

Evaluation of Immunostimulant Activity of the Root and Leaf of *Polyscias Balfouriana Marginata*

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ABSTRACT

The *Polyscias balfouriana* is an ornamental plant widely available throughout the tropical region. In some collections the plant is known as Scutellarium or Dinner Plate Aralia or Balfour Polyscias. It belongs to the family *Araliaceae*. The plant is rich in triterpenoid saponins. In the present work the immunostimulant activity was performed on the leaf and root of the plant. The plant parts were extracted with n-butanol to obtain the saponin fraction; hence it was designated as NBS. The carbon clearance test and the milk induced leukocytosis were determined. Since the family and chemical constituents were similar to *Panax ginseng*, the root powder of it was used as the standard. It was found that the root extract of *Polyscias balfouriana* showed better carbon clearance and milk induced leukocytosis activity than the leaf extract on comparison with the standard ginseng which gave the highest positive response.

Keywords: *Polyscias balfouriana*, immunostimulant, milk induced leukocytosis.

INTRODUCTION

Polyscias balfouriana variety Marginata is an ornamental foliage shrub cultivated in gardens. These plants are popularly known in trades and horticulture nurseries as "aralias" since they belong to the family *Araliaceae*.^[1] The plant is also known as Scutellarium or dinner plate Aralia or Balfour Polyscias. Plants coming under the family *Araliaceae* are mainly constituted by triterpenoid saponins. It is a native of New Caledonia. The triterpenoid saponin content in this family play an important role in the pharmacological activity like stimulation of CNS, anti fatigue and enhancement of non-specific resistance. In the present work the immunostimulant activity of the root and leaf saponin extracts was performed. Ginseng (*Panax quinquefolium*) a tropical plant is one of the few commercially important members of this family.^[2] The crude extracts as well as its pure glycoside (panaxoside) was clinically employed for premature ageing and as revitalize.^[3] Hence in this work ginseng was kept as the standard drug.

The botanical source consists of the leaves and roots of *Polyscias balfouriana* Marginata belonging to the family *Araliaceae*. The order is Umbellales. The plant is available throughout the warmer parts of India, especially in Kerala and Tamil Nadu. It is also available in Irotropical Asia, Malaya.

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MATERIAL AND METHOD

The plants *Polyscias balfouriana* was collected from Tamil Nadu agricultural university, Coimbatore and was identified and authenticated by the Agricultural University, Coimbatore. A herbarium was prepared and submitted in to the University. The complete pharmacological work was explained before the animal ethical committee and the written protocol was submitted. After the approval the animal experiments commenced. All chemicals and reagents used in this work are of analytical grade or above.

Extraction process^[4-5]

The fresh leaves and roots were extracted for 72 hours with 70 % ethanol by hot continuous extraction using Soxhlet apparatus. The extracts obtained were concentrated under vacuum distillation below 60°C. They were diluted with water and further extracted with chloroform to remove the lipid materials. The water extracts left behind were extracted with ethyl acetate and then with n-butanol. The n-butanol layers were separated and evaporated to dryness to give the crude saponin extracts. This was designated as NBS extract.

Preliminary chemical studies^[6]

In the preliminary chemical tests the NBS extract gave highly positive results for triterpenoids and gave good colour reactions for Salkowsky test and Liebermann burchard test.

Acute toxicity studies

Animal studies were conducted after getting clearance from the institutional animal ethical committee. The acute toxicity studies of the NBS extracts of the root and the leaf were performed by the method of Smith(1960)^[7] and it was

proved that the extracts were non toxic and safe up to a dose of 2.5 g/Kg body weight.

