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EFFECT OF ETHANOLIC EXTRACT OF *PARTHENIUM HYSTEROPHORUS* ON HAEMATOLOGICAL PARAMETERS IN RAT

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ABSTRACT
Haematological parameters, such as erythrocyte and leukocyte count, hemoglobin concentration, lymphocyte and neutrophil percentage of blood has been considered as bioindicators of toxicosis in animals exposed to xenobiotics. Ethanolic extract of the plant of *Parthenium hysterophorus* at sub lethal dose, 200 and 400 mg/kg body weight dose when tested against rats in the laboratory reveals a significant decrease in erythrocyte count, hemoglobin concentration, lymphocyte percentage and increase in neutrophil percentage and leukocyte count with respect to time and dose.

INTRODUCTION

Parthenium hysterophorus known as congress grass belongs to the family compositae. Direct contact with plant and plant parts, living or dead, result in dermatitis in mankind. Presence of pollen in air causes diseases, like fever and asthma (Kaur and Sharma, 1986; Lonkar *et al.,* 1974; Rodriguez, 1975; Rodriguez *et al.,* 1976a and b; Shen *et al.,* 1976; Subba Rao *et al.,* 1976, 1978). Live stocks are also allergic and susceptible to *Parthenium hysterophorus*. It is responsible for bitter milk disease in live stock (Narasimhan *et al.,* 1977). Beside these *Parthenium* also shows several prominent biological activities in animal and human models. It contains several important chemical constituents, mainly histamine, saponin, glucosides and triterpene (sesquiterpene), the active ingredient of *Parthenium* is parthenin, a sesquiterpene lactone (Rodriguez, 1975). Sesquiterpene lactones exhibit a wide spectrum of biological activities like cytotoxicity, antitumour, allergic, antimicrobial, antifeedant, phytotoxic, anticancers, hypoglycemic and other pharmacological activities (Rodriguez *et al.,* 1976a). Though, considered nuisance, usage of the biological activities of the plant can make it economically useful. Hence, the present study is designed to evaluate the effects of *Parthenium hysterophorus* on haematological parameters of a mammal.

MATERIALS AND METHODS

Experimental animals

Healthy adult male and female albino rats weighing approximately 100-160g were selected for the experiment.

The albino rats were procured, with the help of local animal supplier. They were kept in large cage with plastic coated wire gauze on all sides, at room temperature 25ºC ± 5ºC. Rats were exposed to photoperiod of 12 hrs per day. The cages were cleaned regularly to avoid rat smell and to maintain proper hygienic conditions. The rats were acclimatized to laboratory conditions for 10 days and fed with rat pellets and water *ad libitum*. The animals were weighed, assigned a number for convenience and divided into groups for experimentation. This work was approved by the departmental ethical committee of DDU Gorakhpur University, Gorakhpur.

Preparation of extract

The plant was collected from the adjacent area of the department of Zoology, DDU Gorakhpur University, Gorakhpur. Five hundred grams of dried aerial parts of the plant was grinded into fine powder and subjected to soxhelet extraction with ethanol for twelve hours. The dark brown extract thus obtained was evaporated to dry in a flash evaporator and the residue was designated as ethanolic extract of *Parthenium hysterophorus* (EEPH) and used as toxicant for further studies.

Sub chronic treatment

The rats were weighed and divided respective to sex into six groups. A group of male rats was administered a sub lethal dose, 200 mg/kg body weight (I) and other group was administered 400 mg/kg body weight (II) of extract for 28 days

orally. Similarly, a group of female rats was administered a sub lethal dose 200 mg/kg body weight (III) and other group was administered 400 mg/kg body weight (IV) of the extract for 28 days orally. The remaining two groups of both male and female rats were orally fed with vehicle of similar dilution without test material and they served as control (V, VI). Each group contained 16 rats. The oral LD_{50} of ethanolic extract of *Parthenium hysterophorus* against rats was found to be 676.647 mg/kg body weight. (Maurya and Kushwaha, 2010)

Haematological studies

Haematological analysis was performed on whole blood, using the Haematological Analyzer Sysmex (Model No. KX 21, Japan). Erythrocyte count (RBC), hemoglobin concentration (Hb), leukocyte count (WBC), neutrophil percentage (NEUT) and lymphocyte percentage (LYM) and WBC were measured in control and treated rats.

