/C/10/CPCSEA. Study approval No-GIPS/IAEC/07). The acute toxicity study was conducted for 24 hrs.

Anticancer activity of DCM extract of *M. pudica*

Experimental protocol

For this study, animals are divided into seven groups each containing six animals. All the animals expect the normal group received Ehrlich ascites carcinoma (EAC) cells 1×10^6 cells/mouse. Group I (normal) received 0.9% normal saline orally, Group II (control) 0.9% normal saline orally, Group III treated with standard methotrexate at 2.5 mg/kg/day p.o, Group IV treated with 250 mg/kg/day p.o of DCM extract, Group V with 500 mg/kg/day p.o of DCM extract, Group VI with 1000 mg/kg/day p.o of DCM extract, and Group VII treated with treated with 2.5 mg/kg/day p.o of isolated compound. All treatments were given for 9 days [9].

Determination of body weight and mean survival time (MST) of the DCM extract treated mice

The body weight and MST of each group consisting of six mice were noted. The antitumor efficacy of *M. pudica* was compared to that of methotrexate. The percentage increase life span (% ILS) of each mouse was calculated using the following equation:

$$\%\,\text{Increase in life span} = \frac{T - C}{C} \times 100$$

Where T=number of days the treated animals survived and C=number of days control animals survived.

Determination of viable and nonviable cell count of the DCM extract treated mice

The viability and nonviability of the cells were checked for the above groups by trypan blue assay. The cells were stained with trypan blue (0.4% in normal saline) dye. On staining, the viable cells did not take the stain while the nonviable cells were stained blue and counted using Invitrogen Auto cell counter.

Determination of hematological parameters of the DCM extract treated mice

At the end of the experimental period, the next day after an overnight fasting, blood was withdrawn from the retro-orbital plexus and used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) count and white blood cell (WBC) count using an automatic analyzer. Half of the animals from each group were sacrificed and checked for tumor volume.

Histopathological study of the DCM extract treated mice

A part of the dissected liver from sacrificed animals of all the groups was cleared off of the surrounding tissues and kept in 10% buffered neutral formalin, dehydrated in alcohol, and then embedded in paraffin. The paraffin blocks were sectioned at a size of 5 µm and stained with hematoxylin and eosin dye and observed under a light microscope for the array of hepatic cords radiating from the central vein and size of sinusoids.

Immunostimulatory activity of DCM extracts of *M. pudica* flower and its isolated compound

Experimental protocol

Mice were divided into seven groups, each containing six animals. Group I (control) was given 1% sodium carboxymethyl cellulose in water (0.3 ml/mouse) for 7 days, Group II was administered with 2.5 mg/kg/day p.o of immunogen and marked as positive standard, Group III was negative standard administered with 2.5 mg/kg/day p.o of cyclophosphamide, and Groups IV-VI were administered with 250, 500, and 1000 mg/kg/day p.o. dosage of DCM extracts for seven days. Group VII was administered with isolated compound at the dose of 2.5 mg/kg/day p.o. At the end of seven days, mice of all the groups were studied for immunostimulatory activity [10].

In vivo carbon clearance test

At the end of 7 days, mice of all the groups were injected via the tail vein the carbon ink suspension (10 µl/g body weights). Blood samples were drawn (in EDTA solution 5 µl) from the retro-orbital vein at intervals of 0 and 15 minutes, a 25 µl sample was mixed with 0.1% sodium carbonate solution (2 ml), and their absorbance was measured at 660 nm. The carbon clearance was calculated using the equation: $(\text{Log}_e\ OD_1 - \text{Log}_e\ OD_2)/15$, where OD_1 and OD_2 are the optical densities at 0 and 15 minutes, respectively [11,12].

