Original Article

Immunomodulatory Activity for Methanolic Extract of *Trigonella foenum graecum* Whole Plant in Wistar Albino Rats

Sneha J Anarthe*, Sunitha, D. Sandhya Rani and M. Ganga Raju

Department of Pharmacology Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad-500090, India

ABSTRACT

The present study was undertaken to evaluate the immunomodulatory activity for methanolic extract of *Trigonella foenum graecum* whole plant with various models as Drug induced myelosuppression, T- cell population, Haemagglutination titer, Delayed hypersensitivity and Carbon clearance at different doses as 100 mg/kg, 300 mg/kg and 500 mg/kg b.w. In drug induced myelosuppression, methanolic extract of *Trigonella foenum graecum* showed restoration of haematological parameters as compared to Azathioprine (negative control) treated group. In T-cell population, methanolic extract of *Trigonella foenum graecum* has shown significant increase in lymphocytes and T-cell rosettes formation when results were compared with standard (Levamisole). At doses 300 mg/kg and 500 mg/kg methanolic extract of *Trigonella foenum graecum* has shown significant potentiation of DTH response after 24h challenge. Methanolic extract of *Trigonella foenum graecum*, has also showed significant increase in antibody titer as compared with standard. In carbon clearance test, the phagocytic index was significantly increased for methanolic extract of *Trigonella foenum graecum*. Thus methanolic extract of *Trigonella foenum graecum* whole plant has showed significant immunostimulatory activity.

Keywords: Immunomodulators, *Trigonella foenum graecum,* Drug induced myelosuppression, Delayed hypersensitivity, T-cell population, Haemagglutination titer, Carbon clearance.

Address for Correspondence

Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India.
E-mail: sneha.pharma@yahoo.co.in

INTRODUCTION

The immune system is involved in the etiology, as well as pathophysiology mechanism of many diseases. Modulation of immune responses to alleviate various diseases has been of interest for many years[1]. Medicinal plants are rich sources of substances which are non-specific immunomodulation of essentially

granulocytes macrophages, natural killer cells and complement functions[1]. Because of the concerns about the side effect of conventional drugs, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades[2].

Medicinal plants as garlic, ginger, mustard, red chili, and fenugreek used as functional foods for instances[3]. Apart from therapeutic properties of various plants like *Viscum album, Panax ginseng, Asparagus racemos, Tinospora cordifolia* etc[4]. also shows immunomodulatory activity (humoral and cell-mediated immunity) while others activate only the cellular components of the immune system, i.e. the phagocytic function, without affecting the humoral or cell-mediated immunity eg., *Prunus amygdalus* (Almond) *and Buchanania lanzan* (Chirronji) significantly stimulated both cell mediated and humoral immunity as *Euryale ferox* (Tel makhana), *Phoenix dactylifera* (Chhohara) *and Zingiber officinale* (Sonth), however, stimulated humoral immunity to a greater extent than CMI. Some of these plants also suppress both humoral and cell-mediated Immunity e.g., *Lagenaria siceraria*[5].

Fenugreek an annual herb belonging to the family Fabaceae commonly known as 'Menthi'. Indigenous to the countries on the eastern shores of the Mediterranean and mostly cultivated in India, Africa, Egypt, Morocco, and occasionally in England. Fenugreek possesses many medicinal properties[6]. Fenugreek is a natural source of iron, silicon, sodium and thiamine and contains mucilagins which are known for soothing and relaxing inflamed tissues. Fenugreek is one of the oldest medicinal plants. Used externally, it can help curing abscesses, boils, carbuncles, fistulas, sciatica, various skin irritations, sores and wounds. Fenugreek seeds and leaves are strongly aromatic and flavorful. Seeds are bitter in taste but lose their bitterness if roasted slightly. They are rich in vitamins such as thiamin, folic acid, riboflavin, niacin, vitamins A, B_6, and C, and are a storehouse of minerals such as copper, potassium, calcium, iron, selenium, zinc, manganese, and magnesium[7].

