

Immunopharmacological activity of flavonoids isolated from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum*

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ABSTRACT

The objective of our present study is to investigate the immunosuppressive activity of variable doses of crude flavonoids (6.25-100 mg/ml; 50 µl) extracted from the leaves of *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* on human whole blood using specific antigen (i.e. hepatitis B vaccine containing surface antigen, 20 µg/ml; 10 µl) is to estimate its blood counts, CD14 monocyte marker and observed its cytotoxicity. The results showed that the crude flavonoids extracted from these three medicinal plants showed maximum inhibition of blood counts, CD14 monocyte marker and cytotoxicity at higher doses. Overall, the data suggests that crude flavonoids extracted from these medicinal plants i.e. *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* showed immunosuppressive activity against HBsAg.

Keywords: Flavonoid; *Mesua ferrea*; *Ficus benghalensis*; *Jasminum auriculatum*.

1. INTRODUCTION

Medicinal plants contained a wide range of bioactive compounds that are present in plant products (leaves, stem, root, seeds etc.) and used as

alternative therapeutic tools for the prevention of many animal and human diseases [1, 2]. Lot of research work related to these medicinal plant products were taken and identified many valuable drugs (e.g. vincristine, vinblastine, taxol etc.) for various immunopharmacological activities e.g. anti-cancer, anti-inflammatory etc. [3-5]. As per literature, extensive research work is available for isolation and characterization of secondary metabolites especially flavonoids from medicinal plant products [6]. These flavonoids are found in higher concentration in legumes, fruits, seeds, vegetables etc. it is estimated that medicinal plant produces at least hundred thousand secondary metabolites during its growth and development [7, 8]. Out of these, more than 4000 flavonoids are being reported. In this regard, researchers focused only those flavonoids (phenolic compounds i.e. flavonones and chalcones) extracted from medicinal plant products and showed as anti-inflammatory, anti-allergic, anti-viral etc. [6-9]. Lot of flavonoids are still reported for various immunopharmacological activities e.g. antileukemic properties, vasodilators (treatment for heart diseases), anti-cancer and anti-aging properties etc. [7, 8].

Mesua ferrea (family *Guttiferae*), medicinal plant used for curing human as well as animal diseases. In India especially Assam use of this

medicinal plant as antiseptic, blood purifier and also showed its medicinal uses in different parts of the plant i.e. leaves and flowers are antidotes for snake bite and scorpion sting, roots are useful in gastritis and bronchitis etc. Moreover, this plant showed number of activities like anti-inflammatory, anti-microbial etc. [10-12].

Ficus benghalensis (family *Moraceae*), medicinal plant showed some medicinal properties e.g. leaves/fruits/bark are used as anti-cancer, anti-microbial properties and also used in the treatment of several diseases e.g. skin, vaginal disorders etc. [13-15].

Jasminum auriculatum (family *Oleaceae*) widely distributed in India, Sri Lanka and Nepal. Extensive research work on medicinal plant products of *Jasminum auriculatum* has already been done related to its immunopharmacological properties against various diseases e.g. roots for skin diseases (ringworm); flowers (aromatherapy, antiseptic, aphrodisiac etc.) and leaves (mouth ulcers) [16-18]. In recent years, further immunopharmacological studies have been carried out related to secondary metabolites especially flavonoid pertaining to explore its potential.

2. MATERIAL AND METHODS

2.1. Plant material

Leaves of medicinal plant were collected from Vidya Pratishthan's School of Biotechnology. For flavonoid extraction using fresh plant leaves samples and these samples were shade dried and prepared powdered form using mortar and pestle.

