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Haematological and immunomodulatory evaluation of aqueous extract of *Parthenium hysterophorus* leaves on wistar rats

Praveen Kumar Vemuri*[1], Bhavana Talluri[1], Vasanthi Sirikonda[2], Tejaswi Padigala[3] and Vijaya Lakshmi Bodiga[5]

[1]*Centre for Genomics and Proteomics, Department of Biotechnology, K L University, Vaddeswaram, Guntur District, Andhra Pradesh, India*
[2]*Department of Bioengineering and Technology, Gauhati University, Assam, India*
[3]*School of Bioengineering and Biosciences, Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, India*
[4]*Department of Molecular Biology, Institute of Genetics & Hospital for Genetic Diseases, Osmania University, Hyderabad, Telangana, India*

ABSTRACT

Parthenium hysterophorus has widely reported traditional uses which include its use for stimulation and purification of platelets as well as body defense. Experiments were carried out to assess the impact of *Parthenium hysterophorus* leaf extract on hematological parameters of wistar rats. The results revealed that the extract (10–100 mg/kg p.o.) produced no significant change in the packed cell volume, haemoglobin, red blood cell count, total leucocyte count, monocyte, basophil, eosinophil, bleeding time and clotting time. The dose of 100 mg/kg p.o. produced a significant ($P<0.05$) increase in neutrophil and decrease in lymphocyte. Platelet count was significantly ($P<0.05$) increased at the dose of 100 mg/kg p.o. The results did not support the traditional use of the plant leaf for stimulation of blood production. It however showed improvement of non-specific immune responses involving phagocytosis and inflammation.

Keywords: *Parthenium hysterophorus*, Haematology, Inflammation, Toxicity, Wistar rats

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INTRODUCTION

Parthenium hysterophorus of Asteraceae family has been reported to have traditional uses in different parts of the world [1]. This noxious weed was first recorded in 1810 at Arunachal Pradesh and Nagaland and then in 1955 at Pune [2]. In India, studies were carried out to elucidate the use of this plant owing to its antioxidant and anti-inflammatory properties [3]. Studies also confirmed about the aqueous extract of *P. hysterophorus* which contains free amino acids, glucose, galactose and potassium chloride [4], promotes platelet count improvement [5], body defense system [6] as well as fertility [7]. Reports have shown that activation of the host's immunological system by any foreign stimulus leads to a spectrum of cellular and humoral events comprising of several effector mechanisms involving several cell types, cell products and soluble serum factors [8]. The origin of these cells are hematopoietic stem cells, therefore shows a positive correlation between the haematologic activities of the body and the immunologic processes. It is an indication that boosting the body's haematological level will possibly boost the immune state of a host [9]. Hence, it is worth to study of *P. hysterophorus* in the field of hematology and immunology concerning their traditional uses. Immunomodulatory therapy could provide an alternative to conventional chemotherapy against variety of diseased conditions to achieve desirable effects [10].

MATERIALS AND METHODS

2.1 Plant material

Leaves of *Parthenium hysterophorus* were collected from Green Fields of K L University, Guntur District, Andhra Pradesh, India. They were then grinded in a mortar with one litre of milliQ water and homogenized for over 3 h period on an orbital shaker (Scigenics, Mumbai) to ensure maximum mixing for preparation of smoothie [11]. The smoothie was spin at 14,000 rpm (Thermo, MicroCL 21 Microcentrifuge) in cold conditions for 12 min and supernatant was removed and stored at 4^0C until further analysis. A yield of 25% w/w extract was obtained for subsequent studies.

2.2 Experimental Animals

Wistar rats weighing between 83.6 – 128.5 g, from S R Biotechnologies, Bangalore and were used for the experimental studies. The animals were maintained under normal environmental temperature (26–28^{o}C), approximately 12 h day and night illumination cycle. The animals were provided with commercial rat feed supplied by Hindustan Lever Ltd, Mumbai [12].

2.3 Acute Toxicity Studies

The estimation of the median lethal dose (LD_{50}) for the extract was done in Wistar rats orally [13]. The extract was administered in biphasic manner using dosages ranging between 10 and 100 mg/kg. The animals were observed for 72 hours for behavioral effects such as nervousness, ataxia, excitement, alertness, dullness and death. The LD_{50} was calculated as the geometric mean of the dose that caused 100% mortality and that which cause 0% mortality.

