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# Evaluation of immunomodulatory effect of methanolic extract of *Mirabilis jalapa* L. tuber on mice.

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**ABSTRACT**

The aim of the study is to evaluate the Immunomodulatory activity of Methanolic Extract of *Mirabilis jalapa* (MEMJ) L. tuber on swiss albino mice. Preliminary phytochemical screening was analyzed as per standard methods. The MEMJ tuber was administered at the different doses of 100 mg/kg, 200 mg/kg and 400 mg/kg p.o bw. Heamagglutination antibody (HA) titer, delayed type hypersensitivity (DTH), neutrophil adhesion test and carbon clearance test were performed to establish the immunomodulatory activity. Levamisole (50 mg/kg/day, p.o.) is used as standard drug to produce extra immune to mice. Statistical analysis of all the data were analyzed using one way ANOVA followed by Dunnett's comparison test where $p<0.05$ were considered statistically significant when compared to control group. Phytochemical screening of different extracts established the presence alkaloids, carbohydrate, glycosides, steroids, phenolics, flavonoids. Oral administration at doses 200 and 400mg/kg p.o. bw. of the extract significantly increased in humoral immunity lead to increased in antibody titre, phagocytic index, in adhesion of neutrophils, positive DTH response in mice when compared to control group. In conclusion the methanolic extract of MEMJ tuber established as potent immunomodulator on both specific and nonspecific immune mechanisms, which was achieved due to presence of flavonoid in the extract.

**Keywords:** *Mirabilis jalapa* tuber, Immunomodulator, Phagocytic index, Antibody titre, delayed hypersensitivity, % of Nutrophil adhesion.

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## INTRODUCTION

Immunomodulator an agent which can stimulate or weaken the immune system. In immune system the agent can helps in the formation of antibody or provides resistance to white blood cell movement. When these agent stimulates the immune response is term as immunostimulants on other hand these agent weaken the immune response is term as immunosuppressant. The immune system is build up indicates that the body defense mechanism developed against microorganisms and to reduce incidence of disease [1]. Immunostumulators is required to stimulant the immune system. It is reported that a number of herbs have shown immunomodulatory activity by stimulating both specific and non-specific immunity [2]. Additionally, medicinal plants utilized for immunomodulation be capable of propose promising choice against a range of diseases, particularly at the point when host defense system must be initiated under the situation of damaged immune reaction. It have been reported that plant metabolites like flavonoids, glycosides, polysaccharides, peptides have property to alter the immune response [3].

*Mirabilis jalapa* Linn commonly known as marvel of Peru or four o' clock flower belonging to the family Nyctaginaceae is the herbaceous plant. Traditionally the plant is well known for its antidysenteric, antiparasitic, carminative, digestive stimulant properties. Also used as tonic, vermifuge, wound healer, seeds have cathartic, roots and tubers have aphrodisiac, diuretic and purgative properties [4,5]. But the immunomodulatory activity of *Mirabilis jalapa* tuber extract has not yet established scientifically. Therefore, this effort was carried out to evaluate immunomodulatory effect of methanolic extract of *Mirabilis jalapa* tuber in relation with its folklore medicinal properties.

## MATERIALS AND METHODS

### Plant material

Tubers of *Mirabilis jalapa* (Fig .1) were collected from local area of Kamrup district, Assam, India and authenticated by Dr. P. P. Baruah (HOD, Department of Botany, Gauhati University, India). The voucher specimen of *Mirabilis jalapa* tuberous root (Acc no. 18176) were kept at the Department of Pharmacognosy, Girijananda CHowdhury Institute of Pharmaceutical Sciences, Assam for future reference. The plant material was process and subjected to washing with running water, dried in shade for 10- 15 days and stored in air tight containers until used.

