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EFFECT OF ETHANOL AND WATER EXTRACT OF LEAVES OF *NYCTANTHES ARBORTRISTIS LINN.* ON THE HEMATOLOGICAL PARAMETERS OF ALBINO RATS

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ABSTRACT:-

Medicinal plants have been curing various disorders in human being from the time immemorial. Allopathic drugs sometimes show negative effect or side effects but herbs are safer and easy to access. The herbal treatment are cheap as compared to allopathic drugs too. The present study mainly aims to discuss the effect of ethanol and water extract of leaves of Nyctanthes arbortristis Linn. on the hematological Parameters of Albino rats. When the animals were treated with ethanol and water extract of leaves of Nyctanthes arbortristis for 30 days, no significant effect were observed on hematological Parameters. However, the extract showed significant (P<0.05) increase in platelets (40 and 200mg/kg) and WBC (1000mg/kg) counts. Considering WBC differential, at all doses cause significant (P<0.05) reduction in the proportion of lymphocytes and a corresponding increase in the proportion of neutrophils. A significant increase in the proportion of eosinophils was observed only at 1000mg/kg. After gross examination on Hematological Parameters in which changes were seen, did not show any detectable abnormalities. The result suggests the ethanol and water extract of leaves of Nyctanthes arbortristis Linn. is safe to use as powder, paste and decoction for medicinal purpose.

Keywords: *- Abnormalities, Detectable, Eosinophils, Immemorial, Lymphocytes, Neutrophils and Nyctanthes arbortristis Linn.*

INTRODUCTION:

Scientific interest in medicinal plants has been increasing in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of conventional medicine. Medicinal plants have been curing various disorders in human being from the time immemorial. Allopathic drugs sometimes show negative effect or side effects but herbs are safer and easy to access. The herbal treatments are cheap as compared to allopathic drugs too. Diseases are the constant problems of mankind, the curative properties of some plants have been discovered and exploited early in human history. Over the years, the knowledge of herbal medicines grown. In this time, a wide range of plants have been sought for their healing affects and used more and more. In many developing countries, the traditional medicine is still the mainstream of health care, and most of the drugs come from the plants. At present there are many medicinal plants that have been promoted to use in primary health care and classified according to their pharmacological actions such as treatment of stomach pain, respiratory problems, antidiabetic, anti-inflammatory etc. Most of the traditionally used medicinal plants have not been sufficiently evaluated for their pharmaceutical and toxicological effects. It is surprising that Ayurvedic system of medicine using indigenous herbs has still been a part and parcel of Indian culture. Some of these herbs are in fact a house hold name in Indian villages. Even today different primitive tribes and native inhabitants of remote interior villages use different types of herbs or herbal preparation to cure their ailments effectively. A lot of studies concerning detail information of individual plant have been done. These include the knowledge of their origin with geographical sources and scientific name, methods of cultivation, collection, physical and chemical characteristics and quantitative evaluation of its constituents. Nyctanthes arbortristis means ‘a night flowering sad tree’. It is an indigenous plant, commonly known as Harsinghar in Hindi, belongs to the family Oleaceae (Nyctaginaceae). It is a small tree with a gray or greenish rough bark with delightfully fragrant flowers in Autumn. The fresh leaves are used for the preparation of homoeopathic medicines. It has frequently been used by the ancient Indian physicians for the treatment of all kinds of fevers and sciatica. The Council conducted the trial of the medicine during the period 1980 – 2003 in various potencies. The study confirms the symptoms like anxiety, restlessness, different kinds of fevers specially intermittent and bilious fevers, headache, gastritis, hepatitis, abdominal colic, constipation, diarrhoea, dry cough, rheumatism & sciatica as mentioned in various homoeopathic literatures. Besides, it was also useful in clinical conditions like vertigo, dysmenorrhoea, bronchitis and seasonal fevers etc., which can be inferred as additional clinical symptoms as observed during the trial. The leaves are antibacterial, anti-inflammatory and anthelmintic. It is used in bilious and obstinate remittent fever, sciatica, and rheumatism. It is also very useful in constipation of children. The plant Nyctanthes arbortristis Linn. is a large shrub which is widely cultivated throughout India as a garden plant. The bitter leaves are used in traditional system of medicine for the treatment of rheumatism, sciatica and intestinal worms.