2.4 Haematological Studies

Wistar rats were grouped into four of five rats each, first group received normal saline (10 ml/kg p.o.) and served as the control. The second, third and fourth groups received the extract (10, 50 and 100 mg/kg p.o.) once daily for 14 days. On the 15th day, all the animals were anaesthesized with chloroform, sacrificed and their blood collected in EDTA anti-coagulant bottles. Haematological methods [14] which includes haemoglobin (Hb), packed cell volume (PCV), red blood cell count, total leucocyte count (WBC), differential leucocyte count, platelet count, bleeding time and clotting time were performed.

2.5 Anti-inflammatory Study

Wistar rats used for the investigation were deprived of water during the experiment to ensure uniform hydration and minimize variability in oedematous response [15]. They were divided into five groups (n = 5). The first group received normal saline (20 ml/kg i.p.) and served as negative control. Three doses of the extract (10, 50 and 100 mg/kg) were administered intraperitoneally to the second, third and fourth groups respectively while acetylsalicylic acid (ASA; 100 mg/kg i.p) was given to the fifth group as a reference standard. Inflammation was then induced 30 min post treatment by injecting 0.1 ml of fresh egg albumin into the sub-plantar surface of the right hind paw of each of the rats. Zero readings were taken twice before injection of egg albumin (0 min) and at 20 min intervals after the injection of egg albumin over a 2 h (120 min) period. The edema at every interval was calculated in relation to the mean paw volume before the injection of the egg albumin. Activity for the treated groups was expressed as percent inhibition of inflammation in relation to the control group.

2.6 Statistical Analysis

The results of the studies were expressed as mean ± SEM. The difference between the control and treated means were analysed using one-way analysis of variance (ANOVA). Student t-test was used where ANOVA showed significant difference. Statistical significance was established at $P < 0.05$. Results were presented as tables and diverse charts (histograms, line graphs).

RESULTS AND DISCUSSION

3.1 Acute Toxicity Studies

No overt toxicity signs or death were observed in rats and mice, 72 h post oral treatment with doses between 10 – 1000 mg/kg. Hence, the estimated oral LD_{50} of *P. hysterophorus* leaf base extract in rats is ≥ 1000 mg/kg. The rats treated intraperitoneally (i.p.) with 10 – 1000 mg/kg doses showed no overt toxicity sign or death 24 h post treatment. However, all the rats treated with 1000 mg/kg i.p. dose became recumbent and died within 48 h of the intraperitoneal treatment while those treated with 10 – 400 mg/kg i.p. doses neither showed toxicity signs nor death 72 h post i.p. treatment. Hence, the intraperitoneal LD_{50} based on 24 h and 48 h post treatment observation times were ≥ 1000 mg/kg i.p. and 714.2 mg/kg i.p. in rats. The mice treated intraperitoneally with extract doses ≤ 700 mg/kg showed neither toxicity signs nor death 24 h post treatment. At the dose of 800 mg/kg i.p., the mice were calm, dull, had increased respiratory rate with mortality of 66.7% and 100.0% occurring within 24 h and 48 h of i.p.

treatment respectively. The mice treated intraperitoneally with 1000 mg/kg dose became calm, dull and recumbent with increased respiratory rate. A mortality of 100.0% occurred at this dose within 24 h. The estimated intraperitoneal median lethal dose in mice was 750.0 mg/kg i.p. and 850 mg/kg i.p. for 24 h and 48 h post treatment observations respectively.

3.2 Haematological Studies

Both increases and decreases were observed at all the tested doses for such parameters as haemoglobin (Hb), packed cell volume (PCV), total red blood cells, total leucocyte count and bleeding time. However, none of these changes was significantly different from the control. Increases and decreases were also observed in neutrophil and lymphocyte indices. However, the increase observed for neutrophil was only significant ($P < 0.05$) at the dose of 100 mg/kg p.o. while the decrease observed in lymphocyte was only significantly ($P < 0.05$) different from control at dose of 100 mg/kg p.o. Also, there was a general but non-significant decrease in the monocyte count. The basophil and eosinophil counts replicated the control count. A general but non-significant decrease was observed in the clotting time while there was increased platelet count in all the doses. The platelet count elevation was significant at the dose of 100 mg/kg p.o. (Tables 1 and 2).