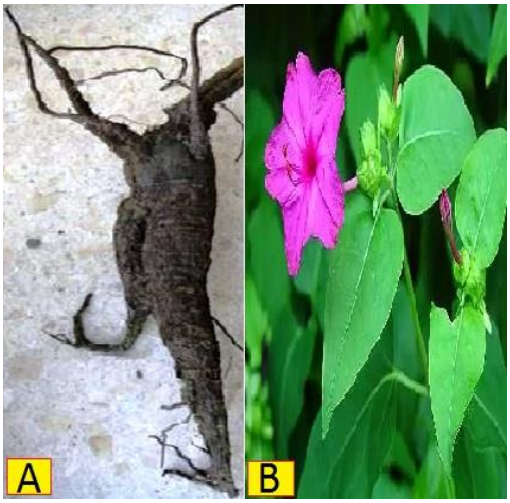


**Figure: 1 Image of Tuberous root (A) and Leaf, flower (B) of *Mirabilis jalapa***

### Preparation of Extract

The dried tubers were subjected to size reduction. About 1kg of crude powder drug was subjected to hot percolation with petroleum ether for 72 hrs to take out fatty material followed by cold maceration at room temperature with different solvent by increasing polarity such as benzene, diethyl ether, chloroform, acetone, ethyl acetate and 95% methanol. for about 48 hrs with frequent shaking up to 6 hrs. Extracts were then

filtered, concentrated under reduced pressure in rotary evaporator (Buchi India Pvt Ltd.) at 40°C and stored in a desiccator until further use Compare to all other extracts methanolic extract shown the maximum quantity i.e 10 gm. [6].

**Preliminary phytochemical screening**

The quantitative chemical tests of different extracts were performed as describe in reference books to identify the presence of different metabolites [6,7].

**Experimental Animal**

Experimental works were carried out with the guidelines set by CPCSEA and were approved by the Institutional Animal Ethical Committee (GIPS/IAEC/Phd/2015/01). Female swiss albino mice of weighed between 20-25 gm were housed and the standard conditions like 22±3 °C of temperature, 50±10% humidity, 12 hrs interval light and dark phase were properly maintained and standard diet were followed as per the CPCSEA guideline.

**Acute toxicity study**

The acute toxicity study was executed as describe in the OECD guideline 425. The methanolic extract of *Mirabilis jalapa* (MEMJ) tuber was administered at of different doses of 2000 mg/kg and 5000 mg/kg bw p.o to overnight fasted experimental mice as suggested in OECD Guidelines 425. The animal behavioral changes and mortality, abnormalities were observed next 24 hrs and then recorded upto 14 days [8,9].

**Experimental method**

**Selection of dose:** After performing the acute toxicity studies it was found that there was no mortality at the dose of 2000 mg/kg bw as well as 5000 mg /kg bw of MEMJ tuber. So the drug was found as safe. Therefore dose optimization was done and 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. were selected for the experimental study.

**Neutrophil adhesion test**

Total thirty number of mice were divided into five groups, six animals (*n*=6) in each group. Group I were served as control received 10 ml/kg bw of normal saline, Group II were served as standard received levamisole (50 mg/kg/p.o) for 14 days. Group III, IV and V were given MEMJ tuber at the dose 100 mg/kg, 200 mg/kg, 400 mg/kg bw/d/p.o accordingly for two weeks. After completion of last dose on the 14$^{th}$ day, all the animals were anesthetized and blood samples were withdrawn from all the groups by puncturing the retro-orbital plexus and stored in disodium EDTA vials. The total leukocyte count (TLC) and differential leukocyte count (DLC) were analyzed and then incubated for 15 min at 37 °C along with nylon fibre (80 mg/ml of blood sample). TLC and DLC were analyzed again with the incubated blood sample to get to neutrophil index (NI) [10]. Percent (%) of neutrophil adhesion was calculated as follows

$$\text{Neutrophil Adhesion (\%)} = \frac{NI_u - NI_t}{NI_u} \times 100$$

Where,

$NI_u$ = Neutrophil Index before incubation with nylon fibre.
$NI_t$ = Neutrophil Index After incubation with nylon fibre.