Humoral antibody (HA) titer test

The animals of all the groups were immunized by injecting 0.1 ml of sheep RBCs (SRBCs) suspension containing 1×10^8 cells intraperitoneally on day 0. Blood samples were collected in microcentrifuge tubes from an individual animal of all the groups by retro-orbital vein puncture on day 8. The blood samples were centrifuged, and serum was separated. Antibody levels were determined by the hemagglutination technique. Briefly, equal volumes of (50 µl) individual serum samples of each group were polled. To serial two-fold dilutions of pooled serum samples made in 50 µl volumes of RPMI-1640 in microtitration plates, 50 µl of 1% suspension of SRBC in RPMI-1640 was added. After mixing, the plates were incubated at 37°C for 1 hr and examined for hemagglutination under a microscope (button formation). The reciprocal of highest dilution, just before the button formation, was observed as the titer values of the test samples [13].

Delayed type hypersensitivity (DTH) test

The experiment of DTH was commenced after the HA titer model in the same animals. On day 8, the thickness of the right hind footpad was measured using vernier caliper. The mice were then challenged

by injection of 1×10^8 SRBCs in the right hind footpad. The footpad thickness was measured again after 24 hrs and 48 hrs of the challenge. The difference between the pre- and post-challenge footpad thickness expressed in mm was taken as a measure of DTH response [14,15].

Neutrophil adhesion test

The mice were pre-treated orally with vehicle or extracts for 14 days as above. At the end of treatment day 14, blood samples were collected from the retro-orbital plexus into heparinized vials and analyzed for differential leukocyte count (DLC). After the initial counts, blood samples were incubated with 80 mg nylon fibers/ml for 15 minutes at 37°C. The incubated blood samples were again analyzed for TLC and DLC, respectively, to give neutrophil index of blood samples. The percent neutrophil adhesion was calculated as follows:

$$\text{Neutrophil adhesion}\,\% = \frac{NI_u - NI_t}{NI_u} \times 100$$

Where NI_u is the neutrophil index of untreated blood samples and NI_t is the neutrophil index of treated blood samples [16,17].

Statistical analysis

Data analysis was performed using Graph Pad Prism software, and the data were expressed as a mean±standard error. The significance level of treatment effect was determined by one-way analysis of variance; $p<0.05$ was considered statistically significant.

RESULTS

Acute dose toxicity study

The DCM extract did not show any toxic reactions and mortality up to a dose of 2000 mg/kg for the extract and 10 mg/kg for the isolated compound. No changes in food consumption, water intake or behavior (tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma) were observed in the mice after dose administration. Hence, DCM 250 mg/kg, 500 mg/kg, and 1000 mg/kg were taken as treatment dose for this study.

Anticancer activity of DCM extract of *M. pudica*

Administration of DCM extract at the doses of 500 mg/kg, 1000 mg/kg and isolated compound at 2.5 mg/kg significantly decreased the tumor volume and viable cell count and increases the nonviable cell count in dose treated mice when compared with control group (Table 1). Further, the MST was increased to 29.76 (% ILS=59.32%) for 500 mg/kg group, 35.36 (% ILS=76.39%) for 1000 mg/kg group, and 36.74 (82.43) for the isolated compound on oral administration. After treatment with DCM extract at the doses, 500 mg/kg, 1000 mg/kg and isolated compound at 2.5 mg/kg in EAC-bearing mice significantly increased the RBC count and Hb content ($p<0.05$) and significantly reduced the WBC count as compared with the EAC control group (Table 2). In EAC treated mice liver section showed dilation and congestion in the central and portal veins of the liver with respect to control mice (Fig. 1a and b). The microscopic examination of liver reveled thickening in hepatic capsule with inflammatory and pigmented cells as well as with diffuse Kupffer cells. The treatment with DCM extracts has reduced most of the pathological alteration observed in EAC control group. The liver section showed few inflammatory cells infiltration in the hepatic parenchymal associated with slight congestion in the central vein (Fig. 1d and e). The treatment group of DCM 1000 and isolated compound shows a normal array of hepatic cord radiating from the central vein and smaller sinusoids (Fig. 1f and g). The anticancer activity of the *M. pudica* at the concentration of DCM 500 and DCM 1000 was found to be equipotent with the standard drug methotrexate at 2.5 mg/kg concentration (Fig. 1c).

