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Immunomodulatory activity of aqueous extract of *Pisonia alba* root with particular reference to splenocytes proliferation and cytokines induction

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ABSTRACT

The goal of this study was to look into the immunomodulatory activity of aqueous extract of Pisonia alba root (PARE) with a focus on splenocyte proliferation and cytokine production. After immunisation with Salmonella antigen, antibody titers were determined by tube agglutination and indirect ELISA assay in four groups of mice: control, antigen alone, and PARE-treated (400 and 800 mg/kg for 21 days), while cellular immunity was studied in three groups of rats (control and PARE-treated - 400 and 800 mg/kg for 21 days). Concanavalin-A (Con-A) was used to stimulate splenocytes from untreated and PARE-treated rats, and the optical density (OD) and stimulation index were measured. To test the effect on splenocyte proliferation, control rats' splenocytes were treated in vitro with PARE (50–1600 g/ml) and Con-A. ELISA kits were used to measure interleukin-2 (IL-2) and IL-6 levels in splenocyte supernatants from control and PARE-treated rats, as well as after in vitro treatment of splenocytes with PARE (50–1600 g/ml). Both approaches revealed a considerable rise in antibody titer in PARE-treated mice and a significant increase in skin thickness in rats after DNCB challenge, indicating that PARE has humoral and cell-mediated immunostimulant potential. After ex vivo and in vitro exposure of splenocytes to PARE and sensitization with Con-A, large increases in OD and stimulation index were found, as well as significant elevations in IL-2 and IL-6 levels in splenocytes supernantant. PARE's humoral and cell-mediated immunostimulant effect appears to be mediated by splenocyte proliferation and enhanced cytokine production, particularly IL-2 and IL-6.

Keywords: Antibody titer, cell-mediated, cytokines, interleukin-2, interleukin-6, Pisonia alba root, splenocytes proliferation.

INTRODUCTION

Presently, diseases, particularly infectious diseases, are on the rise all over the world, necessitating effective body defence mechanisms to regulate them through immunomodulation. Malnutrition and infectious diseases have remained a problem, particularly in underdeveloped countries, because they severely weaken the immune system responses of those who are afflicted [1]. Malnutrition is caused by an insufficient balanced dietary intake, particularly of proteins, and illnesses, which promote each other synergistically [2]. Stress, infectious disorders such as acute respiratory tract infections, diarrheal diseases, yellow fever, hepatitis A and E, TB, and HIV/AIDS are the leading causes of immunodeficiency [3]. Individual diet, on the other hand, strengthens the immune system and defence capabilities of the body [2–5]. Approximately 870 million people were expected to be undernourished globally between 2010 and 2012, accounting for 12.5 percent of the world population, with about 852 million people living in poor nations, where malnutrition is estimated to be 14.9 percent [6]. Malnutrition, on the other hand, has a significant impact on an individual's immune system physiology, and in most cases, it is necessary to boost it in cases of immunosuppression or repress it in cases of overstimulation, such as in autoimmune disease conditions.

Various allopathic drugs or medicines are used to modulate the immune system. However, these drugs are very expensive for poor people, they are not easily accessible, and in most cases, they are associated with adverse drug reactions. As a result, the majority of people especially in the rural areas of the developing world turn to the use of alternative herbal medicines from medicinal plants. *Pisonia alba* which is also

