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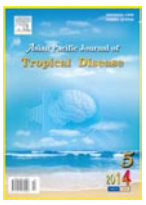

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Immunomodulatory activity of an aqueous extract of *Polygonum minus* Huds on Swiss albino mice using carbon clearance assay

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PEER REVIEW

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Comments

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ABSTRACT

Objective: To explore the immunomodulatory properties of aqueous extract of *Polygonum minus* Huds (*P. minus*), on swiss albino mice using carbon clearance assay.
Methods: The study was performed using *in-vivo* carbon clearance assay or phagocytosis. Aqueous extract of *P. minus* was administered by oral route to Swiss albino mice for seven days. Levamisole, 2.5 mg/kg was used as standard drug.
Results: The phagocytic function of reticuloendothelial system was significantly ($P<0.05$) enhanced by levamisole and *P. minus* aqueous extract groups compared to the control. Treatment with aqueous extract of *P. minus* at 200 and 400 mg/kg body weight resulted in dose dependent increase in phagocytic index (K) where phagocytic index of aqueous extract of *P. minus* at 400 mg/kg body weight is comparable to the standard drug Levamisole at 2.5 mg/kg body weight.
Conclusions: Aqueous extract of *P. minus* has exhibited significant increase in phagocytosis at doses of 200 mg/kg body weight and 400 mg/kg body weight.

KEYWORDS

Carbon clearance assay, Immunomodulatory activity, *Polygonum minus*, Phagocytic index

1. Introduction

Medicinal plant products are known to possess immunomodulatory properties and generally act by stimulating both specific as well as non-specific immunity[1].

Polygonum minus Huds (Polygonaceae) (*P. minus*), known as Kesum in Malaysia is a herb commonly used as a flavoring ingredient. It is a potent natural antioxidant[2-4], and rich in beta-carotene, vitamin A and vitamin C as well as minerals, flavonoids and catechins[5-7]. Since *P. minus* is rich in antioxidants, this study was planned to evaluate its macrophage stimulatory activity by assessing phagocytic index using carbon clearance assay method in Swiss albino mice.

2. Materials and methods

2.1. Chemicals

Carboxy methyl cellulose was purchased from Himedia Mumbai; Rotring drawing ink, from Sanford GmbH, D-22510 Hamburg, Germany; Levamisole from Sigma Aldrich, Bangalore. All other reagents used were of analytical grade.

2.2. Plant material

Aerial parts including stems and leaves of *P. minus* Huds plant was procured from Biotropics Malaysia Berhad, Malaysia and identified on the basis of exomorphic

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characters and review of literature by Mr. Shamsul Khamis, Taxonomist from Institute Bioscience, University Putra Malaysia. The voucher specimen of the sample (SK 2077/12) was deposited in the Herbarium of Institute Bioscience, University Putra Malaysia, Malaysia.

2.3. Extraction and fractionation-phytochemicals

The aerial parts of the plants including stems and leaves were dried by oven for 48 h at a temperature of 40 °C and shredded to 2 to 5 cm in size; then subjected to percolation using purified water and extracted at a temperature of about 80 °C with an extraction ratio of approximately 1:10. The extract was further filtered, concentrated using rotary evaporator with water bath temperature at 65 °C and freeze-dried. The recovery yield was 12%.

2.4. Animals

Male/female Swiss albino mice, 8-10 weeks old and weighing 20-30 g, obtained from Ethix Pharma, Chhattisgarh, India, were used in four groups, for the study. All animal experiments complied with the approval of Institutional Animal Ethics Committee (approval No. BIO-IAEC-479, dated September 2012).

The animals were housed under standard laboratory conditions (temperature 20-23°C), relative humidity of (53%-64%), air-conditioned with air supply (12-15 air changes per hour), 12:12 h light:dark cycles and fed with Nutrilab rodent feed (Provimi Animal Nutrition Pvt. Ltd., India) and water was given *ad libitum*. Drugs for oral administration were freshly prepared as a homogenized suspension of aqueous extract at doses of 200 and 400 mg/kg body weight in 0.5% carboxy methyl cellulose and administered orally, once daily for 7 d to Swiss albino mice[8]. Levamisole at the dose of 2.5 mg/kg body weight (*p.o.*) was used as a standard immunostimulant drug[9].

2.5. Experimental protocols

All experimental protocols and the number of animals used for the experimental work were duly approved by Institutional Animal Ethics Committee.