Immunostimulatory activity of DCM extracts of *M. pudica* flower and its isolated compound

The results revealed that animals treated with doses of the extracts at 500 and 1000 mg/kg show much increase rate of carbon clearance (Fig. 2) from blood (0.03986±0.0017 and 0.04328±0.0035, respectively). The isolated compound also shows increase in carbon clearance rate (0.05462±0.0043). Treatment of mice with DCM extracts of the plant at 250-1000 mg/kg doses and isolated compound at 2.5 mg/kg doses shows much increase in HA titer value as evident from hemagglutination after incubation of serum with SRBCs (Fig. 3). In DTH response test the extract showed statistically significant increase in mean paw edema in dose-dependent manner (0.37±0.04 for 24 hrs, and 0.28±0.02 for 48 hrs with 1000 mg/kg, 0.34±0.05 for 24 hrs and 0.25±0.03 for 48 hrs with 500 mg/kg and 0.28±0.03 for 24 hrs, and 0.27±0.03 for 48 hrs with 250 mg/kg doses of DCM extract of *M. pudica*) in mice, as compared with (0.42±0.21 for 24 hrs and 0.34±0.08 for 48 hrs, respectively) the standard drug immunogen at a concentration of 2.5 mg/kg (Table 3).

Table 1: Effect of DCM extract of *M. pudica* on tumor weight, MST and life span of EAC-bearing mice

S. No.	Treatment	Tumor weight (g)	MST (days)	% increase in life span
1.	EAC control	7.29±0.256	16.44±0.016	
2.	Methotrexate 2.5 mg/kg	2.46±0.087	38.98±0.032	87.32±1.265
3.	DCM 250	5.76±0.018	20.32±0.127	36.98±0.986
4.	DCM 500	4.91±0.023	29.76±0.098	59.32±0.098
5.	DCM 1000	2.98±0.189*	35.36±0.012*	76.39±0.786*
6.	Isolated compound 2.5	2.93±0.134*	36.74±0.122	82.43±0.124

Values are mean±SEM of 3 replicates. *p<0.05. DCM 250: Dichloromethane extract of *M. pudica* at 250 mg/kg, DCM 500: Dichloromethane extract of *M. pudica* at 500 mg/kg, DCM 1000: Dichloromethane extract of *M. pudica* at 1000 mg/kg. SEM: Standard error of mean, MST: Mean survival time, EAC: Ehrlich ascites carcinoma, *M. pudica: Mimosa pudica*

Table 2: Effect of DCM extracts of *M. pudica* on hematological parameters of EAC treated mice

S. No.	Treatment	RBC count (1×10^6 cells/mm^3)	WBC count (1×10^3 cells/mm^3)	Viable cells (1×10^7 cells/mm^3)	Nonviable cells (1×10^7 cells/mm^3)	Hemoglobin (g/dl)	Tumor volume (ml)
1.	EAC control	2.84±0.072	26.31±0.237	7.52±0.172	1.52±0.012	4.37±0.237	8.23±0.014
2.	Methotrexate 2.5 mg/kg	8.76±0.078	12.98±1.012	2.60±0.043	5.32±0.98	13.27±0.086	1.94±0.139
3.	DCM 250 mg/kg	3.18±0.059	25.73±0.068	5.98±0.217	2.74±1.243	6.79±0.165	5.14±0.025
4.	DCM 500 mg/kg	6.14±0.019*	15.72±0.142	3.74±1.078	4.67±0.189	8.63±0.156	3.46±0.135
5.	DCM 1000 mg/kg	7.46±0.139*	13.47±0.037*	2.36±0.062*	6.74±0.072*	11.97±0.337	2.25±0.153*
6.	Isolated compound 2.5 mg/kg	8.23±0.136*	11.92±0.145*	2.25±0.128*	4.93±0.0125	12.32±0.271	1.84±0.012*