2.2. Phytochemical analysis

Preliminary studies of medicinal plant products especially leaves of *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* were carried out pertaining to determined its secondary metabolites especially flavonoids in the aqueous leaves extract through various phytochemical analysis. After confirmation of these flavonoids in the aqueous leaves extract, our group isolated the crude flavonoids from leaves of these medicinal plants. For flavonoid extraction using fresh plant leaves (5 g) were macerated in liquid nitrogen to

prepare fine leaves powder. Thereafter, reflux with 50 ml of 80% methanol along with leaves powder of three medicinal plants in different sets for 2h at 100 °C. Finally, collect the filtrate (after cooling down the solution using whatman filter paper) containing constituents of leaves powdered solution and then add ethyl acetate (10 ml) and distilled water (20 ml) in the ratio of 1:2. Incubate the solution for 4-5 h at room temperature and observed its two different layers i.e. upper layer containing ethyl acetate (evaporate it) and lower layer containing dried extracts (i.e. flavonoids) settled at the bottom [6]. For confirmation of its flavonoid content of three different medicinal plants using lead acetate test, small quantity of lead acetate solution is added and yellow precipitation appears. This yellow colour precipitation showed the presence of flavonoid.

2.3. Flow cytometric estimation on human blood samples pertaining to blood counts and CD14 monocyte surface marker

In order to determine the effect of crude flavonoid extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* using variable doses (6.25-100 mg; 50 µl) on human whole blood (samples received from Mangal Pathology lab, Baramati) for analysing its blood counts (lymphocytes, monocytes and granulocytes count) and estimated its CD14 monocyte surface marker using flow cytometer. For these studies, EDTA human whole blood (50 µl) was taken and then stained with or without CD14 FITC monocyte surface marker in presence of HBsAg (20 µg/ml; 10 µl) along with addition of variable concentration of crude flavonoid (6.25-100 mg; 50 µl) extracted from the leaves of *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum*. Incubate the samples for 2-3 h at 37°C, 5% carbon dioxide incubator. After incubation, add FACS lysing solution (2-3 ml) and incubate it for 8-10 minutes. Centrifuging and washing the samples with buffer PBS (two times). Collect the pellet and dissolved in PBS. Afterwards, the samples were prepared and finally processed through FACS Calibur, flow cytometer (BD Biosciences) [19, 20].

2.4. Cytotoxicity assay

For these studies, lysed human whole blood was cultured for 24 h in presence of variable doses (6.25-100 mg; 50 μ l) of crude flavonoids extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* along with HBsAg (20 μ g/ml; 10 μ l) and proceeds for cytotoxicity assay. After 24 h incubation, add MTT solution (2.5 mg/ml; 10 μ l) to each well of 96 well (flat bottom, tissue culture) plates. Incubate the plate for another 4 h in carbon dioxide incubator. Plates were centrifuged at 1800 rpm for 5 minutes and the supernatant was removed. Add DMSO (100 μ l solution) to the formazon crystals (purple coloured, observed under the microscope) and dissolved it. Finally, absorbance was evaluated in an ELISA reader (Perkin Elmer) at 570 nm [19, 20].

2.5. Statistical analysis

To determine its statistical analysis using one-way ANOVA (Boniferroni multiple comparison) test for determine the comparison between the control and treated groups of crude flavonoids extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum*.

3. RESULTS

3.1. Total blood count and CD14 monocyte surface marker

The effect of variable doses of crude flavonoids of *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* on total blood counts and CD14 monocyte surface marker of human whole blood using HBsAg. The results showed that there is dose dependent decline in total blood counts (Table 1) of human whole blood at higher doses as compared to control. Similarly, same response is reported in case of CD14 monocyte surface marker (Fig. 1). Over all, the data of total blood counts and CD14 monocyte surface marker at higher doses showed immunosuppressive activity.

3.2. Cytotoxicity assay

The effect of variable doses of crude flavonoids on lysed human whole blood pertaining

to its cytotoxicity as shown in Fig. 2. Human lysed blood cells were cultured for 24 h in the presence of variable doses of crude flavonoid along with or without HBsAg. The results showed that there was a significant cytotoxicity at higher doses as compared to control.

4. DISCUSSION

Flavonoids may be categorized into various types i.e. aglycones (monomeric); bi or tri or oligo flavonoids or with different levels of O-methylation or with one or more saccharide units that includes various types of acyl substituents [7, 8]. Recently, extensive work on flavonoids has been done or in progress but these are normally present in crude form especially in case of medicinal plant products [7-9]. In other words, the amounts of flavonoids present in most medicinal plant products are relatively small even though the visual impression is quite striking.