2.6. In-vivo phagocytosis (carbon clearance method)

The mice were divided into four groups of 8 animals each (4 male and 4 females). Group I (control) was given 0.5% carboxy methyl cellulose in water (10 mL/kg body weight, orally) for 7 d. Mice in Group II-IV were given the standard drug (levamisole 2.5 mg/kg body weight, *p.o.*), aqueous extract of *P. minus* at doses of 200 mg/kg body weight and 400 mg/kg body weight, *p.o.*, for 7 d respectively. At the end of 7 days, after the gap of 24 h, the mice were injected, via the tail vein, with 0.1 mL 1.6% (v/v) rotring drawing ink suspension in 1% (w/v) gelatin in saline. Blood samples were drawn, from retro orbital vein at interval of 0 and 15 min. A 50 μL blood sample was mixed with 0.1% sodium carbonate solution (4 mL) and absorbance was measured at 660 nm. The carbon clearance was calculated using the following equation: (Log_e OD_1-Log_e OD_2)/15, where OD_1 and OD_2 are optical densities of blood sample at 0 and 15 min, respectively.

2.7. Statistical analysis

Data were expressed as mean±SEM and statistical analysis was carried out employing the ANOVA followed by Dunnett post-hoc test, which compared the test groups and standard group with the control group.

3. Results

Aqueous extract of *P. minus* exhibited significant phagocytic activity ($P<0.05$) at doses of 200 mg/kg body weight (0.045±0.006) and 400 mg/kg body weight (0.062±0.005) compared to the control group (0.027±0.002) and was dose dependent (Figure 1). The phagocytic activity of the aqueous extract dosed at 400 mg/kg was comparable to the standard (0.060±0.004) levamisole ($P<0.05$) at 2.5 mg/kg body weight.

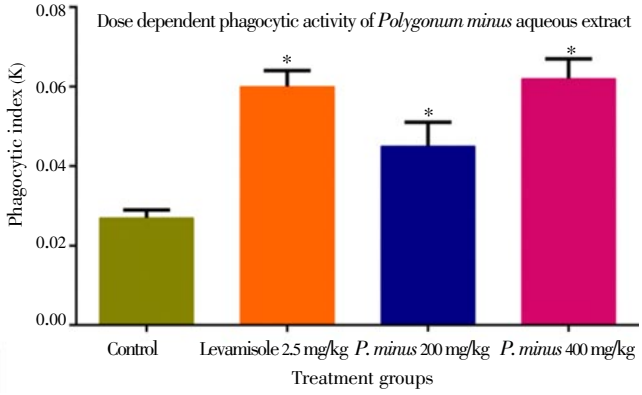

Figure 1. Dose dependent phagocytic activity of *P. minus* aqueous extract.
*: $P<0.05$ compared to control.

4. Discussion

When ink containing colloidal carbon is injected intravenously, the macrophages engulf the carbon particles of the ink and the rate of clearance of ink (carbon particles) from blood is known as phagocytic index. Aqueous extract of *P. minus* stimulates the reticuloendothelial system by significantly increasing the phagocytic index. Phagocytes play an integral role in the host defense and produces reactive oxygen species by macrophages reaction to foreign agents into the body. Phagocytic cells are sensitive to oxidative damage thus affecting the balance between pro-oxidant production and antioxidant defense in cell function. Disturbance in this balance in oxidants represents an oxidative stress[10]. *P. minus* is known for its antioxidant flavanoids[11]. In recent radical absorbance capacity evaluations, *P. minus* aqueous extracts has recent radical absorbance capacity values in between 16 000 to 35 000

(Biotropics Malaysia Berhad, unpublished data, 2012). The study on the mechanism of action is planned for the future. The plant can be explored for its medicinal utilization in treatment of immunodeficiency diseases and as combinational therapy with antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

This study evaluate the immunostimulatory effect of *P. minus* aqueous extract on macrophage phagocytosis using *in vivo* carbon clearance test. Only two concentrations of extract and one positive control (levamisole) were used in this study. This is a very preliminary study.

Research frontiers

This is the first report on the immunostimulatory effect of *P. minus* aqueous extract on macrophage phagocytosis. However, as mentioned in the first column, this study is too preliminary. If author collect the serum and carry out the serum antioxidant evaluation the manuscript can be strengthened.

Related reports

P. minus was well studied for its antioxidant, antimicrobial and antiulcer effect previously (Scopus search showed 38 match references). Besides, acute toxicity was previously published. However, the immunostimulatory effect of this plant was not previously reported.

Innovations & breakthroughs

This paper has shown the potential of *P. minus* as immunostimulant to activate phagocytosis of macrophage in dosage dependent manner. However, this study is too preliminary.

Applications

P. minus was shown by the author as immunostimulant. Thus, this result should encourage the author to further evaluate the detail immunostimulatory mechanism of this plant extract. Furthermore, isolation of active compounds that responsible for this effect should be the author's next focus. With these, standardized *P. minus* can be the potential immunostimulant to strengthen the immunity.

Peer review

Generally, this study showed the potential of *P. minus* to activate macrophage phargocytosis. The result is interesting. The study is helpful for the researchers to further evaluate the detail immunostimulatory mechanism of this plant extract.

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