Values are mean±SEM of 3 replicates. *p<0.05. DCM 250: Dichloromethane extract of *M. pudica* at 250 mg/kg, DCM 500: Dichloromethane extract of *M. pudica* at 500 mg/kg, DCM 1000: Dichloromethane extract of *M. pudica* at 1000 mg/kg. SEM: Standard error of mean, EAC: Ehrlich ascites carcinoma, *M. pudica: Mimosa pudica*, RBC: Red blood cell, WBC: White blood cell

Table 3: Effect of DCM extract of *M. pudica* and isolated compound 2 on delayed type hypersensitivity

S. No.	Treatment	Dose (mg/kg)	Mean of right footpad thickness (mm)	
			24 hrs	48 hrs
1.	Control	-	0.16±0.05	0.16±0.02
2.	Sensitized control	-	0.26±0.12	0.23±0.02**
3.	+ Std	2.5	0.42±0.09*	0.34±0.08
4.	− Std	2.5	0.17±0.07	0.17±0.04*
5.	DCM 250 mg/kg	250	0.28±0.03	0.27±0.03
6.	DCM 500 mg/kg	500	0.34±0.05**	0.25±0.03**
7.	DCM 1000 mg/kg	1000	0.37±0.04*	0.28±0.02*
8.	Isolated compound 2.5 mg/kg	1000	0.39±0.15*	0.32±0.12*

All values are expressed as mean±SEM of three observations. *p<0.05, **p<0.01 comparing with the control. DCM 250: Dichloromethane extract of *M. pudica* at 250 mg/kg, DCM 500: Dichloromethane extract of *M. pudica* at 500 mg/kg, DCM 1000: Dichloromethane extract of *M. pudica* at 1000 mg/kg. SEM: Standard error of mean, *M. pudica: Mimosa pudica*

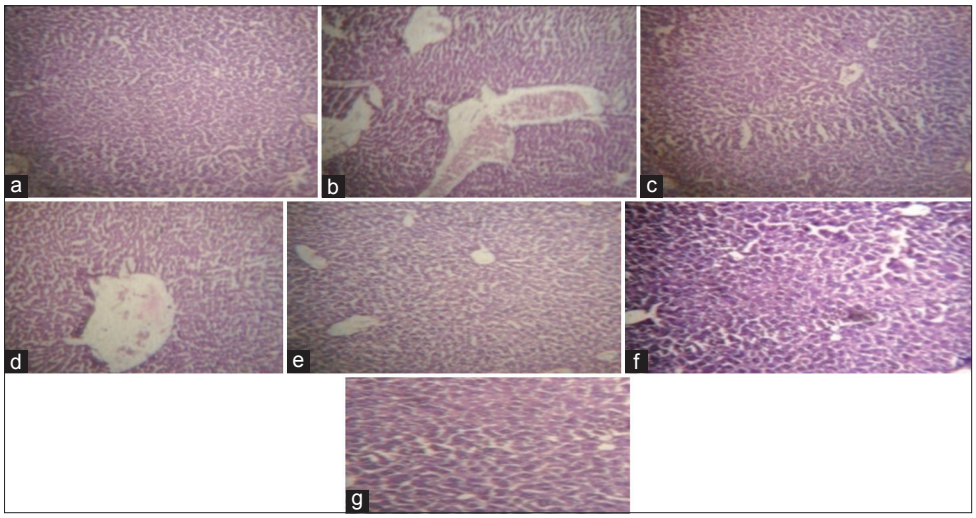

Fig. 1: Histopathological interpretation of dichloromethane (DCM) extracts and compound 2 from *Mimosa pudica* flower on the liver section of Swiss albino mice. (a) Control: Normal nucleus, hepatocytes are radiating outward from a central vein in the center. (b) Ehrlich ascites carcinoma control: Cellular inflammatory infiltration, nuclear hypertrophy, debris in the central vein, hemorrhages and wide sinusoids. (c) Standard: Cytoplasmic degeneration has been reduced, mild cellular inflammatory infiltration and nuclei of hepatic cells are better. (d) DCM 250: Cellular inflammatory infiltration, moderate nuclear hypertrophy, little debris in the central vein and wide sinusoids. (e) DCM 500: Cytoplasmic degeneration has nearly reduced mild cellular inflammatory infiltration and normal array of hepatic cords radiating from the central vein. (f) DCM 1000: Relatively small sinusoids, cellular hemorrhage is not very prominent, no debris in the central vein and mild cellular inflammatory infiltration. (g) Compound 2. Very small sinusoids, cellular hemorrhage is not very prominent, no debris in the central vein and mild cellular inflammatory infiltration

