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Impact of *Prosopis cineraria* (L.) Druce leaves on hematological parameters against induced sub-acute toxicity of *Parthenium hysterophorus* L. in wistar albino rats

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Abstract
An ameliorative effect of *Prosopis cineraria* leaves on hematological parameters against induced sub-acute toxicity of *Parthenium hysterophorus* L. in Wistar albino rats was studied. A total of eighty clinically healthy adult albino rats between 2 and 3 months of age of either sex were divided in eight experimental groups each comprising of ten rats. Parthenium toxicity was induced by oral feeding of ethanolic extract of Parthenium at 150, 300 and 450 mg/kg body weight in group II, III and IV respectively for 28 days. Group V, VI and VII were fed with ethanolic extract of Parthenium at 150, 300 and 450 mg/kg body along with 200 mg/kg body weight of methanolic extract of leaves of *Prosopis cineraria*. Group I serve as control while group VIII was kept as treatment control and fed only methanolic extract of leaves of *Prosopis cineraria* at 200 mg/kg body weight. The treated rats showed a significant ($P \leq 0.05$) increase in total leukocyte count, granulocytes percentage and platelet count receiving the different dose of parthenium which indicated a deleterious effect of parthenium on blood cells. Significant reduction of total erythrocyte count, hemoglobin, lymphocytes percentage, mean platelet volume, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular volume was observed in group II, III and IV. *Prosopis cineraria* could restore the above in group treated with Parthenium at low dose level.

Keywords: ameliorative effect, parthenium toxicity, *Prosopis cineraria*, wistar albino rats

Introduction
Parthenium hysterophorus L. is an aggressive and exotic weed of family Asteraceae, at present has occupied almost all parts of India. It is native to subtropics of North and South America and was accidentally introduced in India in the year 1956 as a contaminant in imported wheat [1-5]. The toxic chemical in plant parts including trichomes and pollens contain sesquiterpene lactones. The major components of toxin being 'Parthenin' and other phenolic acids such as caffeic acid, vanillic acid, anisic acid, chlorogenic acid, parahydroxy-m-benzoic acid and p-anisic acid which are lethal to human beings and animals [6-9]. During scarcity of fodder cattles, sheeps and goats are forced to eat parthenium which can taint their meat and make diary milk unpalatable due to its irritating odor. Those animals can face-off rashes on their body and udders, alopecia, loss of skin pigmentation, allergic skin reactions, dermatitis, diarrhea, anorexia, pruritus, and death [10-12]. When human beings frequently come in contact with this weed, it may cause allergy, dermatitis, eczema, black spots and blisters around eyes, burning rings and blisters over skin, redness of skin and asthma [13-16]. *Prosopis cineraria* (L.) Druce{Khejri}which is a state tree of Rajasthan has been traditionally used by the rural community for treatment of various ailments such as helminthiasis, leprosy, dysentery, bronchitis, asthma, leucoderma, piles, tremors of the muscles and wandering of the mind. Its leaves are fodder for camels, goats and donkeys. Leaf paste of plant is applied on boils and blisters, including mouth ulcers in livestock and leaf infusion on open sores on the skin [17-20]. The present work is formulated to study sub-acute toxicity of *Parthenium hysterophorus* L. in Wistar albino rats and to evaluate the protective property of *Prosopis cineraria* (L.) Druce during Parthenium toxicity.

Materials and Methods

Experimental animals

Eighty (80) clinically healthy adult albino rats between 2 and 3 months of age of either sex, weighing about 100-150 g are used in this study. The animals are kept in polypropylene cages and acclimatized for one week prior to the experiment to alleviate any non- specific stress, in the experimental lab under standard managemental conditions [at a temperature of 25 °C (± 5 °C), with natural 12 hours light/12hours dark cycle]. Standard rat feed and water provided ad libitum throughout the experimental period. The necessary Institute Animal Ethical Committee approval was obtained.

Preparation of extract

Collection of plant Parthenium was done from the surrounding area of CVAS Navania, Udaipur and Prosopis was collected from Desert area of Shekhawati region (Rajasthan). Authentication (Identification) of plant materials was done from Botanical Survey of India, Jodhpur (Rajasthan). Five hundred grams of dried aerial parts of the plant *Parthenium hysterophorus* and Two hundred and fifty grams of dried leaves of the plant *Prosopis cineraria* was grinded into fine powder and subjected to soxhlet extraction with ethanol for Parthenium and methanol for Prosopis for twelve hours and evaporated by using rotary vacuum evaporator.