Present study is an attempt to find out the immunomodulatory activity of *Trigonella foenum graecum* whole plant.

MATERIALS AND METHODS

Plant material collection and extract preparation

Trigonella foenum graecum whole plant was collected from local market Hyderabad, Telangana (India) in the month of January, 2014 and was identified and authenticated from Botany department of Osmania University Hyderabad. The plant material was cleaned, made into small pieces, dried under sun and coarsely powdered and stored. The coarsely powdered plant material (500g) was subjected to extraction with methanol using simple distillation. The extract was concentrated to semisolid mass and stored in air tight containers.

Animals used

Wistar albino rats (Approx 150 to 180 g) were procured from Gentox Bioservices, Hyderabad. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India (Reg. No.1175/ac/08/CPCSEA).

Antigens

Sheep Red Blood Cells (SRBC) were collected in Alsever's solution from NIN slaughter house Hyderabad, India. SRBC were washed 3-4 times with large quantity of sterile and pyrogen free saline[8].

Acute toxicity studies

The methanolic extract of *Trigonella foenum graecum* whole plant was tested for acute toxicity studies as per procedure given in OECD guidelines 425 and limit test method was followed. Mice were starved for 4h and fed orally with MeOH extract of *Trigonella foenum graecum* at doses 2000 and 5000 mg/kg b.w. animals were observed for 14 days for mortality.

Quantitative estimation

Estimation of Total phenolic and flavonoid content

Total phenolic content of methanolic extracts of *Trigonella foenum graecum* was determined by using Folin-Ciocalteu reagent[9]. The blue color formed due to the polyphenol content in the extract was measured at 760 nm. Total soluble phenolic of methanolic extract was determined with Folin-ciocalteu reagent. 1mL of extract solution in the test tube was added to 0.2 mL of Folin Ciocalteu reagent (1:2 in distilled water) and after 20 min, 2 mL of purified water and 1 mL of sodium carbonate (15%) was added. Allowed to react for 30 min and absorbance was measured at 765 nm.

The concentration of total phenolic component in the extract was determined as microgram of Gallic acid equivalent. Total flavonoid of methanolic extract was determined using the method of Liu *et al*[10] with some modifications. In brief, the extract was diluted with 80% aqueous ethanol (0.9 mL). Aliquots of 0.5 ml of extract were added to test tube containing 0.1 ml of 10% aluminum nitrate, 0.1 ml 1M aqueous potassium acetate and 4.3 ml of 80% ethanol. The reaction tubes were set aside for 40 min at room temperature. At the end of this time, optical density of each sample was determined at 415 nm using a UV spectrophotometer. Total flavonoids content was calculated by interpolation on a standard curve established with a reference standard, quercetin. Quercetin and Folin-Ciocalteu reagent were obtained from Sigma-Aldrich, Germany.

In-vitro antioxidant assays

In-vitro antioxidant assays for methanolic extract of *Trigonella foenum graecum*: DPPH radical scavenging activity

The hydrogen donating ability of extracts was examined in the presence of DPPH stable radical. One milliliter of 0.3 mM DPPH methanolic solution was added to 2.5 mL of test solution of different concentrations and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 517 nm. Methanol (1.0 mL) and plant extract solution (2.5 mL) was used as blank, DPPH solution (1.0 mL, 0.3 mM) and methanol (2.5 mL) served as negative control. Ascorbic acid was used as standard[11].

NBT reduction assay

A reaction mixture (3mL) per tube was prepared with 1.4 mL of 50 mM KH_2PO_4-KOH pH 7.4 containing 1mM EDTA, 0.5 mL of 100 µM hypoxanthine, 0.5mL of 100µM NBT. The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100µL of phosphate buffer and 0.5 mL of test extract in saline. The subsequent rate of NBT reduction was determined by spectrophotometric method at 560nm. Ascorbic acid was used as standard. The results were expressed as the percentage inhibition of NBT[11].

