

IMMUNOMODULATORY ACTIVITIES OF LEAF EXTRACT OF *PHOENIX SYLVESTRIS* & STUDY OF HAEMATOLOGY *IN VI VO* STUDY

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ABSTRACT

Plant extracts have been widely evaluated for biological properties. In the present study, the Immunomodulatory effect of leaf extracts of phoenix silvestris was investigated in mice against mefenamic acid induced immunomodulation model. The first group was treated with normal saline, second group was treated with mefenamic acid (100mg/kg) and last group was treated with mefenamic acid 100mg/kg + extract (100mg/kg). The blood sample from the each group was withdrawn. The result shows that the mefenamic acid lower the cell count which was observed in group second, leaf extract of phoenix

silvestris increase the cell count which was observed in group III which was treated with extract and mefenamic acid.

KEYWORDS: Immunomodulation, Phoenix Silvestris, Cell count, mefenamic acid.

INTRODUCTION

Investigation of compounds with immunomodulatory activities has been going on for some times now. Most of the search has been concentrated on lower organisms. A number of compounds have been isolated from bacterial cell wall and shown to possess immunomodulatory activities.^[1] The hot water extract of *Pleurotus ostreatus* mycelium has been shown to possess immunostimulatory effect on cyclophosphamide treated mice.^[2] The extract of the edible mushroom *Agaricus blazei* has been demonstrated to significantly increase serum IgG levels, T-cell populations and polymorph nuclear neutrophils leucocytes phagocytic activity in mice.^[3]

In recent times, many researchers have been turning to higher plants as a source of immunomodulators. Many traditional herbal plants have been used since ancient times because of their antiallergenic, anti-inflammatory, and antipruritic activities. Among 250,000 known species, only a small percentage have been investigated phytochemically and even a smaller portion have been submitted to biological and pharmaceutical screening. Unfortunately, the effects and mechanisms of the activities of plant derived drugs on the immune system have not been well defined.^[4] It is only in the most recent times that scientific literature is continuously reporting plant drugs having immunomodulatory activity. Most of the leads for this activity are from traditional medicines from different parts of the world.^[5, 6, 7]

The aim of this research work is to determine the Immunomodulatory effect of leaf extracts of *Phoenix sylvestris* against mefenamic acid induced immunomodulation in mice.

Extraction

Phoenix sylvestris (fresh leaves) was collected from local area around from Bhopal in the month of September. The material was washed thoroughly in running tap water, rinsed in distilled water.^[8] Leaves were shade dried for one week in open air, cut in to small piece, reduced leaves using laboratory blender for 5 min at high speed and then stored in airtight closed bottles for two days. 500g of phoenix sylvestris (100g in each batch) dried crushed leaves was taken in a paper cone and placed into soxhlet apparatus. 500 ml of solvent (ethanol) was taken in the round bottom flask and the extraction was carried up to the six cycles at 40-50 °C. Solvent got vaporized and raised up-to the condenser where it condensed and falls into the plant sample and extract compound falls into the RBF by successive solvent extraction method.

Phytochemical Screening

Test for carbohydrates

0.5g. of each powder was dissolved separately in 5ml of distilled water and filtered. Few drops of Molisch's reagent were added to each solution, this was then followed by addition of 1ml of concentrated H₂SO₄ by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5ml of distilled water. Formation of red or dull violet colour at the interphase of the two layers was taken as positive test.

Test of alkaloids

0.1g of each powdered was dissolved in 5ml of methanol separately and the filtered. 2ml of each filtrate from each sample were stirred with 5ml of 1% aqueous HCL on water bath and the filtered. From the filtrate, 1ml was taken individually into two test tube. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. to the second 1ml, Mayer's reagent was added and appearance of buff-coloured precipitate was taken as positive test for the presence of alkaloids.

Test of steroids

0.2g of crude powder of each sample was dissolved in 2 ml of acetic acid separately; the solution was cooled well in ice followed by the addition of concentrated H₂SO₄ carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring.