Table 1: Effect of aqueous extract of *P. hysterophorus* leaf on some haematological indices of rats treated orally for two weeks

Treatment (2 weeks)	Hb (g/dL)	RBC (x $10^{12/L}$)	WBC (x $10^{9/L}$)	Platelet (x $10^{9/L}$)	BT (sec)	CT (sec)
Control	12.72±0.38	4.84±0.16	7.78± 0.59	468.0 ± 14.5	31.4±4.1	43.2 ± 2.7
Test (Ph)						
10 mg/kg	12.16±0.55	4.78± 0.24	8.22 ±1.00	476.0 ± 17.7	38.0±6.4	38.0 ± 2.5
50 mg/kg	12.86±0.60	5.10±0.13	8.28±1.20	491.0 ± 18.7	41.4±6.7	36.4±2.8
100 mg/kg	12.26±0.30	4.86±0.29	7.50±1.10	514.8± 18.9*	23.2±3.1	38.4±3.7

Hb = haemoglobin; WBC = White Blood Cell; p.o. = Per os (per oral); RBC = Red Blood Cell; BT = Bleeding Time; CT = Clotting Time.
**= P<0.05, significantly different from control (One-way ANOVA; Student t-test).*

Table 2: Effect of 70% v/v aqueous extract of *P. hysterophorus* leaf on differential leucocyte count of rats treated orally for two weeks

Treatment (2 weeks)	Differential Leukocyte Count (%)				
	Neu.	Lym.	Mon.	Eos.	Bas.
Control	11.8 ± 1.2	87.8 ± 0.9	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
Test (Ph)					
10 mg/kg	20.0 ± 4.2*	79.8 ± 2.2*	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
50 mg/kg	19.0 ± 4.3	80.8 ± 2.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
100 mg/kg	11.6 ± 1.6	88.2 ± 1.7	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0

Neu = Neutrophils; Lym= Lymphocytes; Mon= Monocytes; Eos= Eosinophils;
*Bas = Basophils. *= P<0.05, significantly different from control (One-way ANOVA; Student t-test).*

3.6 Anti-inflammatory Study

The results revealed that intraperitoneal administration of the extract (10, 50 and 100 mg/kg i.p.) did not inhibit fresh egg albumin-induced inflammation (measured as oedema in cm^3) in rats. Significant increase in oedema was rather recorded at the dose of 100 mg/kg i.p. of the extract. The statistical comparison was done with the normal saline treated group (Figure 1).

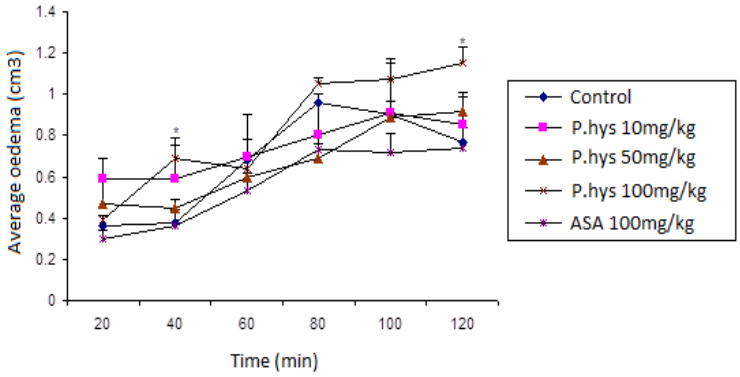

Fig. 1. Effect of 70% v/v aqueous extract of *P. hysterophorus* leaf (10 – 100 mg/kg i.p.) on fresh egg albumin-induced paw oedema in rats.
*** = P < 0.05; statistically different between treated and control group (two-way ANOVA; Student t-test)**

CONCLUSION

The present study evaluated the extract for haematological and immunomodulatory properties of *P. hysterophorus* leaf part of which it is traditionally used. The immunological models adopted in the evaluation took into consideration both specific and non-specific types of immunity. The study revealed that the extract had no significant effect on the haemoglobin (Hb), packed cell volume (PCV), red blood cell count, total leucocyte count, monocytes, basophil, eosinophil, bleeding time and clotting time of rats treated for 14 days. The neutrophils and platelets of the 14-day treated rats however increased significantly ($P<0.05$) increased at 100 mg/kg p.o. and 400 mg/kg p.o. doses respectively, while their lymphocytes decreased significantly ($P<0.05$) at 100 mg/kg p.o.

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