known as *Pisonia alba spanoghe, pisonia umbellifera*, belongs to the family of Nyctaginaceae. It is found on many of the Seychelles Islands that have had habitat restoration and subsequently is a key part of the habitat associated with high biodiversity and a complex food web. It is therefore not as easy as replacing Pisonia with other native tree species; it was discovered by(7) that Pisonia is the most common nest tree for the Seychelles warbler an endemic land bird brought back from near extinction by careful habitat management and translocation, thus showing that careful consideration of the entire island ecosystem is essential. P. alba is a large evergreen shrub. It is originally from the beach forests of Andaman Islands. Leaves: Long, bounty, and fresh green in color. If planted in good sunlight, the leaves may acquire a light yellow color. Flowers: The tree rarely flowers in India. The flowers are small, green, and inconspicuous. Uses: The leaves are edible. Young leaves are used as a vegetable. Leaves make good cattle feed too and are mostly used to treat rheumatism or arthritis. In traditional Indian medicine, they are used as an anti-diabetic; Leaves , of course , are used by natives as cattle feed; They are cooked and eaten for arthritis; The leaves are also carminative; Leaves are an antidote for snake bites; Researches have revealed that favonoids, steroids and phenolic compounds are present in the leaf. (7-11) The aim of this study undertaken is to evaluate and validate the antiurolithiatic potential of hydro-alcoholic extract of P.alba Less against ethylene glycol-induced urolithiasis in rats. This study was undertaken to unravel the of immunomodulatory activity of this test plant with particular reference to splenocytes proliferation and cytokines induction.

MATERIALS AND METHODS

Plant Material

P.alba plant was collected from botanical garden of Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi and nearby areas of Belagavi. The plant material was identified and authenticated by Scientist B, RMRC, Belagavi. The herbarium was prepared and stored at RMRC, Belagavi, Karnataka, India..

Preparation of Extract

By using the hot percolation method, a hot aqueous extract of shade dried and coarsely powdered P.alba (PARE) was prepared in a soxhlet apparatus, and it was concentrated to dryness using a rotatory evaporator at a low pressure and temperature (40°C). For further research, the extract was kept in airtight containers and stored at 4°C.

Experimental Animals

Swiss albino mice (18–25 g) and Wistar rats (100–120 g) of both sexes were obtained from the Laboratory Animal Resource Section of the Indian Veterinary Research Institute in Izatnagar and housed in the laboratory animal house. Before the experiment began, a 15-day acclimatisation period was allowed. The animals had unrestricted access to clean drinking water and pelleted laboratory animal feed, and a nearly 12-hour light-dark cycle was maintained. The Institutional Animal Ethics Committee authorised the experimental protocol.

Humoral immune response

The experimental mice were split into four groups, each with eight mice. Mice in Group I (negative control) were given simply distilled water, while mice in Group II were given Salmonella typhimurium "O" antigen and acted as positive controls. PARE was given orally to Group III and IV mice at 400 mg/kg and 800 mg/kg, respectively, for 21 days. Following that, mice from both groups were inoculated with Salmonella "O" antigen and PARE was continued at 400 and 800 mg/kg. On day 1, the initial dosage of S. typhimurium "O" antigen (0.5 ml) was subcutaneously delivered to 3–4 locations. The first booster of the same antigen was given on the seventh day, and the second booster on the fifteenth day, using the same approach as before. [12-18]

Blood samples were taken from mice in all groups 15 days after the second booster dose using a retro-orbital plexus puncture, and serum was separated for determining antibody titer using a tube agglutination test and an indirect ELISA method using a commercially available kit. Each well's optical density (OD) was measured at 450–570 nm using an ELISA reader (SPAN, India), and the titer was calculated using Trusfield's method. [18]

Cell-mediated immune response

Cell-mediated immune (CMI) response in rats was determined following the method of Tiwary and Goel.[19] Rats of either sex were randomly divided into three groups of five animals each. Group I served as control and received only distilled water. Group II and III animals were orally administered PARE at 400 and 800 mg/kg body weight (b.wt.), respectively and simultaneously, DNCB (1-Chloro, 2,4-dinitrochlorobenzene), a well-established control antigen (allergen) was applied locally. The site of DNCB application was examined for erythema, indurations, and vesicle formation. Thickness of the skin at the DNCB application site was measured at 0, 6, 12, 24, 36, 48, 60, and 72 h post-DNCB challenge using a Vernier caliper. Comparing the pre- and post-DNCB application skin thickness, change in skin thickness was determined and expressed in mm.