Sub chronic treatment

A total of 80 rats will be randomly divided into 8 groups (Group I, II, III, IV, V, VI, VII and VIII). Group-I (n = 10) will serve as control in which 1% Tween 80 suspension (vehicle) will be administered. Treatment group-II will be administered ethanolic extract of Parthenium@ 150 mg/kg b.wt, group-III will receive ethanolic extract of Parthenium@ 300 mg/kg b.wt, group-IV will be administered ethanolic extract of Parthenium@ 450 mg/kg b.wt, group-V will receive ethanolic extract of Parthenium + methanolic extract Prosopis@ 150 mg/kg b.wt and 200 mg/kg b.wt respectively, group-VI will receive ethanolic extract of Parthenium + methanolic extract Prosopis@ 300 mg/kg b.wt and 200 mg/kg b.wt respectively and group-VII will receive ethanolic extract of Parthenium + methanolic extract Prosopis@ 450 mg/kg b.wt and 200 mg/kg b.wt and group-VIII will serve as treatment control and fed only methanolic extract Prosopis 200 mg/kg b.wt orally by gavage. The oral LD_{50} of ethanolic extract of *Parthenium hysterophorus* against rats was found to be 676.64 mg/kg body weight (Maurya and Kushwaha, 2010).

Hematological studies

Blood was collected in dry sterilized vials containing an ethylene diamine tetra acetic acid (EDTA) from retro-orbital sinus of rats at the time of euthanasia. Hematological analysis was performed by using the hematological analyzer Mindray (Model no. BC- 2800 Vet, China). Total leucocytes ($10^3/\mu$l), Lymphocytes (%), Granulocytes (%), Platelet count ($10^3/\mu$l), Mean platelet volume (fl), Total erythrocytes ($10^6/\mu$l), Hemoglobin (g/dl), Packed cell volume or Hematocrit (%), Mean corpuscular hemoglobin (pg), Mean corpuscular hemoglobin concentration (g/dl) and Mean corpuscular volume (fl) were measured in control and treated rats.

Results

The effect of oral administration of ethanolic extract of parthenium and methanolic extract of prosopis on hematological parameters are shown in Table 1a and b respectively. The treated rats showed a significant ($P \leq 0.05$) increase in total leukocyte count, granulocytes percentage and platelet count receiving the different dose of Parthenium. Lymphocytes percentage, mean platelet volume, total erythrocyte count, hemoglobin, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular volume showed significant decrease in II, III and IV group as compared to control whereas group V, VI and VII receiving prosopis showed counteract untoward effect of parthenium. Group VIII receiving methanolic extract of prosopis remained normal throughout the period of experiment. *Prosopis cineraria* supplementation indicated protective role of plant at low dose toxicity in the rats.

Table 1a: Effect on hematological parameters in rats after 28 days oral administration of different dose of ethanolic extract of parthenium and its amelioration with methanolic extract of Prosopis

Groups	Hematological parameters				
	TLC (x $10^3/\mu$l)	Lymphocytes (%)	Granulocytes (%)	Platelet count (x $10^3/\mu$l)	Mean platelet volume (fl)
I	9.45 ± 0.23^{f}	64.6 ± 1.79^{a}	35.54 ± 0.64^{d}	566.3 ± 24.34^{d}	6.71 ± 0.09^{a}
II	11.41 ± 0.36^{de}	56.53 ± 1.04^{cd}	41.71 ± 0.69^{bc}	699.5 ± 11.06^{bc}	6.27 ± 0.08^{bc}
III	13.86 ± 0.35^{bc}	50.87 ± 0.68^{e}	44.05 ± 0.49^{b}	768.0 ± 15.26^{b}	6.08 ± 0.07^{bc}
IV	15.83 ± 0.42^{a}	43.54 ± 1.27^{f}	51.12 ± 0.97^{a}	927.8 ± 37.53^{a}	5.94 ± 0.07^{c}
V	10.73 ± 0.31^{ef}	59.1 ± 1.27^{bc}	38.53 ± 0.70^{cd}	641.8 ± 11.51^{cd}	6.33 ± 0.06^{b}
VI	12.67 ± 0.41^{cd}	53.58 ± 1.16^{de}	43.15 ± 0.82^{b}	722.6 ± 13.81^{bc}	6.15 ± 0.08^{bc}
VII	14.95 ± 0.30^{ab}	45.36 ± 0.92^{f}	49.78 ± 0.87^{a}	897.8 ± 13.18^{a}	5.98 ± 0.07^{bc}
VIII	9.51 ± 0.17^{f}	63.32 ± 1.21^{ab}	36.24 ± 0.89^{d}	564.9 ± 13.20^{d}	6.69 ± 0.09^{a}

