International Journal of Research in Pharmacology & Pharmacotherapeutics (IJRPP)

ISSN:2278-2648

IJRPP |Volume 12 | Issue 1 | Jan - Mar – 2023
www.ijrpp.com

Research article ***Medical research***

Invitro Evaluation of Immunosuppressant Activity of *Moringa Oleifera*

V. Vahini, G. Archita, B. Kavyasri, B. Rachana, D. Kalpana, T. Nandini*, P. Chandrika

Bojjam Narasimhulu Pharmacy College, Road Number 2, Vinayak Nagar, Vani Nagar, Saroor Nagar West, Sayeedabad, Hyderabad, Telangana 500059.

***Corresponding Author: T. Nandini**
Published on: 8.3.2023

ABSTRACT

The immunosuppressive activity of the Methanol extract of bark of *Moringa Oleifera*consisting of a mixture of saponins, flavonoids, tannins, steroids, phenol and glycosides wasstudied on the immune responses in mice.Methanol extract of *Moringa Oleifera*(MO) wasadministered orally at doses of 50, 100 and 150 mg/kg/day to healthy mice divided into fourgroups consisting of six animals each.The assessment of immunomodulatory activity was carriedout by testing the humoral (antibody titre) and cellular (foot pad swelling) immune responses tothe antigenic challenge by sheep RBCs.Furthermore, the effect on hematological parameters aswell as relative organ weight was determined. On oral administration MMO showed a significantdecrease delayed type hypersensitivity (DTH) response whereas the humoral response to sheepRBCs was unaffected. Thus MMO significantly suppressed the cellular immunity by decreasingthe footpad thickness response to sheep RBCs in sensitized mice.With a dose of 100 and 150 mg/kg/day the DTH response was 8.31 ± 1.53 and 6.19 ± 2.34respectively in comparison to correspondingvalue of 15.43 ± 1.69for untreated control group.These differences in DTH response werestatistically significant ($P < 0.05$). The study demonstrates that MMO shows preferential suppression of thecomponents of cell-mediated immunity and shows no effect on the humoral immunity.

Keywords: Immunosuppressive, *Moringa Oleifera*,Delayed type hypersensitivity, Haemagglutinating antibody titre and Methanolic extract.

INTRODUCTION

Immunosuppression involves an act that reduces the activation or efficacy of the immune system. Some portions of the immune system itself have immunosuppressive effects on other parts of the immune system, and immunosuppression may occur as an adverse reaction to treatment of other conditions. Immunosuppressants are used to control severe manifestations of allergic, autoimmune and transplant-related diseases. Some drugs have a diffuse effect on the immune system while others have specific targets. Drugs with diffuse effects are more likely to cause damaging adverse effects, but the effectiveness of the more specific drugs may be reduced if their action can be bypassed by alternative metabolic pathways. Treatment protocols therefore frequently use drug combinations to minimize adverse effects and to prevent resistance to treatment. Although protocols are essential to allow scientific evaluation, the clinician must be prepared to tailor treatment based on the ongoing assessment of drug effects, disease activity and the robustness of the individual patient.[1,2] Deliberately induced immunosuppression is generally done to prevent the body from rejecting an organ transplant or for the treatment of autoimmune diseases such as rheumatoid arthritis or Crohn's disease. This is typically done using drugs, but may involve surgery (splenectomy), plasmapharesis, or radiation. Many of the currently available immunosuppressants were developed for use in oncology or transplantation. As this treatment is potentially life-saving desperate measures can be justified. However, there are now over 80 autoimmune diseases and several common allergic conditions in which immunosuppressants could play a role although they may not be life-saving. Clinically they are used to:[3]

- Prevent the rejection of transplanted organs and tissues (e.g. bone marrow, heart, kidney, liver)
- Treatment of autoimmune diseases or diseases that are most likely of autoimmune origin (e.g. rheumatoid arthritis, myasthenia gravis, systemic lupus erythematosus, Crohn's disease, and ulcerative colitis).
- Treatment of some other non-autoimmune inflammatory diseases (eg. long term Allergic Asthma control).