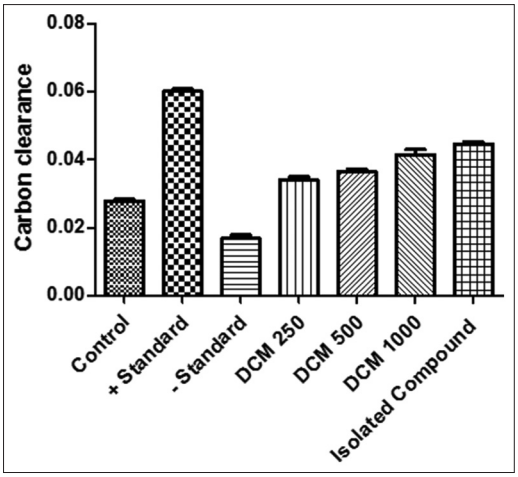

Fig. 2: Results for carbon clearance test (absorbance) for dichloromethane extracts of *Mimosa pudica* and isolated compound. DCM 250: Dichloromethane extract of *M. pudica* at 250 mg/kg, DCM 500: Dichloromethane extract of *M. pudica* at 500 mg/kg, DCM 1000: Dichloromethane extract of *M. pudica* at 1000 mg/kg

Isolated compound also exhibits good response for DTH response (0.39±0.15 for 24 hrs and 0.32±0.12 for 48 hrs). In neutrophil adhesion test, the incubation of blood with nylon fibers produced a decrease in the neutrophil counts due to adhesion of neutrophils to the fibers. All the doses of DCM extract of *M. pudica* showed a significant increase in the neutrophil adhesion when compared to standard drug immunogen and isolated compound (Fig. 4).

The results thus depict a good anticancer and immunostimulatory activity of DCM extracts of flower of *M. pudica* and its isolated compound 11β hydroxy-3 methoxy 1,2 dehydro crinane.

DISCUSSION

Many plant products used in traditional medicine as anticancer agents have been reported to have immunostimulatory activities. The immunotherapeutic properties of these plants extracts initiate the enhancement of anticancer response by inhibiting secretions of inhibitory cytokines and recruitment of immune cells in the tumor site. In this study, we found that DCM extracts of *M. pudica* significantly shows both anticancer and immunostimulatory activity in experimental mice which gives a strong evidence for the traditional usage of immunostimulatory anticancer drugs.

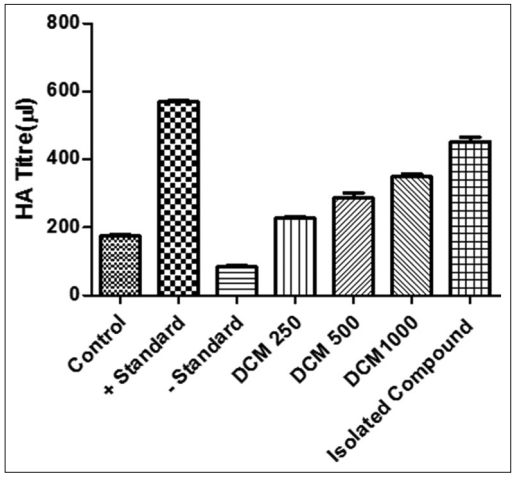

Fig. 3: Results for humoral antibody titer test for dichloromethane extracts of *Mimosa pudica* and isolated compound 2.
DCM 250: Dichloromethane extract of *M. pudica* at 250 mg/kg,
DCM 500: Dichloromethane extract of *M. pudica* at 500 mg/kg,
DCM 1000: Dichloromethane extract of *M. pudica* at 1000 mg/kg