Splenocytes proliferation assay

Ex vivo and in vitro effect of PARE on splenocytes proliferation was determined employing the procedure described earlier.[20]

Ex vivo studies

Adult rats of both sexes were divided into three groups of six animals each at random. For 21 days, rats in Group I (control) were given triple glass distilled water, whereas rats in Groups II and III were given PARE at 400 and 800 mg/kg b.wt., respectively. Animals from all three groups were slaughtered after 21 days, and splenocytes were collected. A cell culture of 200 microliters was put to each well of a flat bottom culture plate. Each well received two micrograms of concanavalin-A (Con-A), with the exception of the blank, which contained simply cell culture. Culture plate was incubated at 37°C in CO2 incubator (5% CO2; 80% relative humidity for 72 h. After incubation, the supernatant was removed, and the plate was air-dried. Cell culture grade dimethyl sulfoxide (50 µl) was added in each well to dissolve the formazone crystals, formation of which is directly proportional to the number of viable cells. Live cells reduce tetrazolium salts to colored formazone compound while the dead cells do not form

formazone crystals. OD of each well of the culture plate was measured at dual wavelengths of 560–670 nm using ELISA reader and percent stimulation index was calculated using the following equation and compared with the OD of control well having no extract.

Stimulation index = (OD of the well with Con-A/OD of the well without Con-A)/OD of the well without Con-A.

In vitro studies

Test extract was filtered through a 0.2 micron membrane filter, and different dilutions (50, 100, 200, 400, 800, and 1600 g) of PARE were added to different wells of a culture plate containing 200 l of spleen cells (2 106 cells/ml in RPMI-1640 medium, except for the control, which contained only splenocytes culture. Con-A (2 g) was likewise applied to all but the blank wells, and the rest of the method was the same as in ex vivo investigations. Cytokines (interleukin-2 and interleukin-6) induction

Ex vivo studies

Adult rats of either sex weighing 100–120 g were randomly divided into three groups of eight animals each for ex vivo investigations. For 21 days, rats in Group I (control) were given triple glass distilled water, whereas rats in Groups II and III were given PARE at 400 and 800 mg/kg b.wt., respectively. On the 22nd day, all three groups' animals were humanely slaughtered, and their spleens were aseptically extracted for splenocyte harvesting. 200 µl spleen cells (2 × 106 cells/ml) in RPMI-1640 medium were placed in each well of culture plate and 2 µg of Con-A each was added in all the wells except blank. Interleukin (IL-2) and IL-6 cytokine levels were determined in the supernatant collected from cultured splenocytes of different treatment groups using the commercially available ELISA kits (Thermo Scientific).

In vitro studies

For in vitro studies on the effect of PARE on cytokines (IL-2 and IL-6) induction in splenocytes, splenocytes were isolated from healthy untreated rats. Nyctanthes extract was filtered through 0.2 micron membrane filter and different concentrations (50, 100, 200, 400, 800 and 1600 µg/ml) of PARE were added into different wells of culture plate containing 200 µl of spleen cells (2 × 106 cells/ml) in RPMI-1640 medium while the control well contained only splenocytes. 2 µg of Con-A each was added in all the wells except blank and levels of cytokines (IL-2 and IL-6) were determined in supernatants collected from cultured splenocytes as described above.

RESULTS

Table 1: Effect of simultaneous administration of Pisonia alba root (400, 800 mg/kg) for 21 days and Salmonella typhimurium O antigen on humoral immune response in mice by tube agglutination test and indirect ELISA assay in mice.

Groups/Treatment	Antibody titer	
	Tube agglutination test	Indirect ELISA assay
Control negative	719.01±292.15a	1649.01±374.55
Control positive (S.typi O antigen)	919.00±142.03	3399.01±706.01
PARE (400mg/kg) (S.typi O antigen)	1999.01±547.89	5999.01±1022.33
PARE (800mg/kg) (S.typi O antigen)	3599.01±454.81	21999.01±7090.64

P<0.01. Data presented are mean±SE of eight animals in each group. Different superscripts in a column indicate significant differences. SE=Standard error, S.typhi-Salmonella typhi, PARE-Pisonia alba root.

[Table 1] summarises antibody titers in mice of various groups against S. typhimurium "0" antigen utilising tube agglutination and indirect ELISA tests. The serum antibody titer was notably, although nonsignificantly, greater in the lower PARE dose (400 mg/kg) group when compared to the negative and positive control groups, as shown in [Table 1]. However, both assay protocols found that it was considerably (P 0.01) greater in the higher dose PARE group (800 mg/kg) compared to the control and 400 mg/kg PARE-treated groups.