Cortisone was the first immunosuppressant identified, but its wide range of side effects limited its use. The more specific azathioprine was identified in 1959, but it was the discovery of cyclosporine in 1970 that allowed for significant expansion of kidney transplantation to less wellmatched donor-recipient pairs as well as broad application of liver transplantation, lung transplantation, pancreas transplantation, and heart transplantation. Some immunosuppressants act through immunodepletion of effector cells, while others are predominantly immunomodulatory, affecting the activity of cells, usually through cytokine inhibition. Immunosuppressive drugs can be classified into five groups:[4,5]

I. Glucocorticoids
II. Cytostatics
III. Antibodies
IV. Drugs acting on Immunophilins
V. Other drug

Innate and Specific immunity

The innate immune system is die first fine of defence that acts immediately to resist against infection. The innate response is characterized by a limited capacity to distinguish between infectious agents and die efficacy to respond is not improved with repeated exposure, due to the absence of memory.

The cells of the innate immune system specifically recognize pathogen-derived antigens by means of pattem-recognition receptors (PRRs), the specificity of these are highly conserved and genetically dictated. This group of receptors belong to the family of Toll-like receptors (TLRs), which recognize pathogen associated molecular patterns (PAMPs) uniquely expressed on cells of microorganisms and absent from host cells. The TLRs are type I transmembrane proteins with an intracellular domain very similar to that of the cytoplasmic domain of the interleukin (IL) -1 receptor (IL-1R) and possibly use an analogous molecular signalling pathway. EL-1 activation involves the triggering of several transcription factors, including nuclear factor kappa B (NF-kB) and c-jun/AP-1 that are crucial for the transcription of several pro-inflammatory cytokines, costimulators and chemokines.

The specific immune response displays specificity, diversity and memory that improves with repeated exposure and seltfnon-self recognition. The specific immune system is capable of recognizing billions of uniquely different structures on foreign antigens via specific receptors on the surface of T and B lymphocytes. The capacity of these cells to distinguish foreign molecules from self is learned during ontogeny. The components involved in the specific immune response include T and B lymphocytes, antigen presenting cells (APCs) and plasma cells.[6]

Lymphocytes Each B and T cell is genetically programmed to encode for a surface receptor and is responsible for the recognition of a particular antigen. Antigen receptor diversity is generated in immunoglobulin (Ig) and T cell receptor (TCR) variable regions by the process of VDJ recombination from a spectrum of germline gene segments to produce a randomly selected lymphocyte receptor with a unique specificity. In T cells subsequent thymic selection governs the composition of the peripheral TCR repertoire. Individual TCR's exhibit a certain degree of cross-reactivity therefore a single TCR can recognize multiple peptide-major histocompatibility complex (MHC) antigens and, vice versa, a single peptide-MHC can be recognized by multiple TCR's. Thus a T cell response to a given individual antigen can involve more than one TCR clonotype.

The adaptive immune response to an antigen can be divided into three phases: firstly the recognition stage, secondly a phase of activation, and finally the effector phase. The first stage involves the binding of antigens to specific B or T cell receptors. This is followed by a sequence of events occurring within the immune cell resulting in the proliferation and expansion of the activated cell and consequently the differentiation of the activated cell and its progeny into effector cells. Effector lymphocytes either directly kill infected body cells or they secrete cytokines. The cytokines act either by directly destroying pathogens or induce other cell types, such as cytotoxic T lymphocytes (CTL) and macrophages to enhance their capacity to eliminate the invading organism.