RESULTS

The effects of oral administration of ethanolic extract of *P. hysterophorus* (EEPH) on haematological parameters of male and female rats are shown in Table 1 and 2 respectively. The treated rats showed a highly significant ($p < 0.01$) increase in total leukocyte count, neutrophil percentage and lymphocyte percentage decreased as compared to control rats with respect to time and dose in both male and female rats. A non significant decrease in erythrocyte count was observed in female rats on 14th and 28th day when exposed to 200 mg/kg body weight of EEPH, while in male rats a non significant decrease in erythrocyte count on 14th day and a highly significantly decrease in erythrocyte count was observed in comparison to control rats on exposure to 200 mg/kg body weight of EEPH. On exposure to 400 mg/kg body weight of EEPH a highly significant decrease in erythrocyte count was observed in both male and female rats. The female rats showed a non significant decrease in hemoglobin concentration on 14th day, while on 28th day they showed highly significant decrease in comparison to control rats on exposure to 200 mg/kg body weight of EEPH, whereas in male rats a highly significant decrease in hemoglobin concentration was observed in comparison to control rats on exposure to 200 mg/kg body weight of EEPH. At 400 mg/kg body weight of EEPH a highly significant ($p < 0.01$) decrease in hemoglobin concentration was observed in both male and female rats.

DISCUSSION

Blood is an overall reflector of the animal health and provides important profiles for the toxicological impacts on animal tissues. A significant decrease in erythrocyte (RBC) count and hemoglobin concentration of treated rats was observed. Adequate hemoglobin percentage is needed for the normal physiology of animals, which depends on the erythrocyte count. EEPH may induce inhibition of RBC formation that reduces the RBC count and leads to a decrease in Hb content. The depletion in RBC count and Hb content can be attributed to defective haemopoiesis (Choudhari and Deshmukh, 2007). Other possible factors affecting adversely may be reduced food intake by animals or internal haemorrhages (Kumar *et al*., 1999).

Goel *et al*. (1982) have reported haemolysis leading to anemia in *Heteropneustes fossilis* after malathion exposure. Panigrahi and Mishra (1978) have reported a decrease in RBC count, Hb percent and increase in WBC in *Colis fasciatus* exposed to metals. Fall in Hb content and RBC count can be correlated with induction of anemia in experimental animals after exposure to toxic compounds (Widmann, 1984; Cella and Watson, 2000). The decrease in Hb content and RBC count can be correlated with paling of animals, weakness and morbidity (Kumar *et al*., 1999; Cella and Watson, 2000; Choudhari and Deshmukh, 2007).

Significant increase in WBC count of treated rats on sub chronic treatment of EEPH observed in the present study can be attributed to the stimulation of immune system (Oluwole, 2001). EEPH induces leucocytosis in rats. Leucocytosis is said to exist when the leukocyte count increases above normal limits. Pathologically it is spoken of as the inflammatory, infectious, post hemorrhagic, toxic and experimental form as well as the type that accompanies malignant disease (Oser, 1965). Leucocytosis is considered as an adaptive value for the tissue pathology under chemical stress of toxicant. The leucocytosis may also be attributed for the removal of cellular debris of necrosed tissue in the rats under the toxic stress (Mc leay and Brown, 1974). An increase in WBC count after chemical stress recorded in the present study is in accordance with various workers (Pandey *et al*., 1976a, 1976b; Goel and Garg, 1980; Sastry and Sharma, 1980; Goel *et al*., 1981, 1982; Agrawal *et al*., 1982; Sharma *et al*., 1984; Tyagi, 1984; Goel and Maya, 1986).