MATERIAL AND METHOD:

Plant Materials:- Plant sample (Leaves) collected, were first washed thoroughly in tap water in order to remove foreign materials like soil, sand etc. and finally rinsed with double (glass) distilled water, then these were spread thinly in fresh polythene sheets in the dry air (under fan) for several hours avoiding any dust contamination. Then they were dried in the thermostatically controlled oven drier at $60^0 80^0$. To avoid metallic contamination tray made of fiber glass used, in which plant samples were thinly spread inside the oven.

After preliminary drying, the sample taken out from the oven drier, brought to room temperature and leaves crushed to coarse powder. 100mg powder of the leaves of *Nyctanthes arbortristis*is taken in the soxhlet and extracted with 450 ml. 95% ethanol at controlled temperature. The collected extracted was concentrated under reduced pressure below 45^0c using rotary evaporator. The complete removal of the solvent from the extract was carried out in the rotary evaporator. The material thus obtained was stored at 4-5^0c until used.

Experimental Animals:-Albino rats of both sexes weighing between 120 to 150 g were used for the experiment. Animal had ad libitum access of standard laboratory diet and water, except during the previous night of experiment. The animals were grouped randomly into control and treated group containing five rats in each group. They were housed under standard environmental conditions of temperature and were allowed to free access to drinking water and pallet diet. The rats were kept in the experimental facilities for the week to allow them to be acclimated prior to dosing. Animals were put on fasting except water up to 16-18 hours prior to giving them medicine at day zero. The experimental protocol as per the WHO and OECD guideline was maintained (WHO, 2000 AND OECD, 2001).

Dose administration:- The ethanol and water extract of roots of Tragiainvolucrata was orally administered at concentration of 20, 40, 200 and 1000mg/kg three subsequently treated group for 30 days (midterm) and 60 days (end term study), the control group was given distilled water only.

Hematological assay:-Blood samples were collected from rats and kept into EDTA bottles. They were analyzed for the determination of packed cell volume (PCV), red blood cell (RBC) count, hemoglobin, platelet count, total and differential WBC count using standard methods (Ghai, 1995). All the hematological tests were performed using Automated Hematology Analyzer of Ranbaxy (Model No. Lablife Noble III).

Statistical analysis: Results were expressed as mean ± standard error of mean (SEM). Statistical significance and post hoc least-significant difference (LSD) test. The data obtained from the study were analyzed using student test. P value less than 0.05 were considered significantly.

Table A

Effect of ethanol extract of Sample No. OP/L/BJ3on hematological parameters in rats.

	Mid term study (30 days)				End term study (60 days)			
	Control	40mg	200mg	1000 mg	Control	40mg	200 mg	1000 mg
PCV%	40.6±1.17	34.6±2.94	40.80±1.9	41.4±2.46	17.2±4.16	33.0±1.38	33.5±1.8	34.1±1.00
RBC(10^6/mm^3	6.64±0.38	5.40±0.85	6.63±0.71	6.52±0.73	6.05±0.87	5.84±0.39	5.84±0.42	6.30±0.32
Hb(g/dl)	13.6±0.39	12.59±1.04	14.64±0.71	14.15±0.56	12.55±1.35	12.43±0.50	20.98±0.50	13.25±0.37
Platelet(10^5/ mm$^{3)}$	1.42±0.37	1.33±0.21	2.33±0.26	1.87±0.49	1.35±0.39	5.16±0.91*	4.70±0.97$^{\#}$	3.11±0.78
WBC (10^3/mm^3)	4.52±0.47	4.00±0.60	5.52±0.88	5.60±0.92	5.02±0.46	3.75±0.68	5.53±1.07	12.50±2.22$^{\#}$
Lymphocytes	63.80±2.63	66.20±5.10	56.40±2.77	56.40±2.42	62.00±3.92	41.40±3.56*	46.40±3.08$^{\#}$	42.20±2.15*
Monocytes	1.00±0.32	0.90±0.20	1.50±0.40	1.10±0.37	1.40±0.24	1.40±0.40	1.40±0.51	1.60±0.24
Neutrophils	35.00±2.90	34.80±4.82	41.00±2.61	42.00±2.47	36.20±3.75	58.60±3.66*	51.40±3.57$^{\#}$	57.00±2.61*
Eosonophils	0.20±0.20	0.20±0.20	0.20±0.20	0.40±0.24	0.40±0.24	0.60±0.24	0.80±0.37	1.20±0.20$^{\#}$
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values are mean ± SME (n=5), #p<0.05, *p<0.01 (significant, student t-test) vs control.