**Carbon Clearance test (CCT)**

The study was performed to evaluate the phagocytic cell activity of macrophase system which was measured by elimination of carbon of macrophase system by carbon clearance test. For this test, the grouping of animals and drug treatments were performed in similar way as describe in the neutrophil adhesion test respectively for 7 days. After 2hrs of last dose on day 7$^{th}$ all the mice were injected via tail vein with carbon ink suspension (10 µl/gm bw). Blood sample were collected about 25 µl (in EDTA solution 5 µl) from retro orbital

plexus under mild ether anesthesia at 0 and 15 min interval and which was then mixed with 2 ml of 0.1% sodium carbonate solution and the absorbance was determined at 675 nm [11]. The phagocytic index (K) was calculated by using the equation:

$$K = \frac{\text{In } OD_1 - \text{In } OD_2}{t_2 - t_1}$$

Where $OD_1$ and $OD_2$ are the optical density at time $t_1$ and $t_2$ respectively.

**Haemagglutination antibody (HA) titer**

For this test, the grouping of animals and drug treatments were carried out in similar way as describe in CCT for 7 days. The MEMJ tuber was administered orally on three days before and three days after of immunization including the day of immunization (i.e -3, -2, -1, 0, +1, +2, +3). All the mice of different groups were immunized with 0.1 ml of 20% SRBC in normal saline i.p. and challenged on day 0. On day 7$^{th}$ blood was withdrawn from retro-orbital plexus from all antigenically sensitized and challenged mice respectively. Blood was centrifuged to separate serum. Antibody levels were determined by the haemagglutination technique. From each group the equal volumes of individual serum samples were pooled. Two fold serial dilutions with pooled serum samples were prepared in 25 µl volumes of normal saline in microtitration plates were added to 25 µl of 1% suspension of SRBCs in saline. The plates were incubated at 37°C for one hour and then observed for haemagglutination under microscope. The highest dilution giving haemagglutination was taken as the antibody titre [12,13,14,17].

**Delayed type hypersensitivity (DTH) response:**

In this method the drug treatment was provided exactly the same as HA titre technique. On the day 0 the animals of different groups from I to V (n=6 in each group) were immunized with SRBCs (0.1ml of 20% SRBC i.p.) in normal saline. On 8$^{th}$ day the thickness of the right hind footpad was measured by using a vernier caliper and animals were challenged with 0.03 ml of 20% SRBCs in subplantar region of right hind paw. After 8, 24 and 48 hrs of challenge the footpad thickness was measured. The difference between the pre- and post challenge footpad thickness were calculated to measure the DTH response was expressed in mm and the mean values of all treatment groups were compared with that of control group [15,3].

**Statistical analysis**

Results were expressed as mean value ± SEM. Data were analyzed incorporating GraphPad Prism 5 software and all statistical comparison were made using one way analysis of variance (ANOVA) followed by Dunnet's test post hoc analysis. Results were articulated where P values <0.05, was considered statistically significant as compared to control group.

## RESULT AND DISCUSSION

**Preliminary phytochemical screening:**

The preliminary phytochemical screening of different extract *Mirabilis jalapa* tuber showed the presence of phytocostituents like alkaloids, carbohydrate, glycosides, Steroids, saponins, phenolics, flavonoids. (Table 1).

**Table 1: Phytochemical Screening of various extracts of *Mirabilis jalapa* tuber**

| Bioactive Constituents | Pet. Ether | Benzene | Diethyl Ether | Chloroform | Acetone | Eth. Acetate | Methanol |
|---|---|---|---|---|---|---|---|
| Alkaloids | – | – | – | – | – | – | + |
| Flavonoids | – | – | – | + | + | + | + |
| Saponins | – | – | – | – | + | + | – |
| Carbohydrate | – | – | – | + | + | + | + |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Glycosides | – | – | – | + | + | + | + |
| Steroids | – | – | – | + | + | + | + |
| Phenolics | – | – | – | + | + | + | + |
| Terpenoids | – | – | – | – | – | – | – |
| Proteins | – | – | – | – | – | – | – |

*Indications:* [+] denotes present; [-] denotes absent

**Acute toxicity study**

The methanolic extract of *Mirabilis jalapa* tuber did not showed any toxic effects or death up to dose of 5000 mg/kg body wt.