Table 1: Effect of different doses of ethanolic extract of *P. hysterophorus* on haematological parameters in male rats

Parameters	Day	Control	Change in parameter when treated with 200 mg/kg body weight Mean ± (change in %)	Change in parameter when treated with 400 mg/kg body weight Mean ± (change in %)
WBC	14th	8.75 ± 0.057	9.9** ± 0.047↑ (13.14%)	12.45** ± 0.110↑(42.28%)
	28th	9.05 ± 0.074	10.5** ± 0.149↑(16.02%)	15.7** ± 0.124↑(73.48%)
NEUT	14th	13.4 ± 0.326	15.725** ± 0.272↑(17.35%)	24.325** ± 0.486↑(81.52%)
	28th	11.2 ± 0.428	25.1** ± 0.169↑ (124.10%)	37.02** ± 0.288↑(230.53%)
LYM	14th	87.025 ± 0.719	77.725** ± 0.331↓(10.68%)	68.1** ± 0.169↓(21.74%)
	28th	85.45 ± 1.568	68.41** ± 0.268↓19.94%)	58.25** ± 0.299↓(31.83%)
RBC	14th	8.65 ± 0.110	8.632 ± 0.039↓(0.20%)	7.662** ± 0.038↓(11.42%)
	28th	8.925 ± 0.144	8.187** ± 0.010↓ (5.18%)	7.117** ± 0.017↓(20.25%)
Hb	14th	14.425 ± 0.098	13.975** ± 0.098↓(3.11%)	13.775** ± 0.098↓(4.50%)
	28th	14.6 ± 0.047	13.05** ± 0.074↓(10.61%)	13.03** ± 0.074↓(10.75%)

*indicates significant ($p < 0.05$) and **indicates highly significant ($p < 0.01$) difference between control and treated groups when student's t-test was applied between treated and control groups

Table 2: Effect of different doses of ethanolic extract of *P. hysterophorus* on haematological parameters in female rats

Parameters	Day	Control	Change in parameter when treated with 200 mg/kg body weight Mean ± (change in %)	Change in parameter when treated with 400 mg/kg body weight Mean ± (change in %)
WBC	14th	7.75 ± 0.057	10.05** ± 0.074↑(29.67%)	10.325** ± 0.128↑(33.22%)
	28th	7.45 ± 0.110	12.6** ± 0.163↑(63.12%)	14.175** ± 0.055↑ (90.26%)
NEUT	14th	11.925 ± 0.698	19.931** ± 0.306↑(67.14%)	24.0** ± 0.286↑(101.25%)
	28th	12.225 ± 1.356	21.325** ± 0.457↑(74.43%)	28.675** ± 0.675↑ (134.56%)
LYM	14th	83.625 ± 0.451	82.0* ± 0.244↓(2.04%)	71.125** ± 0.556↓(14.94%)
	28th	84.475 ± 0.411	72.275** ± 0.190↓(14.44%)	56.0** ± 0.270↓(33.77%)
RBC	14th	6.415 ± 0.051	6.387 ± 0.014↓(0.43%)	6.047** ± 0.017↓(5.72%)
	28th	6.6 ± 0.047	6.542 ± 0.015↓(0.87%)	6.002** ± 0.019↓(9.05%)
Hb	14th	14.075 ± 0.158	13.75 ± 0.072↓(2.3%)	13.22** ± 0.074↓ (6.03%)
	28th	14.7 ± 0.124	11.15** ± 0.074↓(24.12%)	10.7** ± 0.124↓(27.21%)

*indicates significant ($p < 0.05$) and **indicates highly significant ($p < 0.01$) difference between control and treated groups when student's t-test was applied between treated and control groups

Differential leukocyte count (DLC) is an important tool in diagnosing diseases in animals (Hesser, 1960). Mammalian neutrophills are responsible for phagocytosis and disposal of foreign materials or debris of damage tissues. The percentage of lymphocyte decreased significantly with a significant increase in neutrophil count in the present study. Lucky (1977) has reported a decline in lymphocytes associated with an elevation of neutrophil during infection of dropsy. Garg (1981), Mishra and Srivastawa (1979) have also shown similar decrease in lymphocytes associated with an increase in neutrophills in *Channa punctatus* and *Colisa fasciatus*, respectively under chemical stress. The increased neutrophills in present study may also account for the removal of dead and damaged cell debris from the tissues under toxic stress of the compounds present in EEPH.

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