Table 2: Effect of oral administration of Pisonia alba root (400, 800 mg/kg) for 21 days on di-nitrochloro-benzene-induced delayed type hypersensitivity response in rats.

Stick thickness in(mm)	Groups / Treatment		
	Control	PARE (400mg/kg)	PARE (800mg/kg)
Before DNCB application	0.97±0.01 [a]	1.07±0.10 [a]	0.99±0.01 [a]
Before last DNCB application (0h)	1.14±0.11 [a]	1.26±0.18 [a]	1.37±0.16 [a]
6h after last DNCB challenge	1.63±.0.11 [a]	1.76±0.12 [a,b,β]	2.55±0.18 [c, β]
12	2.17±0.08 [a]	2.24±0.16 [a]	2.50±0.17 [a]
24	1.59±0.13 [a]	2.53±0.19 [a,b]	2.11±0.09 [β,b]
36	1.39±0.13 [a]	1.51±0.14 [a]	1.57±0.13 [a]

48	1.21±0.07 [a]	1.23±0.10 [a,b,α]	1.67±0.06[c,β]
60	1.03±0.03 [a]	1.29±0.11 [a]	1.27±0.09 [a]
72	0.97±0.01 [a]	1.11±0.04 [a]	1.23±0.11 [a]

[α]P<0.05, [β]P<0.01 Data presented are mean±SE of five animals in each group. Different superscripts in a column indicate significant differences. SE=Standard error, S.typhi-Salmonella typhi, PARE-Pisonia alba root.

[Table 2] shows skin thickness data from mice in the control and PARE-treated groups (400 and 800 mg/kg) at 0 and 6, 12, 24, 36, 48, 60, and 72 hours following the last DNCB treatment. According to statistical analysis of the results, skin thickness in the PARE-treated (800 mg/kg) group differed substantially (P 0.01) from that of the control group at 6, 24, and 48 hours, as well as between the 400 and 800 mg/kg PARE-treated groups at various time intervals, as shown in [Table 2].

Table 3: Effect of oral administration of Pisonia alba root (400, 800 mg/kg) for 21 days on ex vivo splenocytes proliferation in rats.

Parameters	Control	PARE (400mg/kg)+Con-A (5mg/ml)	PARE (800mg/kg)+Con-A (5mg/ml)	PARE (mg/kg)	
				400	800
OD	0.141±0.02[a]	0.61±0.003[e,*]	0.663±0.02 [b,*]	0.59±0.02 [e,d,*]	0.67±0.02 [b,d,*]
Stimulation index	-	4.34±0.024 [a]	4.67±0.07 [b,*]	4.20±0.034 [a,c,*]	4.77±0.03 [b,*]

[*]P<0.001Data presented are mean±SE of six animals in each group. Different superscripts in a column indicate significant differences. SE=Standard error, S.typhi-Salmonella typhi, PARE-Pisonia alba root.

Ex vivo effects [Table 3] shows the effect of oral feeding rats PARE (400 and 800 mg/kg) for 21 days on splenocyte proliferation before and after Con-A sensitization. PARE significantly (P 0.01) increased splenocyte proliferation at both dose levels (400 mg/kg and 800 mg/kg) when compared to the control group, as evidenced by a considerable (P 0.05) rise in OD and stimulation index in both groups, which was similar to that seen after sensitization with Con-A.

Table 4: Effect of in vitro treatment of splenocytes with Pisonia alba root at 50, 100, 200, 400, 800, and 1600 µg/well on rats splenocytes proliferation.