**Neutrophil adhesion test**

The rate of percentage of neutrophil adhesion in animals with control group was found to be 14.37±1.01, while MEMJ-treated groups dosing from 200 to 400 mg/kg revealed significant increase in neutrophil adhesion (26.89±2.99 and 27.16±1.24 respectively) as compared to control where as dose 100 mg/kg bw was observed less significant (24.89±1.34) compared to high doses. (Table 2, Fig. 2)

**Table 2: Effect of Methanolic extract of *Mirabilis jalapa* (MEMJ) tuber on carbon clearance assay, HA titre test and % of Neutrophil adhesion test.**

| Group | Treatments (Dose) | % of neutrophil Adhesion | carbon clearance assay | HA titre |
|---|---|---|---|---|
| I | Control | 14.37±1.01 | 0.0113±0.0019 | 79.4733±5.1884 |
| II | Std | 38.55±2.31*** | 0.0304±0.0013*** | 482.7433±12.7394*** |
| III | 100mg/kg (MEMJ) | 24.89±1.34* | 0.0176±0.0005ns | 129.3700±4.5133** |
| IV | 200mg/kg (MEMJ) | 26.89±2.99** | 0.0196±0.002* | 236.4800±9.2033*** |
| V | 400mg/kg (MEMJ) | 27.16±1.24** | 0.0245±0.0027** | 297.0733±6.9503*** |

Values are mean±SEM, (n=6). One way ANOVA followed by Dunnett's test *$p$<0.05, **$p$<0.01, ***$p$<0.001 significant; ns = not significant when compared with control group.

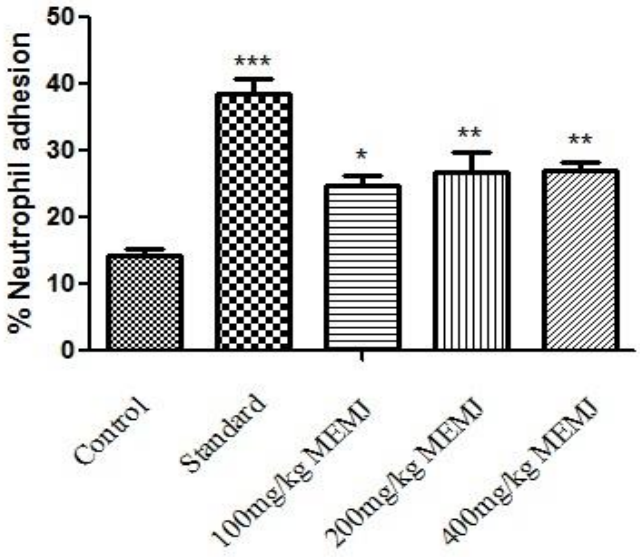


**Fig. 2: Effect of % of Neutrophil adhesion against treated groups**

Values are mean±SEM, ($n$=6). One way ANOVA followed by Dunnett's test *$p$<0.05, **$p$<0.01, ***$p$<0.001 significant; ns = not significant when compared with control group.

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**Carbon Clearance test:**

Enhance in phagocytic activity is considered when the removal of carbon is faster from blood circulation. The result was showed that MEMJ tuber treated groups with dose ranging from 200 to 400 mg/kg showed the significant increased of phagocytic index (0.0196±0.002 and 0.024 ±0.0027 respectively) when compared to control group (0.0113±0.0019) but it was observed that at the dose of 100 mg/kg no significant increased in phagocytic index (0.0176±0.0005). (Table 2, Fig. 3).