Human T cell subsets T cells are important in the recognition and eradication of a wide variety of microorgansims. Once they have been stimulated by peptide antigen presented on HLA class II DR molecules the T cells begin to produce IL-2 and clonally expand and subsequently differentiate to express new patterns of genes essential for immune effector function. The Th cells express CD4+ antigen and can differentiate into different subsets including ThO, Thl and Th2 subsets which are distinguished by the array of cytokine genes they express. It should be noted here that with the identification of novel molecules, further reclassifications of T cell subsets are possible, and the descriptions provided below are based on the current understanding of the field from several different laboratories. Additionally, for the purpose of this thesis only the CD4+ subsets will be discussed.[7, 8]

Medicinal Plants as Immunosuppressive Agents [9]

Cannabis suppresses the hyperactivity of immune system by acting on cannabinoid receptors such as cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). CB1 receptors are mostly present in the brain whereas CB2 receptors are abundant in immune cells. So, the cannabinoids are used for the treatment of cancer and inflammatory disorders.

Brasilicardin-A is isolated from the cultured broth of Nocardia brasiliensis IFM0406 and exhibit immunosuppressive activity. It contains the active chemical constituents such as amino acid and perhydro-phenanthrene moiety with a sugar rhamnose.

Green tea is a product of the dried leaves of Camellia sinensis of family Theaceae and is used for treatment of autoimmune arthritis. The polyphenolic compound (catechin) present in green tea possesses antiinflammatory properties.

Artemisia annua has been extensively used for the treatment of autoimmune diseases such as systemic lupus

erythematoses and rheumatoid arthritis. Artemesinin as well as its derivatives artesunate and artemether possess immune-modulatory effect.

Sarsaparilla plant contains steroids such as sarsasapogenin, smilagenin, sitosterol, stigmasterol and saponins like sarsasaponin, smilasaponin. Sarsaparilla is beneficial for the treatment arthritis due to its ability to inhibit TNF-α induced NFk-β activation.

Ashwagandha (Withania somnifera) is widely used in the treatment of psoriasis, arthritis and rheumatism. The chemical constituents present in Ashwagandha are alkaloids (isopelletierineanaferine), steroidal lactones (withanolides, withaferins), saponins (sitoindoside VII and VIII) and withanolides. These constituents produce immunosuppressive action on B and T cell activity in hyper-immune states.

Ginger (Zingiber officinale) is used to decrease pain in arthritis. The chemical constituent zingiberene is mainly responsible to reduce pain and inflammation by inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) pathways.

Stephania tetrandra is used for the treatment of autoimmune disease and rheumatic arthritis. Tetrandrine is the active constituent which produces immunemodulating effects by inhibiting.

Salvia miltiorrhiza of Labiatae family is recognized for the treatment of immunological disorders. The chief active constituent of Salvia miltiorrhiza is Tanshione IIA (TSN). TSN produces its action by reducing inflammatory cytokines such as IL-2, IL-4 and TNF-α.

Liquorice (Glycyrrhiza glabra) belongs to Fabaceae family which exhibits immune-modulatory properties. Glycyrrhizin and glycyhrritinic acid are the active constituents which have antiinflammatory effects by inhibition of calcineurin activity and T-cell proliferation.

Parthenolide is a major sesquiterpene lactone present in extracts of Tanacetum parthenium of Asteraceae family and used for the treatment for rheumat.oid arthritis. It inhibits the release of pro-inflammatory mediators such as nitric oxide, prostaglandin (PG) E2 and TNF-α.

Berberis vulgaris of Berberidaceae family has immunosuppressive action. Berbamine is the active constituent which has selective inhibitory effect on STAT-4 expression and production of IFN-γ in cells.

Andrographispaniculata of family Acanthaceae is recommended for the relief of rheumatoid arthritis and autoimmune disease. The main constituent Andrographis paniculata is andrographolide. It produces inhibitory effects on NF-κB trans-activation activity.

MATERIALS

Moringa Oleifera Local market, Methanol Merck, Petroleum ether Merck

METHODOLOGY

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal (s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

Drugs and Chemicals

Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal (s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

Drugs and Chemicals

Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Preparation of methanolic extract of Moringa Oleifera

Plant leaves of *Moringa Oleifera* were obtained from the Local market. The dried and coarsely powdered bark (400 g) extracted successively with 1.5 ml each of petroleum ether (60 – 80ºC), methanol and in a Soxhlet extractor for 72 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40 – 50ºC). The petroleum ether extract yielded a pale brown sticky semisolid, weighing 3 g (3%) The methanol extracts yielded reddish brown and semi-solid residues, weighing 7.0 g (7.0%). Methanolic extract of Moringa Oleifera has been selected for further studies.