Treatment	OD	Stimulation Index
Control (Without Con-A)	0.141±0.02[a]	-
Con-A (2µg/well)	0.716±0.05	5.05±0.43a
PARE (50µg/well)	0.577±0.003c,*	4.07±0.02b,α
PARE (100µg/well)	0.631±0.024b,c,*	4.44±0.17a
PARE (200µg/well)	0.613±0.023b,c,*	4.32±0.16a
PARE (400µg/well)	0.651±0.013b,c,*	4.61±0.088a
PARE (800µg/well)	0.663±0.01b,c,*	4.67±0.14a
PARE (1600µg/well)	0.681±0.01b,c,*	4.78±0.133a

*[α]P<0.05, *P<0.001. Data presented are mean±SE of six observations. Different superscripts in column differed significantly. Con-A=Concanavalin-A, OD=Optical density, SE=Standard error, PARE=Pisonia alba root*

[Table 4] summarises the effects of in vitro exposure of splenocytes to various concentrations of PARE (50–1600 g/well) and Con-A (2 g) on OD and stimulation index compared to those in control groups. The results showed that, when compared to the OD of the control group, all of the PARE-treated splenocytes wells had a significant (P 0.05–0.01) rise in OD, which was practically equivalent to that caused by Con-A. Similarly, the stimulation index after PARE exposure was found to be almost identical to that after Con-A exposure, but the stimulation index in different PARE-treated groups did not differ significantly from one another, indicating that no concentration-dependent effect was observed, as evidenced by the data in [Table 4].

Table 5: Effect of oral administration of Pisonia alba root (400, 800 mg/kg) to rats for 21 days on interleukins-2 and interleukins-6 induction in splenocytes

Parameters	Control	PARE-treated group	
		400 mg/kg	800 mg/kg
IL-2 (pg/ml)	947.57±6.39[a]	984.81±7.87[b,β]	1011.42±7.38c[*,α]
IL-6 (pg/ml)	714.66±10.94[a]	777±15.31[b,β]	781.17±12.70[b,β]

*α P<0.05, β P<0.01, *P<0.001. Data presented are mean±SE of six animals in each group. Different superscripts in a row differed significantly. IL=Interleukin(s), SE=Standard error, PARE=Pisonia alba root*

[Table 5] shows the levels of cytokines (IL-2 and IL-6) in the splenocytes of rats treated with PARE (400 and 800 mg/kg) and a control group. PARE-treated splenocytes (400 mg/kg and 800 mg/kg) had IL-2 levels of 985.81 7.85 and 1012.41 7.39, respectively, which were considerably (P 0.001) greater than the control group (948.58 6.39). Even the higher dose treatment group (800 mg/kg) had a considerably greater IL-2 value (P 0.01) than the 400 mg/kg treatment group [Table 5]. IL-6 values were likewise considerably (P 0.01) higher in PARE-treated groups (400 and 800 mg/kg) than in the control group, although unlike IL-2, the effect on IL-6 values was not dose-dependent [Table 5].

Table 6: Effect of in vitro exposure of rat splenocytes to different concentrations of Pisonia alba root on interleukins-2 and interleukins-6 cytokines induction

Treatment	IL-2 (pg/ml)	IL-6 (pg/ml)
Control (Without Con-A)	947.87±6.38[a]	715.68±10.93[a]
PARE (50µg/well)	982.5±11.63b,[c,α]	764.80±11.08[b,c,α]
PARE (100µg/well)	977.27±5.01[b,c]	793.67±8.93b,*
PARE (200µg/well)	1014.95±9.68b,[d,*]	837.97±17.42[b,d,*]
PARE (400µg/well)	1002.54±7.72b, [*]	833.64±8.76[b]
PARE (800µg/well)	992.82±6.63[b, β]	782.21±4.82 [b]
PARE (1600µg/well)	997.66±5.85 [b, β]	773.84±10.42 [b]

*α P<0.05, β P<0.01, *P<0.001. Data presented are mean±SE of six animals in each group. Different superscripts in a row differed significantly. IL=Interleukin(s), SE=Standard error, PARE=Pisonia alba root*

[Table 6] shows the results of in vitro treatment of rat splenocytes with various doses of PARE (50, 100, 200, 400, 800, and 1600 g/well) on IL-2 and IL-6 levels in splenocytes. The analysis of the data revealed that there was a significant rise in IL-2 and IL-6 IL-2 levels (pg/ml) in comparison to control at practically all of the concentrations utilised, although the effect was not concentration-dependent.