Table 1b: Effect on hematological parameters in rats after 28 days oral administration of different dose of ethanolic extract of parthenium and its amelioration with methanolic extract of Prosopis

Groups	Hematological parameters					
	TEC (x $10^6/\mu$l)	Hb (g/dl)	PCV (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)
I	8.972 ± 0.09^{a}	15.03 ± 0.21^{a}	45.06 ± 0.83^{a}	18.44 ± 0.19^{a}	34.94 ± 0.10^{a}	54.82 ± 0.41^{a}
II	8.067 ± 0.09^{bc}	13.23 ± 0.17^{bc}	39.79 ± 0.28^{bc}	17.24 ± 0.18^{bc}	33.76 ± 0.18^{bc}	52.95 ± 0.20^{b}
III	7.887 ± 0.11^{bc}	11.95 ± 0.19^{d}	37.78 ± 0.58^{c}	16.96 ± 0.18^{bcd}	33.55 ± 0.20^{bc}	52.51 ± 0.32^{bc}
IV	7.538 ± 0.14^{c}	9.84 ± 0.38^{e}	33.92 ± 0.96^{d}	16.45 ± 0.17^{d}	32.98 ± 0.18^{c}	51.22 ± 0.34^{d}
V	8.345 ± 0.15^{b}	13.83 ± 0.23^{b}	41.92 ± 0.43^{b}	17.64 ± 0.14^{b}	34.01 ± 0.21^{b}	53.48 ± 0.21^{b}
VI	7.970 ± 0.11^{bc}	12.31 ± 0.23^{cd}	39.91 ± 0.68^{c}	17.25 ± 0.11^{bc}	33.74 ± 0.21^{bc}	52.84 ± 0.23^{b}
VII	7.655 ± 0.13^{c}	10.06 ± 0.31^{e}	34.77 ± 0.47^{d}	16.53 ± 0.13^{cd}	33.12 ± 0.23^{c}	51.43 ± 0.27^{cd}
VIII	8.983 ± 0.18^{a}	15.13 ± 0.21^{a}	45.15 ± 0.68^{a}	18.47 ± 0.19^{a}	34.96 ± 0.11^{a}	54.86 ± 0.16^{a}

All values are represent Mean ± SEM; n=10 in each group; values bearing different superscript in the same column differ significantly between groups at P≤0.05 in Tukey's multiple comparison post hoc test.

Discussion

The blood cells are the mobile units of the body's protective system and it provides important profiles for the toxicological impact on animal tissue [21, 22]. Significant increase in total leukocyte count, granulocytes percentage and platelet count of treated rats on sub chronic treatment of ethanolic extract of parthenium observed in the present study may be due to the stimulation of immune system [23]. Ethanolic extract of parthenium induces leucocytosis in rats, which may be due to the removal of cellular debris of necrosed tissue in the rats under the toxic stress [22, 24]. A significant decrease in total erythrocyte count, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, hemoglobin, lymphocytes percentage and mean platelet volume was observed in treated rats. Hemoglobin percentage is needed adequately for the normal physiology of animals. Ethanolic extract of parthenium may induce inhibition of RBC formation that reduces the erythrocyte count and leads to a decrease in hemoglobin content. This depletion of erythrocyte count and hemoglobin content can be attributed to defective hemopoiesis. Other possible factors may be reduced food intake by animals, internal hemorrhages, paling of animals, weakness and morbidity [22, 25]. The protective role of methanolic extract of *Prosopis cineraria* supplementation may be due to its antioxidant property which can reduce the free radical- mediated oxidative stress [26, 27].

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