In vivo models

Experimental protocol

The experiments was done according to the CPCSEA guidelines and approved by the Institutional Animal Ethical Committee.

The methanolic extract was dissolved acacia in distilled water and the dose was selected at 100, 300 and 500 mg/kg body weight. The methanolic extract was administered to animal for 14 days by oral gavage. Albino rats were divided into groups comprising of six animals each.

Group I served as control and was administered vehicle only.

Group II received standard drug (Immunostimulant) Levamisole.

Group III received standard drug (Immunosuppressant) Azathioprine acts as negative control.

Group IV received 100 mg/ kg b.w. methanolic extract of *Trigonella foenum graecum.*

Group V received 300mg/ kg b. w. of methanolic extract of *Trigonella foenum graecum.*

Group VI received 500 mg/kg b. w. of methanolic extract of *Trigonella foenum graecum.*

Delayed type hypersensitivity

Antigen Challenge: On 0th day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, i.p. Animals were divided into different groups each containing 6 animals each.

Group I - Control, 1% Gum acacia suspension in saline.

Group II - Standard, Levamisole, 50 mg/kg b.w, p.o (1st to 7th day).

Group IV, V, VI – methanolic extract of *Trigonella foenum graecum* at doses 100, 300, and 500 mg/kg b.w, p.o respectively (1st to 7th day).

On 7th day prior to injection, right hind footpad thickness was measured with Micrometer screw gauge (Mitutoyo Digimatic). Then animals were challenged by injecting 1% SRBC (20 µL) into the right hind footpad. On 8th and 9th day footpad thickness of animals was again measured and mean difference of pre and post was calculated and reported[12].

Antibody (HA) titre response to SRBC

On 0th day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, i.p. Animals were divided into different groups each containing 6 animals each.

Group I - Control, 1% Gum acacia suspension in saline.

Group II - Standard, Levamisole, 50 mg/kg b.w, p.o (1st to 7th day).

Group IV, V, VI – methanolic extract of *Trigonella foenum graecum* at doses 100, 300, and 500 mg/kg b.w, p.o respectively (1st to 7th day).

On 7th day before challenge, blood was withdrawn from retro-orbital plexus of each animal. Blood was centrifuged, and serum was separated. Serial two fold dilutions were made (50 µL of serum was added to 1st well of 96-well micro titre plate containing 50 µL normal saline. 1% SRBC (50 µl) dissolved in normal saline was added to 1st well, 50 µl of diluted serum was added to 2nd well containing 50 µL normal saline and 50µL 1% SRBC. Such serial dilutions were done till 24th well. Plates were incubated at 37ºC for 1 h., highest dilution that has shown visible agglutination was considered as haemagglutination antibody[13].

Carbon clearance assay (Phagocytic activity)

Antigen Challenge: On 0th day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, i.p. Animals were divided into different groups each containing 6 animals.

Group I - Control, 1% Gum acacia suspension in saline.

Group II - Standard, Levamisole, 50 mg/kg b.w, p.o (1st to 8th day).

Group IV, V, VI – methanolic extract of *Trigonella foenum graecum* at

doses 100, 300, and 500 mg/kg b.w, p.o respectively (1st to 8th day).

Carbon clearance test was performed after completion of the drug pretreatment. Animals were stabilized for 2 days (9th day and 10th day). On 11th day, the treated rats received an intravenous injection (tail vain) of carbon suspension (1:50 dilution of Indian ink, Camel) in a dose of 0.5 mL/100 g body weight[14]. Blood was withdrawn from the retro-orbital venous plexus before injection, at 5 min and 15 min after injection of carbon suspension. 0.05 mL of blood was lysed with 4 mL of 0.1% Na_2CO_3 and the optical density was measured spectro-photometrically at 650 nm wavelength.

The phagocytic index K was calculated using the following equation.

$K = \log (OD_0) - \log (OD_t)/t$ Where OD_0 is the OD at 0 min and OD_t is the OD at t min[15].