Preliminary phytochemical screening

Preliminary phytochemical screening of the plantextract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids as per the standard methods.

Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b).Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c).Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth

Iodide). Formation of red precipitate indicates the presence of alkaloids.
d).Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution).Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of Carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
a).Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
b).Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
c).Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of saponins

a). FrothTest: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for15 minutes. Formation of 1cm layer off a am indicates the presence of saponins.
b).FoamTest:0.5gm of extract was shaken with 2ml of water. If foam produced persists forten minutes it indicates the presence of saponins.

Detection of steroids

a).Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
b).Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of Tannins

Gelatin Test: To the extract,1 %gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of Flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
Leadacetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Animals

Healthy male albino mice (25 – 30 g) were selected for the study. Animals were housed in standard isolation cages (45×35×25cm) under environmentally controlled conditions with 12 - h light/12 - h dark cycle. Mice were allowed free access to water, standard laboratory rat chow (Hindustan Liver Pvt. Ltd, Mumbai) throughout the experiment. Fresh sheep red blood cells (SRBC) in Alsever's solution were prepared in host department after collecting fresh sheep blood from local slaughter house.

Antigen

SRBC collected in Alsever's solution, were washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5 × 109 cells/ml for immunization and challenge.

Treatment

The animals were divided into four groups consisting of six animals each. A group of six untreated rats were taken as control (Group I). The methanolic extract of *Moringa Oleifera* was fed orally for 14 days at a dose of 50 mg/kg/ day (Group II), 100 mg/kg/day (Group III) and 150 mg/ kg/day (Group IV) for assessment of immunomodulation effect. The animal experimental protocols were approved by the Institute Animal Ethics Committee.

Haemagglutinating antibody (HA) titre

Haemagglutinating antibody titre was determined according to the method of Puri et al (1993). Mice of group II, III and IV were pretreated with MMO for 14 days and each mouse was immunized with 0.5 × 109 SRBC/mouse by i.p. route, including control mice. The day of immunization was referred to as day 0. The animals were treated with MMO for 14 more days and blood samples were collected from each mouse on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBC (0.025 × 109 cells). The microtitre plates were incubated at room temperature for two hours and examined visually for agglutination. The highest number dilution of serum showing haemagglutination has been expressed as HA titre On 15th day of treatment, all the mice were sacrificed and blood was collected in heparinized vials. Blood samples for animals of each group were subjected for hematological studies such as total WBC count and spleen leukocyte count. Spleen and thymus were dissected out and embedded in 10% formalin solution to record their weight.

Delayed type hypersensitivity (DTH) response

Six animals per group (control and treated) were immunized on day 0 by i.p. administration of 0.5 × 109 SRBC/mouse and challenged by aintraplanter administration of 0.025 × 109 SRBC/ml into right hind foot pad on 7th day. The MMO was administered orally from day 1until day 7. DTH response was measured at 24 h after SRBC challenge on day 8 and expressed as mean percent decrease in paw volume (plethysmometrically).

STATISTICAL ANALYSIS

The data were analysed using One-way analysis of variance (ANOVA) followed by Dunnett test. P values < 0.05 were considered significant. But unfortunately immune-suppressants are suffers from a number of serious adverse effects among which nephrotoxicity, hepatotoxicity, induction of diabetes.

RESULTS AND DISCUSSION

Phytochemical screening test

The phytochemical screening of the MMO indicated the presence of saponins, flavonoids, tannins, steroids, phenol and glycosides. The results of HA titre and DTH response are shown in Table 1.

Table 1: Result of chemical group tests of the Methanolic extract of *Moringa Oleifera* leaves.