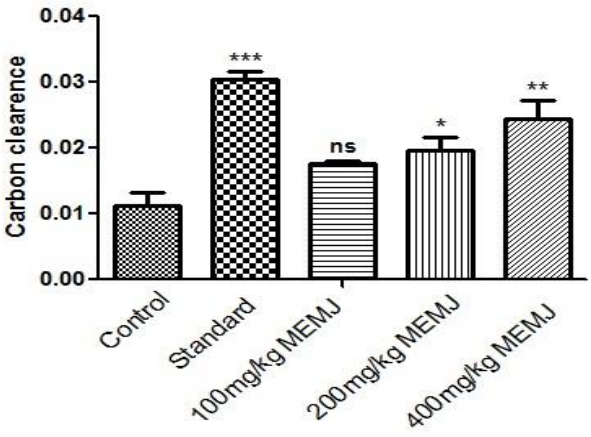


**Fig. 3: Effect of carbon clearance against treated groups**

Values are mean±SEM, (*n*=6). One way ANOVA followed by Dunnett's test **p*<0.05, ***p*<0.01, ****p*<0.001 significant; ns= not significant when compared with control group.

**Haemagglutination antibody (HA) titer**

Haemagglutination antibody titer was determined to establish the humoral response against SRBC. Oral administration of MEMJ tuber showed significant rise in HA titre after 1 hour of incubation with SRBCs when compared to control group. MEMJ with different doses, i.e. 100, 200 and 400 mg/kg respectively (129.3700±4.5133, 236.4800±9.2033 and 297.0733±6.9503 respectively) shown high significant in HA titre where as control group showed 79.4733±5.1884 (Table 2, Fig. 4) which signified the immunostimulation property of extract through humoral immunity.

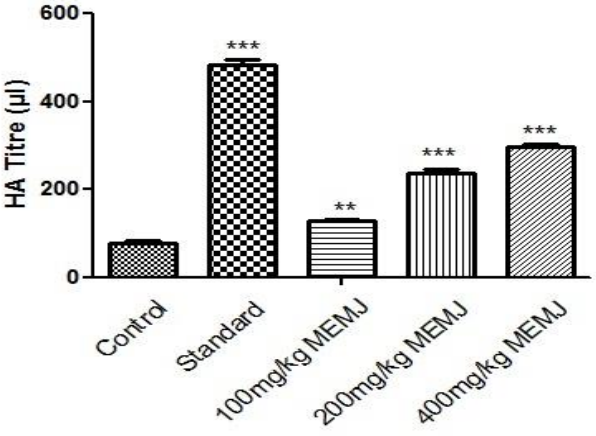


**Fig. 4: Effect of HA titre against treated groups**

Values are mean±SEM, (*n*=6). One way ANOVA followed by Dunnett's test **p*<0.05, ***p*<0.01, ****p*<0.001 significant; ns = not significant when compared with control group.

**Delayed type hypersensitivity (DTH) response:**

DTH reaction, i.e. foot pad reaction was carried out to evaluate the cell mediated immune response of MEMJ tuber. As shown in Table 3, the MEMJ tuber at high doses, i.e. 200 and 400 mg/kg bw respectively produced a significant dose-related increase in DTH reactivity in rats when compared with control group. The thickness of right foot pad was measured after 8, 24 and 48 hrs. The experimental study proved that the enhancement of DTH reaction in mice by SRBC which established the stimulatory effect of methanolic extract. (Table 3, Fig 5, 6 and 7).