Drug induced myelosuppression

Antigen Challenge: On 0th day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, i.p. Animals were divided into different groups each containing 6 animals each.

Group I - Control, 1% Gum acacia suspension in saline.

Group II - Standard, Levamisole, 50 mg/kg b.w, p.o (1st to 13th day).

Group III - Negative control, Azathioprine 3 mg/kg b.w, p.o (11th, 12th, 13th day).

Group IV, V, VI – methanolic extract of *Trigonella foenum graecum* at doses 100, 300, and 500 mg/kg b.w, p.o respectively (1st to 13th day).

On 0th day, blood was withdrawn from retro-orbital plexus of animals of each group and subjected to haematological parameter determination. Methanolic extract of *Trigonella foenum graecum* was administered upto 13 day. Azathioprine (3 mg/kg, b.w) is given to all animals on 11th, 12th and 13th day, 1 h after extracts administration except control and standard group. On 14th day, blood was withdrawn from retro- orbital plexus of animals of each group and subjected to hematological parameters determination and restoration of parameters were observed[16].

T-cell population

Antigen Challenge: On 0th day, all groups were sensitized with 0.1 mL of SRBC containing 1×10^8 cells, i.p. Animals were divided into different groups each containing 6 animals.

Group I - Control, 1% Gum acacia suspension in saline.

Group II - Standard, Levamisole, 50 mg/kg b.w, p.o (1st to 10th day).

Group IV, V, VI – methanolic extract of *Trigonella foenum graecum* at doses 100, 300, and 500 mg/kg b.w, p.o respectively (1st to 10th day).

On 11th day, blood was collected from the retro-orbital plexus and anticoagulated with Alsever's solution in separate test tubes. Test tubes containing blood were kept in sloping position (45°) at 37^oC for 1 h. RBCs were allowed to settle at bottom and supernatant was collected from each test tube by using micropipette which contains lymphocytes. 50 µL of lymphocyte suspension & 50 µL SRBC were mixed in test tube and incubated. Resultant suspension was centrifuged at 200 rpm for 5 min and kept in a refrigerator at 4^0C for 2 h. The supernatant fluid was removed and one drop of cell suspension was placed on a glass slide. Total lymphocytes were counted and a lymphocyte binding with three or more erythrocytes was considered as rosette and number of rosettes was counted[17].

Statistical analysis

Graph Pad prism 3 software and MS Excel was used for statistical analysis of data. All the results were expressed as

mean± standard error of mean (S.E.M.), analyzed for ANOVA and post hoc Dunnet's t-test (Multiple). Differences between groups were considered significant at $p<0.05$, $p<0.01$ levels.

RESULTS

Acute toxicity studies

No behavioural changes and no mortality were observed for methanolic extract of *Trigonella foenum graecum* upto 5000 mg/kg b.w. after 14 day. Methanolic extract was evaluated for total phenolic and flavonoid content. The concentration of total phenolic and total flavonoid content in the methanolic extract was found to be 31µg % and 81µg % respectively.

Methanolic extract of *Trigonella foenum graecum* was evaluated for *in vitro* antioxidant assay with DPPH radical scavenging assay and NBT inhibition assay. Methanolic extract of *Trigonella foenum graecum* has shown significant *in-vitro* antioxidant activity with DPPH assay and NBT inhibition assay with IC_{50} 5.4 µg/mL and 32.4 µg/mL when results were compared with standard as ascorbic acid (3.85 µg/mL) and gallic acid (3 µg/mL) respectively (Table 1, 2).

Haemagglutination titer test

In Haemagglutination titer test, agglutination was compared with control,. Group-IV (100 mg/kg b.w.), Group-V (300 mg/kg b.w.) and Group-VI(500 mg/kg b.w.) treated with methanolic extract of Fenugreek 100 mg/kg, 300 mg/kg, 500 mg/kg shown agglutination titer in X:16, X:32 dilutions, X: 64, X:156 dilutions, X:256, X:520 dilutions respectively (Table 3).