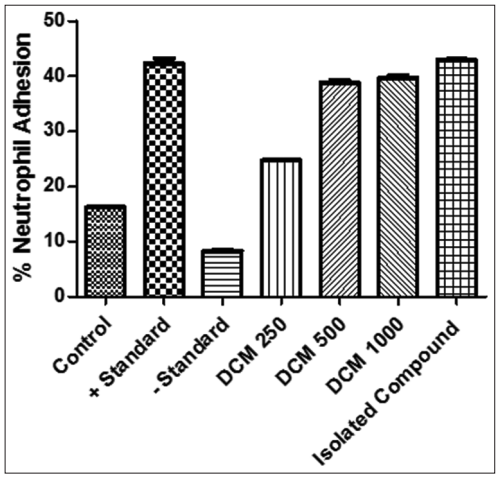

Fig. 4: Results for % neutrophil adhesion for dichloromethane extracts of *Mimosa pudica* and isolated compound.
DCM 250: Dichloromethane extract of *M. pudica* at 250 mg/kg,
DCM 500: Dichloromethane extract of *M. pudica* at 500 mg/kg,
DCM 1000: Dichloromethane extract of *M. pudica* at 1000 mg/kg

The results from the acute dose toxicity study reveals that the animals treated with the DCM extract at a higher dose of 2000 mg/kg and isolated compound at 2.5 mg/kg, did not produce any significant toxicity signs, behavioral changes, body weight changes, or macroscopic findings during the observational period. Hence, the LD_{50} of DCM extract of *M. pudica* and isolated compound should be more than 2000 mg/kg and 10 mg/kg, respectively.

This study indicated that the DCM extracts of flowers of *M. pudica* have significantly enhanced the erythrocyte count and Hb level when compared to that of EAC control indicating the apoptosis-inducing capacity of the extract. Viable cell count was decreased, and nonviable cell count was increased in intraperitoneal fluid by the plant extracts in a dose-dependent manner. This indicated a toxic effect on these cells that resulted in cell death. It signifies that the drug was absorbed directly by the EAC cells in the peritoneal cavity and causes the lysis of the EAC cell by the direct cytotoxic mechanism. The antitumor activity of the plant extracts at 500 and 1000 mg/kg and isolated compound at 2.5 mg/kg body weight dose was effective as the standard drug methotrexate suggesting its potent activity as an antitumor agent. The histopathological examination of the liver section revels that the extract treated mice group shows normal array of hepatic cord radiating from the central vein and smaller sinusoids in a dose-dependent manner which signifies the replacement of cancerous cells with normal cells (Bhadoriyal and Mandoriya, 2012).

Further, the study reveals that the extracts (250-1000 mg/kg) and isolated compound (2.5 mg/kg) evoked a significant increase in percent neutrophil adhesion and rate of carbon clearance. This may potentially help in increasing immunity of body against microbial infections by upregulation of β_2 integrins present on the surface of the neutrophil. The high values of hemagglutinating antibody titer obtained in the case of the plant's extracts and isolated compound have indicated that immunostimulation was achieved through humoral immunity. The increase in DTH reaction in mice in response to SRBC antigen revealed significant paw edema ($p<0.05$) in the dose-dependent manner (250-1000 mg/kg) for the plant's extracts and isolated compound at 2.5 mg/kg. In this study, we found that DCM extracts and isolated compound modulate both cellular and humoral immunity in experimental mice. Thus, the DCM extract of flowers of *M. pudica* and isolated compound 11β hydroxy-3 methoxy 1,2 dehydro crinane can be a promising anticancer agent with immunostimulant property.

CONCLUSION

In this work, it is observed that anticancer activity of *M. pudica* Linn. (Fabaceae) is due to the stimulation of macrophages which are the important cells for the immune system. Thus, this study can further be proceed for a better understanding of anticancer and immunostimulatory mechanism of *M. pudica* which can be a boon for the human society.

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