**Table 3: Effect of Methanolic extract of *Mirabilis jalapa* (MEMJ) tuber on DTH response using SRBCs as an antigen in rats**

| Group | Treatments (Dose) | Mean Right footpad thickness (mm) | | |
|---|---|---|---|---|
| | | After 8 hrs | After 24 hrs | After 48 hrs |
| I | Control | 0.0617±0.0031 | 0.0635±0.0316 | 0.0639±0.0305 |
| II | Std | 0.1200±0.0221$^{*}$ | 0.4350±0.0473$^{***}$ | 0.3367±0.0390$^{***}$ |
| III | 100mg/kg (MEMJ) | 0.0817±0.0166$^{ns}$ | 0.2083±0.0199$^{*}$ | 0.1783±0.0180$^{*}$ |
| IV | 200mg/kg (MEMJ) | 0.0833±0.0161$^{ns}$ | 0.2800±0.0169$^{***}$ | 0.2183±0.0255$^{**}$ |
| V | 400mg/kg (MEMJ) | 0.1083±0.0095$^{ns}$ | 0.3233±0.0329$^{***}$ | 0.2717±0.0325$^{***}$ |

Values are mean±SEM, (n=6). One way ANOVA followed by Dunnett's test *$p$<0.05, **$p$<0.01, ***$p$<0.001 significant; ns = not significant when compared with control group.

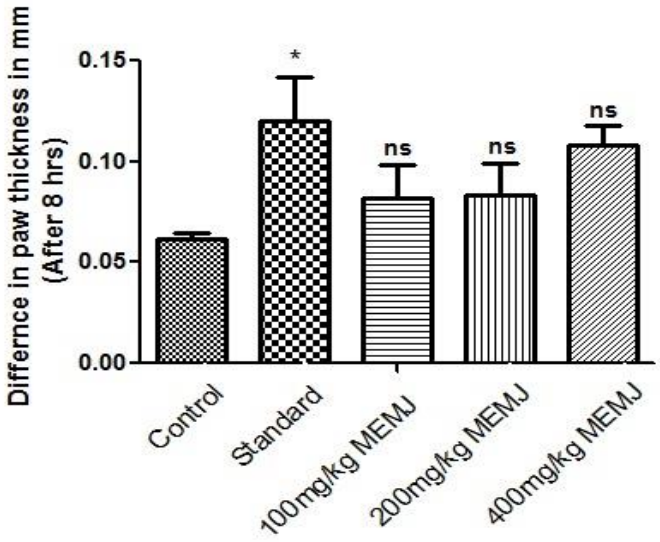


**Fig. 5: Effect of DTH reaction after 8hrs against treated groups**

Values are mean±SEM, ($n$=6). One way ANOVA followed by Dunnett's test *$p$<0.05, **$p$<0.01, ***$p$<0.001 significant; ns = not significant when compared with control group.

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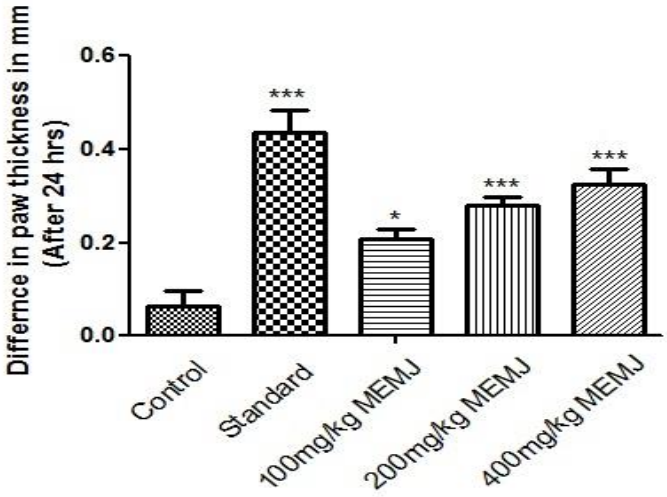


**Fig. 6: Effect of DTH reaction after 24hrs against treated groups**

Values are mean±SEM, (*n*=6). One way ANOVA followed by Dunnett's test **p*<0.05, ***p*<0.01, ****p*<0.001 significant; ns = not significant when compared with control group.