Drug induced myelosuppression

Azathioprine treatment for the period of 3 days showed significant reduction in Hb and WBC count ($p<0.01$) and thereby exerted immunosuppressant effect when compared to control animals. Combined treatment of extracts and myelosuppressive drug at all doses showed restoration of WBC count when compared to azathioprine treated groups. Levamisole, a standard immunomodulatory drug, has not shown marked difference in Hb and RBC count and WBC count. Hematological parameters were restored in all treated group ($p<0.01$) (Table 4, 5, 6).

Carbon clearance

The significant increase in Phagocytic index was observed at all doses (100 mg/kg, 300 mg/kg, 500 mg/kg b.w.) compared with standard ($p<0.01$) (Table 7).

Delayed hypersensitivity

The result indicates that there was significant decrease in the foot paw thickness at doses of 100 mg/kg ($p<0.01$), 300 mg/kg ($p<0.01$) and 500 mg/kg ($p<0.05$) methanolic extract of treated group when compared against normal control and standard. All the results are shown in terms of mean difference, in the foot paw thickness, when compared against control and standard. The drug influences cell mediated immune response in dose dependent manner (Table 8).

T- cell population

In Present study increasing doses has showed significant increase in lymphocytes and rosettes when results were compared with control. At dose 300 mg/kg, significant increase in lymphocytes ($p<0.05$) was observed when compared with standard, at dose 500mg/kg showed ($p<0.05$) increase in rosettes and lymphocytes (Table 9, 10).

DISCUSSION

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune

reactions it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immuno-suppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factor[18].

Antibody production to T-dependent antigen SRBC requires co-operation of T and B-lymphocytes and macrophages. The high values of haemagglutinating antibody titer obtained in case of methanolic extract of fenugreek whole plant have indicated that immunostimulation was achieved through humoral immunity[19]. In the present study the methanolic extract of fenugreek whole plant may be capable to influence the role of immunoglobulins results activation of pre B cells and/or dendritic cells results in activation of antibodies which give the higher agglutination titer against SRBC's antigens.

Increase in rosette formation and lymphocyte formation in T-cell population test indicate effect of methanolic extract of fenugreek whole plant on cell mediated immunity. Methanolic extract of fenugreek showed dose dependant immunomodulatroy activity. Methanolic extract of fenugreek may activate the CD4 and CD8 cells which influence T-cell mechanism results increase in T-cell immune response significantly. The R.E.S. is the best defined functionally by its ability to scavenge debris or other foreign matter and forms first line of defense. The rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen, from the bloodstream is a measure of reticuloendothelial phagocytic activity[20]. In carbon clearance test, methanolic extract treated groups, exhibited significantly high phagocytic index. This indicates stimulation of the reticulo-endothelial system by methanolic extract of fenugreek. Methanolic extract of fenugreek may influence the mechanism of phagocytosis, largely distributed monocytes macrophages or R.E.S. which result in significant increase in the phagocytic index with carbon clearance test. DTH reaction is antigen specific and causes erythematic and indurations at the site of antigen injection in immunized animals when encountered with activated Th1 (T-helper cells) cells by certain antigens, via SRBC. DTH comprises of two phases, an initial sensitization 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 antigen induces DTH response, where Th1 cells secrete a variety of cytokines and other non specific inflammatory mediators[21]. Myelosuppression is a decrease in the production of blood cells[22]. Azathioprine immunosuppressive agent which act at various levels on cells involved in defense mechanism against various invaders by inhibiting both cell mediated and humoral immunity[14]. Azathioprine significantly decreases the Hb, RBC, and WBC counts. Azathioprine treatment for the period of 3 days showed significant reduction in Hb, WBC count and RBC count and thereby exerted immunosuppressant effect when compared to control animals. Combined treatment of extracts and myelosuppressive drug at all doses showed restoration of all haematological parameters ($p<0.01$) when compared to azathioprine treated groups. Levamisole, a standard immunomodulatory drug, has not shown marked difference in Hb and RBC count, but WBC count was increased.