Flavonoid isolated from these three medicinal plants and determined its immunobiological activity against HBsAg and showed marked decline in case of total blood counts and CD14 monocyte surface marker at higher concentrations. In this study we show that CD14 expression may be used to identify monocytes that are present in human whole blood samples. The capacity to enumerate monocytes in human whole blood as part of an existing lymphocyte immunophenotyping platform, is an important addition to the screening repertoire [19, 20]. In the present study our group focused on immunobiological activity of these flavonoids from these medicinal plants that are responsible to inhibit CD14 surface marker and total blood counts in a concentration-dependent manner. HBsAg was used as the standard and exhibited enhancement of total blood counts and CD14 monocyte surface marker when compared to the control.

In contrast, comparing the human blood profile using flow cytometry pertaining to blood counts (lymphocytes, monocytes and granulocytes) to assess immunosuppressive potential against HBsAg. From the results that are shown in Table 1; it can be concluded that human whole blood is well suited for the evaluation of its immunosuppressive activity of flavonoid extracted from three medicinal plants.

Table 1. Effect of variable doses of flavonoids on human blood counts using flow cytometry.

Plant material	Doses (mg/ml; 50 μ l)	Lymphocytes	Monocytes	Granulocytes
<i>Mesua ferrea</i>	Control PBS	5.46 $\pm$ 0.78	3.86 $\pm$ 0.14	38.6 $\pm$ 2.18
	HBsAg, 20 μ g/ml; 10 μ l	11.94 $\pm$ 1.56	8.16 $\pm$ 0.52	52.14 $\pm$ 3.78
	6.25	9.18 $\pm$ 1.04	5.18 $\pm$ 0.92	46.8 $\pm$ 5.44
	12.5	8.76 $\pm$ 0.88	4.84 $\pm$ 0.78	39.4 $\pm$ 4.88
	25	7.48 $\pm$ 0.62	4.12 $\pm$ 0.34	36.2 $\pm$ 3.98
	50	6.14 $\pm$ 0.82	3.21 $\pm$ 0.18	32.2 $\pm$ 4.02
	100	4.24 $\pm$ 0.56	3.86 $\pm$ 0.14	28.12 $\pm$ 3.24
<i>Ficus benghalensis</i>	Control PBS	5.46 $\pm$ 0.78	3.86 $\pm$ 0.14	38.6 $\pm$ 2.18
	HBsAg, 20 μ g/ml; 10 μ l	11.94 $\pm$ 1.56	8.16 $\pm$ 0.52	52.14 $\pm$ 3.78
	6.25	12.24 $\pm$ 1.56	6.16 $\pm$ 0.24	38.2 $\pm$ 4.88
	12.5	10.14 $\pm$ 0.98	5.02 $\pm$ 0.58	32.18 $\pm$ 3.86
	25	7.42 $\pm$ 1.04	4.78 $\pm$ 0.82	29.6 $\pm$ 3.24
	50	6.24 $\pm$ 0.78	3.64 $\pm$ 0.76	24.6 $\pm$ 2.78
	100	5.18 $\pm$ 0.24	2.18 $\pm$ 0.18	20.18 $\pm$ 1.98
<i>Jasminum auriculatum</i>	Control PBS	5.46 $\pm$ 0.78	3.86 $\pm$ 0.14	38.6 $\pm$ 2.18
	HBsAg, 20 μ g/ml; 10 μ l	11.94 $\pm$ 1.56	8.16 $\pm$ 0.52	52.14 $\pm$ 3.78
	6.25	9.16 $\pm$ 1.46	6.88 $\pm$ 0.98	44.2 $\pm$ 5.78
	12.5	7.84 $\pm$ 1.12	6.16 $\pm$ 0.78	34.8 $\pm$ 4.84
	25	6.78 $\pm$ 0.76	5.94 $\pm$ 0.72	31.2 $\pm$ 3.16
	50	5.44 $\pm$ 0.86	4.54 $\pm$ 0.66	27.4 $\pm$ 2.88
	100	4.89 $\pm$ 0.28	3.12 $\pm$ 0.22	19.6 $\pm$ 1.88

Flow cytometric analysis of crude flavonoids (6.25-100 mg/ml; 50 μ l) extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* for determine its effect in human whole blood against HBsAg containing lymphocytes, monocytes and granulocytes count. Data acquisition of 10000 events and fraction or separation of cell populations representing different phenotypes analysed using cell quest software.