DISCUSSION

The considerable (P 0.01) increase in serum antibody titer in mice against S. typhimurium "O" antigen after treatment with PARE suggests that Nyctanthes aqueous extract has immunomodulatory potential. Other researchers have reported an increase in humoral immune response to sheep RBCs and macrophage migration index in Balb/c albino mice after treatment with ethanolic extracts of Nyctanthes, seeds, and roots, as well as an increase in antibody titers, numerical values of immunocytes, and functions of phagocytes after treatment with Nyctanthes leaves extracts [2],[15],[16],[21]. [16],[22] The carbon clearance test in cyclophosphamide-induced myelosuppression in mice[17], against systemic candidiasis [21], and pesticide toxicity have all been used to demonstrate the immunomodulatory potential of Nyctanthes leaves, seeds, and flowers. [22]

CMI response data, as measured by skin thickness in control and PARE-treated rats, revealed a significant (P 0.01) increase in skin thickness in 800 mg/kg PARE-treated animals compared to those in the control group at different time intervals, implying that PARE has promising cell-mediated immunity potential. Our findings on the delayed form of hypersensitive reaction corroborate those of other researchers. [15],[16],[21],[22]

Interleukins are immunological signals that travel between cells and augment both local and systemic host responses; thus, an increase in interleukins is a biomarker for an elevated immune response. T and B cells require cytokines to be stimulated. Th1 cells are responsible for pro-inflammatory cellular immunity and produce IL-2, whereas Th2 cells express IL-6 and mediate humoral immunity by differentiating B-cells to plasma cells to create antibodies [23],[24]. When the levels of cytokines in PARE treatment groups were compared to those in the control group, PARE caused a significant (P 0.05–0.001) rise in IL-2 and IL-6, which corresponded to significant increases in skin thickness and antibody titers, respectively. IL-2 stimulates the action of big granular lymphocytes and natural killer cells [25], whereas IL-6, a multifunctional lymphokine, modulates the humoral immune response by activating B-lymphocytes and their development into plasma cells. [23],[24]

Significant (P 0.05–0.001) increases in IL-2 and IL-6 levels in PARE-treated splenocytes (50–1600 g/well) in this investigation, compared to controls, further substantiates PARE's immunomodulatory capabilities, even at extremely low concentrations (50 g). As a result, the findings of our ex vivo and in vitro experiments clearly indicate that cytokines signal/modulate PARE's immunostimulant effect. Ex vivo and in vitro investigations on splenocyte proliferation found that PARE therapy resulted in a considerable increase in optimum density and stimulation index, which was practically identical to that seen with Con-A. This finding also lends credence to PARE's ability to operate as an immunostimulant by promoting splenocyte growth.

Diterpenoids, nyctanthin, nyctanthoside, rengyolone, astragalin, flavonoids, anthocyanins, d-mannitol, essential oils, glycosides, carotenoids, and carotenoids are abundant in Nyctanthes extract, and the majority of these phytoconstituents have potent antioxidant and free radical scavenging activity, as well as overall immunostimulant. [17]

CONCLUSION

As a result, based on the literature and our findings, Nyctanthes appears to be an ideal candidate for the formulation of a safe and effective herbal immunomodulator, and/or it can even be considered a constituent of functional foods in the modern world, as herbal immunomodulators are becoming increasingly popular in the world of natural health because they normalise immunity rather than boost it. However, more research into immunobioactive mechanisms is needed, particularly the cross-talk mechanism(s) between splenocytes, cytokines, and cells that mediates humoral and cell-mediated immunity.

REFERENCES

1. UNICEF. Young child survivial and development: UNICEF Annual Report 2008. UNICEF; Available at: http://www.unicef.org/publications/files/UNICEF_Annual_Report_2008_EN_072709.pdf.
2. Calder PC, Field CJ, Gill HS. Nutrition and immune function. Br J Nutr. 2003;90:239–41.