CONCLUSION

The results obtained in the present study conclude that *Trigonella foenum graecum* whole plant is a potent immunostimulant, stimulating specific and non-specific immune mechanisms. It may be due to the presence of various phytoconstituents present in *Trigonella foenum graecum* like phenolics, flavonoids, tannins, and alkaloids, are already reported to possess immunomodulatory activity.

REFERENCES

1. Sharma P. Charaka Sanhita, Chikitsasthana. Chaukhamba Orientalia, Varanasi (1983) 2.
2. Fong HH. Integration of herbal medicine into modern medical practices: issues and prospects. Integr: *Cancer Ther*. (2002)1: 287-293.
3. Singh Virendra Kumar, Sharma Pramod Kumar, Dudhe Rupesh, Kumar Nitin. "Immunomodulatory effects of some traditional medicinal plants". *J. Chem. Pharm. Res*., 2011, 3(1):675-684.
4. Sainis KB, Sumariwalla PF, Goel A, Chintalwar GJ, Sipahimalani AT and Banerji A. "Immunomodulatory properties of stem extracts of *Tinospora cordifolia*: cell targets and active principles". In: Upadhyay SN. (ed) Immnunomodulation. Narosa Publishing house, New Delhi (1997) 95.
5. K. Gulati, A. Ray, P. K. Debnath, S. K. Bhattacharya "Immunomodulatory Indian medicinal plants". *J. Nat. Remedies*. 2002:2 (2):121–131.
6. Rashmi yadav, Rahul Kaushik, Dipeeka Gupta. "The Health Benefits of *Trigonella foenum- graecum:* A Review". *Int. J. Eng. Res. App.*1 (1): 032-035.
7. Atefeh Sheikhlar, 2013. "*Trigonella foenum-graecum* L. (Fenugreek) as a Medicinal Herb in Animals Growth and Health". *Sci. Int*. 1:194-198.
8. Youcef Necib, Ahlem Bahi, Sakina zerizer, Cherif Abdennour and Mohamed Salah Boulakoud. "Immunomodulatory activity of argan oil (*Argania spinosa l*)*" Am. J. Immunol*. 2013; 9 (3): 85-87.
9. Maryam Zahin, Farrukh Aqil and Iqbal Ahmad. "The *In vitro* antioxidant activity and total phenolic content of four Indian medicinal plants". *Int. J. Pharm. Pharm. Sci*. 2009; 1(1):88-95.
10. Liu F, Ng TB. "Anti oxidative and free radical scavenging activities of selected medicinal herbs". *Life Sci*. 2000; 6: 725–735.
11. Bisht.D.S. Padalia R.C., Joshi S.C., Tewari A., Mathela C.S. "Constituents of *Nepeta clarket hook. f.* and their antioxidant activity". *Ind. J. Chem*., 2010; 49(B): 807-811.
12. Switi B. Gaikwad, Dr. G. Krishna Mohan, Kavitha J. Reddy. "*Moringa oleifera* leaves: Immunomodulation in wistar albino rats". *Int. J. Pharm. Pharm*. Sci. 2011; 3(5):975-1491.
13. S. Sathianarayanan, A. Rajasekaran. "Immunomodulatory activity of ethanolic extract of *Wrightia tinctoria* leaves". *Int. J. Pharm. Pharm. Sc*i. 2012; 4(4):975-1491.
14. Ziauddin, Neeta Phansalkar, Pralhad Patki, Sham Diwanay D, Bhushan Patwardhan. "Studies on the immunomodulatory effects of Ashwagandha" *J. Ethnopharmacol*. 1996; (50): 69-76.
15. Yashraj Yadav, P.K Mohanty and S B Kasture. "Evaluation of Immunomodulatory activity of hydroalcoholic extract of *Quisqualis indica* Linn. Flower in wistar rats". *Int. J. Pharm. Life Sci*.2011; 2(4): 976-7126.
16. Singh MP, Ahirwar J, MuthaL N. "Evaluation of Immunomodulatory activity of aqueous extract of *Ficus bengalensis* aerial roots in wistar rats": *Asian. J Pharm. Clini. Res*., 2011; 4(1):974-2441.
17. Youcef Necib, Ahlem Bahi, Sakina zerizer, Cherif Abdennour and Mohamed Salah Boulakoud. "Immunomodulatory activity of argan oil (*Argania spinosa* L)*". Am. J. Immunol*. 2013; 9 (3): 85-87.
18. N. Makare, S. Bodhankar, V. Rangari. "Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice". *J Ethnopharmacology*, 2001; 78: 133-7.
19. Subhadip Hajra, Archana Mehta and Pinkee Pandey "Immunostimulating activity of methanolic extract of *Swietenia mahagoni* seeds". *Int. J. Pharm. Pharm. Sci*., 2012; 4 (1): 0975-1491.