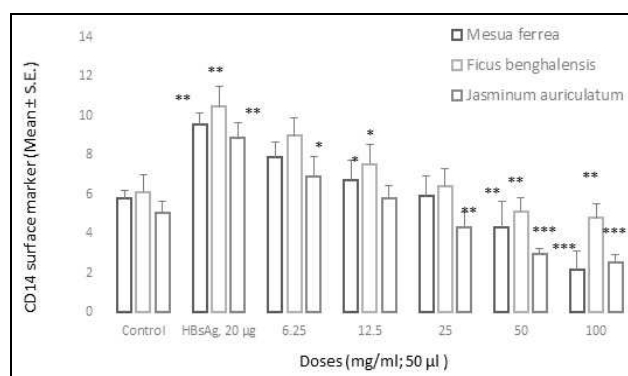


Figure 1. Effect of variable doses of flavonoid on CD14 monocyte surface marker. Lysed human whole blood were treated with variable doses of flavonoid along with HBsAg (described in materials and methods section) and then lysed and wash the cells with phosphate buffered saline and analysed through flow cytometer (FACS, Calibur) using forward and side scatter gating applied for data acquisition of 10000 events of cell populations representing different phenotypes analysed using cell quest software. *P* values: **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compared to control.

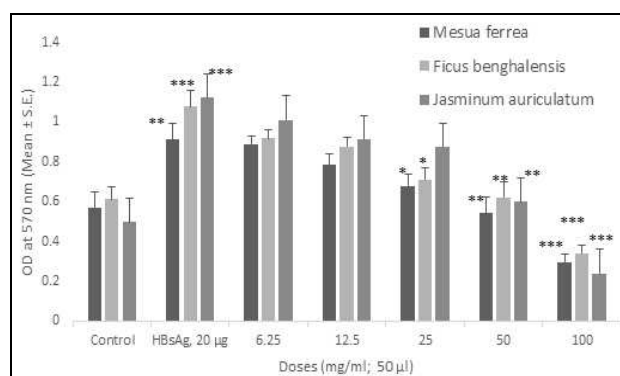


Figure 2. Cytotoxicity assay. Lysed human whole blood were cultured with HBsAg along with variable doses of flavonoid or HBsAg alone. After 24 h, proliferation was measured by MTT assay. The results are presented as Mean $\pm$ S.E. *P* values: **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compared to control.

In summary, flavonoids extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* were shown to suppress the release of mediators related to immunosuppression at higher doses in the form of CD14 monocyte surface marker and blood counts. The result obtained from the experiment it is concluded that flavonoids extracted from *Mesua ferrea*, *Ficus benghalensis* and *Jasminum auriculatum* having immunosuppressive activities. The results support the presence of biologically active components which may be worth further investigation and elucidation.

The effect of the crude flavonoid isolated from these medicinal plants at various concentrations on lysed human whole blood and was evaluated its cytotoxicity against HBsAg using MTT assay. The optical density of proliferation of lymphocytes at different concentrations were given in Fig. 2 and the results of this experiment showed that the flavonoid isolated from these medicinal plants inhibited HBsAg stimulations at higher concentrations, indicating its potential as an effective immunosuppressive compound.

5. CONCLUSIONS

These results, in assemblage with the potent inhibitory activity of crude flavonoid isolated from these medicinal plants in human whole blood against HBsAg. Further studies are necessary to isolate, characterize and elucidate the structures of these flavonoids that are present in crude form and should be responsible for eliminating several diseases.

AUTHOR'S CONTRIBUTION

This work was carried out in collaboration between two authors. AG performed the experimental work, collect the preliminary data and produced the initial draft. SRC managed the literature survey and approved the final manuscript. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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