Saponins	++
Flavonoids	+
Flavonoid	++
Tannins	+
Steroids	+++
Phenol	++
Glycosides	+

Table 2: Effect of *Moringa Oleifera* on HA titre and DTH response to antigenic challenge by sheep RBCs in mice

Groups	HA titre	DTH response (% decrease in paw volume)
I (Untreated)	5.31 ± 1.61	15.43 ± 1.69
II (50 mg/kg, p.o.)	5.10 ± 2.92	11.62 ± 0.63*
III (100 mg/kg, p.o.)	5.21 ± 0.51	8.31 ± 1.53**
IV (150 mg/kg, p.o.)	4.81 ± 0.73	6.19 ± 2.34**

*The values are mean ± SD of 6 mice in each group. One-way ANOVA followed by Dunnet multiple comparisons test; *P < 0.05, **P < 0.01 Vs group I.*

Table 3: Effect of *Moringa Oleifera* on WBC, spleen leukocytes count and relative organ weight in mice

Groups	WBC (thousand/cmm)	Spleen leukocyte (thousand/cmm	Thymus weight (g/100 g B.W)	Spleen weight (g/100 g B.W)
I (Untreated)	13.5 ± 1.69	49.2 ± 7.1	0.09 ± 0.01	0.36 ± 0.034
II (50 mg/kg, p.o.)	11.5 ± 0.13ns	41.5 ± 6.3ns	0.08 ± 0.02ns	0.33 ± 0.053ns
III (100 mg/kg, p.o.)	9.6 ± 0.69*	33.2 ± 5.0*	0.06 ± 0.01*	0.27 ± 0.061*
IV (150 mg/kg, p.o.)	8.91 ± 1.34**	27.3 ± 2.4**	0.04 ± 0.02**	0.21 ± 0.026*

*The values are mean ± SD of 6 mice in each group. One-way ANOVA followed by Dunnet multiple comparisons test; nsP> 0.05,*P < 0.05, **P < 0.01 Vs group I.*

Even with the administration of increasing doses of *Moringa Oleifera*, the HA titre did not show any significant increase as compared to untreated control group indicating that the *Moringa Oleifera* has no effect on the humoral immunity. The DTH response to SRBC which corresponds to cell mediated immunity showed a significant dose dependent decrease due to treatment with *Moringa Oleifera* with dose of 100 mg/kg/ day and 150 mg/kg/day. The DTH response was 8.31 ± 1.53 and 6.19 ± 2.34 respectively in comparison to corresponding value of 15.43 ± 1.69 for untreated control group. The dose dependant differences in DTH response were statistically significant (P<0.05). Thus MML treatment induced marked inhibition of DTH response to SRBC in the animals. Finally, the effects of *Moringa Oleifera* on WBC, spleen leukocytes count and relative organ weight in mice are shown in Table 2. *Moringa Oleifera* at the dose of 100 mg/kg and 150 mg/kg., p.o caused a significant reduction in the WBC, Spleen leukocyte counts as well as relative spleen weight and thymus weight. But the effect was more pronounced at dose of 150 mg/kg (P < 0.01) as comp

CONCLUSION

In the present study, the immunosuppressant activity of *Moringa Oleifera*, an important plant in indigenous medicinal practice was explored. Administration of *Moringa Oleifera* was found to decrease total WBC count and spleen leukocyte count significantly indicating that the extract could suppress the non-specific immune system. Moreover there was decreased in the relative spleen weight and thymus weight supports these findings. *Moringa Oleifera* has been shown to contain *Moringa Oleifera* A and B and steroidal lactones. At

present we do not know whether these compounds are responsible for the immunosuppressant activity produced by extract. Further studies using isolated compounds are in progress.