Test for saponins

1g of crude powder of each sample was boiled with 5ml of distilled water separately and then filtered. To each filtrate, about 3ml of distilled water was further added and shaken vigorously for about 5 minute. Frothing which persisted on warming was taken as an evidence for the presence of saponins.

Test for flavonoids

About 0.5 g of each powder was dissolved in 5 ml of ethanol separately, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of concentrated HCL. A pink, orange or red to purple colouration indicates the presence of flavonoids.

Test for tannins

About 0.5g of each portion of crude powder was stirred with about 10 ml of distilled water separately and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of each filtrate and occurrence of a blue- black, green or blue-green precipitate indicates the presence of tannins.

Detection of Phenol

When 0.5 ml of FeCl₃ (w/v) solution was added to 2 ml of test solution, formation of an intense colour indicated the presence of phenols.

In-vivo study

Experimental Animals Current study utilized 18 albino mice (45-50gm) aging between 6 and 8 weeks. Mice were housed in the animal house of NRI institute of pharmaceutical sciences, at standard environmental conditions (ambient temperature of $25 \pm 2^\circ\text{C}$). Mice were maintained at standard pellet diet and water ad libitum. All the experiments were approved by the Institutional Animal Ethics Committee, NRI institute of pharmaceutical sciences, Bhopal.

Grouping of animals

The 18 animals were divided in to 3 grouped.(49)

I Group	Control (normal saline)
II group	Treated with mefenamic acid (100 mg/kg orally)
III group	Treated with extract + mefenamic acid (100mg/kg+100mg/kg)

1ml Blood sample of mice after the dosing was collected by cardiac puncture method in the three different heparinised tubes for CBC test and 2 ml blood sample was taken in 3 different tubes for RFT test.

The kidneys removed. A section was fixed in formalin for 24hr before processing for histological examination.

Haematology

Sample was given to the Department of pathology Balaji fracture hospital Bhopal

RESULT AND DISCUSSION***Phytochemical screening***

The phytochemical screening of the ethanolic extract of leaves of phoenix sylvestris was performed for various phytochemical constituent and the constituent was given in table.

Hematology

The mefenamic acid suppresses the immune system when given to the animal. In group 1st the Hematological parameter was found as normal as compare to group 2nd. The WBC and other parameter was decrees in group 2nd were animal was treated with mefenamic acid. It shows that the mefenamic acid decrees the hematological parameter. The 3rd group treated with mefenamic acid and extracted and the result shows that WBC and other hematological parameter was increased.

Table no. 1: Phytochemical screening of leaf extract of phoenix sylvestris.

S.No	Test	Positive	Negative
1.	Carbohydrates	+	-
2.	Alkaloids	+	-
3.	Steroid	+	-
4.	Saponins	-	+
5.	Flavonoids	+	-
6.	Tannins	+	-
7.	Phenol	-	+

The following parameter was observed in complete blood count (CBC) test.

Table no.2: Complete Blood Count.

Sr.no	Complete Blood Count	Sample 1	Sample 2	Sample 3
1.	Hemoglobin (gm/dl)	11.5±0.147	11.3±0.089	11.4±0.141
2.	R.B.C. count (mil/cmm)	4.39±0.029	4.29±0.02	4.37±0.152
3.	Total WBC count (/cmm)	3700±85.75	1500±107	2500±0.42
4.	Packed cell volume (%)	34.9±0.35	34.1±0.12	34.5±0.36
5.	Mean corpuscular volume(cu micron)	79.5±1.41	79.49±0.41	78.95±0.97
6.	Mean corpuscular hemoglobin (picogra.)	26.2±0.28	26.49±0.84	26.09±0.28
7.	Mean corpuscular Hb con.(g/dl)	32.95±0.84	33.14±0.69	33.04±1.32
8.	Neutrophils (%)	67±1.41	52±2.36	61±0.141
9.	Lymphocytes (%)	26±2.35	38±0.47	32±0.85
10	Eosinophil (%)	04±0.47	04±0.16	02±0.06
11	Monocytes (%)	03±0.89	06±0.45	05±0.23
12.	Basophils (%)	00	00	00

Where n= 6/group (value was expressed in mean and standard deviation)

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