Table B

Effect of water extract of Sample No. OP/L/BJ3on hematological parameters.

	Mid term study (30 days)				End term study (60 days)			
	Control	40mg	200mg	1000 mg	Control	40mg	200 mg	1000 mg
PCV%	40.6±1.17	36.6±2.94	40.60±1.9	40.4±2.46	17.2±4.16	31.0±1.38	32.5±1.8	33.1±1.00
RBC(10^6/mm^3	6.64±0.38	5.50±0.85	6.53±0.71	6.42±0.73	6.05±0.87	5.64±0.39	5.84±0.42	6.20±0.32
Hb(g/dl)	13.6±0.39	12.29±1.04	13.64±0.71	14.05±0.56	12.55±1.35	10.43±0.50	10.98±0.50	11.25±0.37
Platelet(10^5/ mm$^{3)}$	1.42±0.37	1.31±0.21	2.13±0.26	1.77±0.49	1.35±0.39	5.15±0.91*	4.70±0.97$^{\#}$	3.11±0.78
WBC (10^3/mm^3)	4.52±0.47	4.00±0.60	5.22±0.88	5.50±0.92	5.02±0.46	3.75±0.68	5.53±1.07	12.50±2.22$^{\#}$
Lymphocytes	63.80±2.63	65.20±5.10	58.40±2.77	58.40±2.42	62.00±3.92	40.40±3.56*	46.40±3.08$^{\#}$	42.20±2.15*
Monocytes	1.00±0.32	0.80±0.20	1.40±0.40	1.20±0.37	1.40±0.24	1.40±0.40	1.40±0.51	1.60±0.24
Neutrophils	35.00±2.90	33.80±4.82	40.00±2.61	40.00±2.47	36.20±3.75	57.60±3.66*	51.40±3.57$^{\#}$	57.00±2.61*
Eosonophils	0.20±0.20	0.20±0.20	0.20±0.20	0.40±0.24	0.40±0.24	0.60±0.24	0.80±0.37	1.20±0.20$^{\#}$
Basophils	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values are mean ± SME (n=5), #p<0.05, *p<0.01 (significant, student t-test) vs control.

RESULTS:

The effect of the ethanol and water extracts of the leaves of N. arbortristis was studied on the haematological parameters of the rats. All the results were represented in the Table No. A and B. It was also observed with leaves extracts that after 30 days administration of the leaves extracts on different doses show no significant effect on hematological parameters. However, in respect of 60 days daily administration, the extract showed significant (P < 0.05) increase in platelet (40 and 200 mg/kg) and WBC (1000mg/kg) counts.

Considering WBC differential, at all doses caused significant (P < 0.05) reduction in the proportion of lymphocytes and a corresponding increase in the proportion of neutrophils. A significant increase in the proportion of eosinophils was observed only at 1000 mg/kg.

CONCLUSION:

A possible risk from an elevation of platelet count is thrombosis which invokes a predisposition to stroke and heart attack. However, all animals in this group appeared normal and healthy and no mortality was recorded all through the experiment. The proportion of neutrophils was increased with an accompanying reduction in the proportion of lymphocytes. Neutrophils are the first defense responders to microbial infections (bacterial or fungal) and are associated with other inflammatory processes.

Lymphocytes on the other hand generate antibodies that bind to pathogens to enable their destruction and are more involved in defense against intracellular bacteria, virus infected cells and tumor cells.

The extracts at this dose can be said to enhance immediate response to microbial attack and inflammatory processes.

After gross examination on Hematological Parameters in which changes were seen, did not show any detectable abnormalities. The result suggests the ethanol and water extract of leaves of *Nyctanthes arbortristis* Linn. is safe to use as powder, paste and decoction for medicinal purpose.

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