REFERENCES

1. Abbas AK, Lichtman AH. Basic immunology: functions and disorders of the immune system. Philadelphia: W B Saunders Company; 2001.
2. Web link. Available from: http://www.nih.gov/niams/healthinfo/lupusguide/chppis15.htm.
3. Reiff A. A review of Campath in autoimmune disease: biologic therapy in the gray zone between mmunosuppression and immunoablation. Hematology. 2005;10(2):79-93. doi: 10.1080/10245330400026139, PMID 16019453.
4. Sompayrac LM. How the immune system works. Boston: Blackwell Publishing Science; 1999.
5. Guidelines for vaccination of solid organ transplant candidates and recipients. Am J Transplant. 2004;4;Suppl 10:160-3.
6. Barriga OO. Immunomodulation by nematodes: a review. Vet Parasitol. 1984;14(3-4):299-320. doi: 10.1016/0304-4017(84)90098-0, PMID 6382784.
7. de Macedo MS, Mota I. Antigenic competition in IgE antibody production: I. Establishment of parameters involved in primary and secondaryresponses. Immunology. 1980;40(4):701-8. PMID 7429547.
8. Urban JF Jr, Madden KB, Svetić A, Cheever A, Trotta PP, Gause WC et al. The importance of Th2cytokines in protective immunity to nematodes. Immunol Rev. 1992;127:205-20. doi: 10.1111/j.1600-065x.1992.tb01415.x, PMID 1354652.
9. Sahoo BM, Banik BK. Medicinal plants: source for immunosuppressive agents. Sahoo Banik Immunol Curr Res. 2018;2:1.
10. Available from: https://my.clevelandclinic.org/health/drugs/10418-immunosuppressants.
11. Kamatani T, Otsuka R, Murata T, Wada H, Takahashi T, Mori A et al. Evaluation of immunosuppression protocols for MHC-matched allogeneic iPS cell-based transplantation using a mouse skin transplantation model. Inflam Regen. 2022;42(1):4. doi: 10.1186/s41232-021-00190-7, PMID 35105370.
12. Rehberger K, Escher BI, Scheidegger A, IngeWerner, Segner H. Evaluation of an in vitro assayto screen for the immunotoxicpotential of chemicals to fsh. 2021;11:3167.
13. Zimmermann-Klemd AM, Reinhardt JK, Morath A, Schamel WW, Steinberger P, Leitner J et al. Immunosuppressive Activity of Artemisia argyi Extract and Isolated compounds. Front Pharmacol. April 08 2020;11:402. doi: 10.3389/fphar.2020.00402, PMID 32322200.
14. Lu J, Guan S, Shen X, Qian W, Huang G, Deng X et al. Immunosuppressive activity of 8-gingerol on immune responses in mice. Molecules. 2011;16(3):2636-45. doi: 10.3390/molecules16032636, PMID 21441866.
15. Veletzky L, Rehman K, Lingscheid T, Poeppl W, Loetsch F, Burgmann H et al. *In vitro* activity of immunosuppressive drugsagainst Plasmodium falciparum. Veletzky et al. Malar J. 2014;13:476. doi: 10.1186/1475-2875-13-476, PMID 25476467.
16. Hamdy S, Haddadi A, Shayeganpour A, Alshamsan A, Montazeri Aliabadi H, Lavasanifar A. The immunosuppressive activity of polymeric micellar formulation of cyclosporine A: in vitro and in vivo studies. AAPS J. 2011;13(2, June):159-68. doi: 10.1208/s12248-011-9259-8, PMID 21336534.
17. Olson RD, Headley MB, Hodzic A, Walsh GM, Wingett DG. In vitro and in vivo Immunosuppressive Activity of a Novel anthracycline, 13-deoxy, 5-iminodoxorubicin. Int Immunopharmacol. 2007 June;7(6):734-43. doi: 10.1016/j.intimp.2007.01.010, PMID 17466907.
18. Song Bocui, Wang Z, Liu Y, Xu Sisi, Huang Guoren, Xiong Y et al. Immunosuppressive activity of daphnetin, one of coumarin derivatives, is mediated through suppression of NF-kB and NFAT signaling pathways in mouse T cells. PLoS One. May 2014;9(5) | Issue 5 | e96502:e96502. doi: 10.1371/journal.pone.0096502, PMID 24800925.
19. Bian L, Guo Z-K, Wang HX, Wang J-S, Wang H, Li QF et al. In vitro and in vivo ImmunosuppressiveCharacteristics of hepatocyte growth factor-modified murine mesenchymal stem cells. In Vivo. 2009;23(1):21-7. PMID 19368120.