Carbon clearance test^[8-9]

Swiss albino mice of either sex weighing between 25-30g were used for the screening. They were divided into 7 groups of 5 animals each, and then the animals were marked for their identity. Animal groups I-IV received NBS extracts of root and leaf of *Polyscias balfouriana* dissolved in 2 % saline at doses 250 mg/kg and 500mg/kg body weight. Group V and VI received root powder of white *Panax ginseng* at a dose of 250 mg/kg and 500 mg/kg. The group VII animals received 2 % saline. The scheduled doses were administered to all the groups orally for 15 days prior to the injection of carbon particles. On the 16th day the animals were injected 0.1ml of carbon suspension (Pelikan Tuschea ink, Germany) intravenously through tail vein. Blood samples were collected from the retro-orbital plexus immediately at 3, 6, 9, 12 and 25 minutes after injection of carbon suspension. Blood samples of 25 mcl were taken from all the animals and were analyzed with 2 ml of 0.1% acetic acid and then the samples were measured for absorbance spectrophotometrically at wavelength of 675 nm. The graph of absorbance against time was plotted for each animal in respective group. The rate of carbon clearance, termed as Phagocytic index is the slope of time concentration curve obtained from the graph. The mean phagocytic index was calculated for each group and is tabulated in Table 1.

Milk induced leukocytosis^[10]

Swiss albino mice of either sex weighing between 25-30 g were used for screening. They were divided into four groups of 5 animals each. The animals were marked for their identity. Milk was injected (0.1ml subcutaneously) to induce leukocytosis. Blood samples were collected from the tail vein of the animals and leukocyte count was determined. Now the animals of group I and II received NBS root and leaf extracts dissolved in 1 % CMC at doses of 50, 100 and 250 mg/kg body weight respectively for 3 days. Group III received root powdered of white *Panax ginseng* dissolved in 1 % CMC at dose of 50, 100 and 250 mg/kg body weight respectively. Group IV animals received 1 % CMC. All the groups of animals received their doses scheduled orally with the milk injection (0.1ml subcutaneously) for a period of 3 days. The dose of NBS extracts required for 50 % reduction in leukocytosis was determined. The results are tabulated in Table 2.

RESULT

The effect of NBS extracts of leaf and root of *Polyscias balfouriana* and root powder of white *Panax ginseng* on carbon clearance test on mice was studied. It was confirmed that the root powder of white *Panax ginseng* gave very good carbon clearance activity at a dose of 500 mg/kg (phagocytic index=0.0911) and NBS root extract of *Polyscias balfouriana* gave a better carbon clearance activity at a dose of 500 mg/kg body weight (phagocytic index=0.0619) than the other doses of root and leaf extracts. It was observed that root and leaf extract of *Polyscias balfouriana* possess good anti-leukocyte activity .the best activity was found to be at a dose of 136 mg/kg of root extract.

DISCUSSION

Adaptogens are the substances which help to increase resistance of the body towards noxious influence. *Panax ginseng* is claimed to be the "elixir of life" in the traditional chinese system of medicine. Thereafter ginseng has been

extensively investigated, experimentally and clinically and has been claimed to be a good immunostimulant. Studies have proven that environmental pollution and routine habits has an influence in the oxidative stress and immunodeficiency syndromes in homosapiens. Therefore importance of a drug to boost the immunity to acquaint with the present environment has been identified and a sincere attempt has been done to identify the immunostimulant and carbon clearance activity of *Polyscias balfouriana*. Milk induced leukocytosis and carbon clearance test after parenteral route of administration of cow milk and carbon suspension subsequently in mice has proved that *Polyscias balfouriana* root and leaf extracts have a comparable result with that of ginseng in the study based on the immunostimulant activity. Hence it can be used as a suitable substitute for ginseng.

Table 1: Determination of Phagocytic index

Animal group	Drug	Dose mg/kg	Phagocytic index
Group1	PBML	250mg/kg	0.0266
Group2	PBML	500mg/kg	0.0412
Group3	PBMR	250mg/kg	0.0452
Group4	PBMR	500mg/kg	0.0619
Group5	ginseng	250mg/kg	0.0721
Group6	Ginseng	500mg/kg	0.0911
Group7	2 % saline	2ml/kg	0.0103

Table 2: Determination of milk induced leukocytosis

Animal group	Drug	1 st day dose (mg/kg)	2 nd day dose (mg/kg)	3 rd day dose (mg/kg)	Reduction in 50% leukocyte count	Actual dose for 50% reduction in dosage (mg/ml)
Group1	PBMR	50	100	250ml	2200	136
Group2	PBML	50	100	250ml	3125	200
Group3	Ginseng	50	100	250ml	2875	99
Group4	1%CMC	2ml	2ml	2ml	4500	4500

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