20. Smriti Tripathi, Anup Kumar Maurya, Monica Kahrana, Annapurna Kaul and Ram Kumar Sahu., "Immunomodulatory activity of ethanolic extract of *Trigonella foenum graecum* leaves on mice". *Sch. Res. Lib.*, 2012; 4 (2): 708-713.
21. N.L. Dashputre, N.S. Naikwade., "Immunomodulatory Activity of Abutilon *indicum* Linn. on Albino Mice". *Int. J. Pharm. Sci. Res.*, 2010; 1(3): 178-184.
22. Bilal Bin-Hafeez, Rizwanul Haque, Suhel Parvez, Suwarna Pandey, Iqbal sayeed, S Raisuddin., "Immunomodulatory activity of aqueous extract of *Trigonella foenum graecum"*. *Int. Immunopharmacol*. 2003; 3(2): 257-265.

Table 1. DPPH radical scavenging activity for methanolic extract of *Trigonella foenum graecum*

S. No.	Test extract	Dose (µg/ml)	% inhibition	IC_{50} (µg/ml)
1	MET	2.5 5 10	25±0.33 45±1.45 80±0.88	5.4
2	Ascorbic acid	1 2.5 5	22±0.078 43±0.274 78±0.136	3.85

Values are expressed as Mean ± SEM

Table 2. NBT inhibition assay for methanolic extract of *Trigonella foenum graecum*

S. No.	Test extract	Dose (µg/ml)	% inhibition
1	MET	2.5 5 10	5±0.02 8±0.15 19±1.15
2	Gallic acid	1 2.5 5	57.44±0.005 63.40±0.008 84.09±0.005

Values are expressed as Mean ± SEM

Table 3. Haemagglutination titer test for methanolic extract of *Trigonella foenum graecum*

Group	Treatment	Dose	Antibody titre Mean ± SEM (n=6)
I	Control		17±3.21
II	Levamisole	50mg/kg	384±8.92
III	MET	100mg/kg	37±35.7$^{ns, **}$
IV	MET	300mg/kg	149±53.97$^{ns, **}$
V	MET	500mg/kg	341±57.24$^{a, ns}$

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.001, b=p<0.05), (**= p<0.01, *= p<0.05), ns- non significant.

Table 4. WBC count for methanolic extract of *Trigonella foenum graecum*

Group	Treatment	Dose	WBC count ($\times 10^3$/mm) mean ± SEM (n=6)	
			0 day	14 day
I	Control		9±0.41	9±0.14
II	Levamisole	50mg/kg	10±0.21	10±0.24
III	NC	3mg/kg	9±0.22$^{ns, ns}$	6±0.44$^{a, **}$
IV	MET+A	100mg/kg	6±0.27$^{ns, ns}$	5±0.16$^{a, **}$
V	MET+A	300mg/kg	7±0.21$^{ns, ns}$	5±0.32$^{a, **}$
VI	MET+A	500mg/kg	8±0.27$^{ns, ns}$	6±0.21$^{a, **}$

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.001, b=p<0.05), (**= p<0.01, *= p<0.05), ns- non significant.