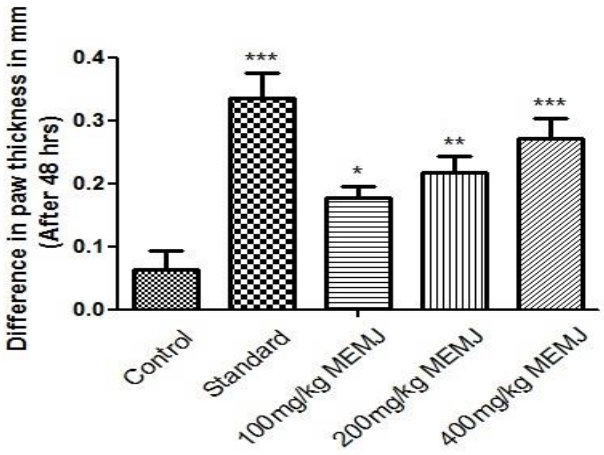


**Fig. 7: Effect of DTH reaction after 48hrs against treated groups**

Values are mean±SEM, (*n*=6). One way ANOVA followed by Dunnett's test **p*<0.05, ***p*<0.01, ****p*<0.001 significant; ns = not significant when compared with control group.

In this study the traditional claim of *Mirabilis jalapa* for its medicinal value was proved scientifically. Phytochemical screening of methanolic extract of *Mirabilis jalapa* tuber showed the presence of flavonoids, phenolic compounds which might be responsible for the activity.

Neutrophils play the vital role in innate immune system. It circulates in the blood around the body and they signal if an infectious agent is present, they are the first cells to move to the site of the infection to start to kill the microorganism by formation of oxygen radicals [16,17]. In the present study, methanolic extract of *Mirabilis jalapa* tuber inducing a significant increase in neutrophils to nylon fibres, which correlates the increase in percent neutrophils at high dose (200 and 400 mg/kg, p.o.). This may potentially help in increasing immunity of body against microbial infections. The initial focus of the greater part of the immunomodulatory compound is accepted to be macrophages which have a major role in modulating the immune system [18]. To evaluate the effect on reticuloendothelial cell mediated phagocytosis, the carbon clearance assay was performed [19]. At the point when colloidal ink containing carbon particles are infused specifically into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is coordinated by an exponential condition is known as phagocytic index [20]. Methanolic extract of *Mirabilis jalapa* tubers at dosing 200 and 400mg/kg enhanced the phagocytic capacity by showing the improvement in

the clearance rate of carbon by the cells of the RES. Heamagglutination antibody titer was determined to establish the humoral response against SRBC. Antibody particles, a result of B lymphocytes and plasma cells, are fundamental to humoral insusceptible reactions; IgG and IgM are the significant immunoglobulins which are included in the supplement enactment, opsonization, balance of toxins and so forth. Humoral immunity to SRBCs increased by administration of methanolic extract of *Mirabilis jalapa* tubers, which is proved by rising up the antibody titre in animals (Table 2) also signify the role of T and B lymphocyte in the antibody synthesis [21]. The high values of haemagglutinating antibody titre obtained in the case of MEMJ tuber have shown that increase in humoral immune response is directly proportionate to immunostimulation. Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). CMI reactions are vital to defense against infectious agents, infection of foreign matters and delayed-type hypersensitivity reactions. Delayed hypersensitivity is a major mechanism of defense against various intracellular pathogens, including mycobacteria, fungi, and certain parasites, and it occurs in transplant rejection and tumor immunity [22]. Therefore, enhance the DTH response in mice in light of Immune system microorganism subordinate antigen uncovered the stimulatory impact of methanolic extract of *Mirabilis jalapa* tuber on white blood cells.

## CONCLUSION

The present study concluded that the immune response was increased in methanolic extract of *Mirabilis jalapa* tuber might be due to flavonoids content in the tuberous part. Therefore the study suggested that the methanolic extract is a potent immunostimulant on both specific and nonspecific immune mechanisms. So, the methanolic extract has therapeutic potential, thus it might be served as an effective natural immunomodulatory agent.

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