3. Cooper MD, Schroeder HW. Primary immune deficiency diseases. In: Harrison TR, editor. Harrison's Principles of Internal Medicine. 16. New York: Mc Graw-Hill; 2005. pp. 1939–41.
4. Chandra RK. Nutrition and the immune system: an introduction. Am J Clin Nutr. 1997;66:460S–3S.
5. Calder PC, Jackson AA. Undernutrition, infection and immune function. Nutr Res Rev. 2000;13:3–29.
6. FAO, WFP, IFAD. Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition: The State of Food Insecurity in the World 2012. FAO; Rome: 2012. pp. 1–65. Available at: http://www.fao.org/docrep/016/i3027e/i3027e.pdf.
7. Singha S, Bawari M, Choudhury MD. Hepatoprotective and antipyretic effect of bark of Nyctanthes arbortristis Linn. Int J Pharm Pharm Sci 2014;6 Suppl 2:110-4.
8. https://commons.wikimedia.org/wiki/File. retrived 2019.
9. Esau, K. 1964. Plant anatomy. Jhon Wiley Publications, Newyork. Pp. 767.
10. Kokate, C.K., "Practical Pharmacognosy" 2002, p: 107-114, 123-125, 130. 2002: 1st edition; 405-406, 426-489.
11. Kokate, C.K., Evaluation of crude drug, Practical Pharmacognosy, 1985; Ist edition; 122-135.
12. Khanapur M, Avadhanula RK, Setty OH. In vitro antioxidant, antiproliferative, and phytochemical study in different extracts of Pisonia alba root . Biomed Res Int 2014;2014:291271.
13. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Yadev VR, Park B, et al. Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases: Reverse pharmacology and bedside to bench approach. Curr Drug Targets 2011;12:1595-653.
14. Ratnasooriya WD, Jayakody JR, Hettiarachhi AD, Dharasiri MG. Sedative effects of hot flower infusion of Pisonia alba root on rats. Pharm Biol 2005;43:140-6.
15. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant activity of Pisonia alba root L. J Ethnopharmacol 1994;42:31-7.
16. Kannan M, Singh RA, Kumar A, Jegatheswari P, Subburayalu S. Studies on immune-bioactivities of Pisonia alba root (Oleaceae). Afr J Microbiol Res 2007;1:88-91.
17. Sravani C, Kumar TM, Krishna KL, Mahalakshmi AM, Ramprasad KL, Bhavana V. Evaluation of immunomodulatory activity of Pisonia alba root Linn. Flower. Int J Pharm Sci 2014;4:480-8.
18. Trusfield M. Diagnostic testing. In: Veterinary Epidemiology. 3rd ed. : Blackwell Publication, Oxford, U.K.; 2007. p. 306-7.
19. Tiwary BK, Goel MC. Contact sensitivity to DNCB in normal and cell-mediated-immunity deficient chickens: In vivo detection and correlation with lymphocyte transformation and graft-versus-host reaction. Vet Immunol Immunopathol 1985;8:329-39.
20. Jayanthi M, Garg SK, Yadav P, Bhatia AK, Goel A. Some newer marker phytoconstituents in methanolic extract of Moringa oleifera leaves and evaluation of its immunomodulatory and splenocytes proliferation potential in rats. Indian J Pharmacol 2015;47:518-23.
21. Khan ZH, Manglani A, Shukla PK, Puri A, Saxena SC, Tandon JS. Immunomodulatory effect of plant extracts and iridoid glucoside from Pisonia alba root against systemic candidiasis in mice. Pharm Biol 1995;33:297-304
22. Bhatia A, Kaur J. Nyctanthes arbour-tristis leaves estract as antagonize or immunotoxic effects of chemical pesticides (experimental study). Int J Environ Stud 2001;58:197-215.
23. Rogge L. A genomic view of helper T cell subsets. Ann N Y Acad Sci 2002;975:57-67.
24. D'Elios M, Del Prete G. Th1/Th2 balance in human disease. Transplant Proc 1998;30:2373-7.
25. Waldmann TA. The biology of interleukin-2 and interleukin-15: Implications for cancer therapy and vaccine design. Nat Rev Immunol 2006;6:595-601.