Table 5. RBC count for methanolic extract of *Trigonella foenum graecum*

Group	Treatment	Dose	RBC count ($\times 10^6$/mm) mean ± SEM (n=6)	
			0 day	14 day
I	Control		7±0.12	7±0.08
II	Levamisole	50mg/kg	7±0.28	8±0.14
III	NC	3mg/kg	7±0.11$^{ns, ns}$	4±0.36$^{a, **}$
IV	MET+A	100mg/kg	7±0.11$^{ns, ns}$	5±0.22$^{a,, **}$
V	MET+A	300mg/kg	7±0.11$^{ns, ns}$	5±0.26$^{b, *}$
VI	MET+A	500mg/kg	7±0.16$^{ns, ns}$	6±0.13$^{ns, ns}$

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.001, b=p<0.05), (**= p<0.01, *= p<0.05), ns- non significant.

Table 6. Hb count for methanolic extract of *Trigonella foenum graecum*

Group	Treatment	Dose	Hb count (g/dL) mean ± SEM (n=6)	
			0 day	14 day
I	Control		12±0.12	11±0.15
II	Levamisole	50mg/kg	12±0.21	12±0.33
III	NC	3mg/kg	11±0.22[ns, ns]	7±0.44[a, **]
IV	MET+A	100mg/kg	11±0.17[a, *]	10±0.14[b, ns]
V	MET+A	300mg/kg	11±0.28[a, ns]	10±0.24[a, **]
VI	MET+A	500mg/kg	12±0.18[ns, ns]	11±0.21[ns, ns]

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.001, b=p<0.05), (**= p<0.01, *= p<0.05), ns- non significant.

Table 7. Phagocytic index for methanolic extract of *Trigonella foenum graecum*

Group	Treatment	Dose	Phagocytic index mean ± SEM (n=6)
I	Control		0.045±0.006
II	Levamisole	50mg/kg	0.073±0.005
III	MET	100mg/kg	0.049±0.006[ns, **]
IV	MET	300mg/kg	0.032±0.007[ns, **]
V	MET	500mg/kg	0.053±0.004[ns, **]

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.001, b=p<0.05), (**= p<0.01, *= p<0.05), ns- non significant.

Table 8. DTH response for methanolic extract of *Trigonella foenum graecum*

Group	Treatment	Dose	DTH Response Mean ± SEM (n=6)	
			24hrs	48hrs
I	Control		5±0.44	4±0.45
II	Levamisole	50mg/kg	8±0.55	7±0.56
III	MET	100mg/kg	5±0.39[ns, **]	5±0.56[a, **]
IV	MET	300mg/kg	7±0.38[ns, *]	5±0.41[a, **]
V	MET	500mg/kg	7±0.49[a, ns]	6±0.36[a, ns]

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.01, b=p<0.05), (**= p<0.01,*= p<0.05), ns- non significant.

Table 9. Lymphocytes for methanolic extract of *Trigonella foenum graecum*

Group	Treatment	Dose	Lymphocytes mean ± SEM (n=6)
I	Control		142±3.84
II	Levamisole	50mg/kg	208±3.68
III	MET	100mg/kg	140±5.27[ns, **]
IV	MET	300mg/kg	199±4.55[a, *]
V	MET	500mg/kg	200±8.69 [a, ns]

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.01, b=p<0.05), (**= p<0.01,*= p<0.05), ns- non significant.

Table 10. Rosettes for methanolic extract of *Trigonella foenum graecum*

Group	Treatment	Dose	Rosettes Mean ± SEM (n=6)
I	Control		10 ±0.58
II	Levamisole	50mg/kg	23±1.87
III	MET	100mg/kg	10±0.42[ns, **]
IV	MET	300mg/kg	20±1.95[a, ns]
V	MET	500mg/kg	22±1.98 [a, ns]

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.01, b=p<0.05), (**= p<0.01,*= p<0.05), ns- non significant.