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Effects Of Aqueous extract Of *Tephrosia purpurea* on Haematological parameters in Snakehead Fish *Channa punctatus*

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Abstract

The aim of the present study was to evaluate the ichthyotoxic effects of aqueous extract of biopesticide plant *Tephrosia purpurea* on snakeheaded freshwater fish *Channa punctatus*. The survival number and survival percentage of fish were recorded after 24 hr, 48 hrs, 72 hrs and 96 hrs with the test concentrations of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 gm per litre. The TLm (LC_{50}) value for aqueous extract of *T. purpurea* were 4.5 gL^{-1} in 72 hrs and 4 gL^{-1} in 96 hrs. Haematological parameters such as TEC was decreased by 5.63% and 14.86%; TLC was increased by 13.65% and 903.75%, HBC was decreased by 4.59% and 6.84% ESR was increased by 3.97% and 6.78% and specific gravity was decreased by 6.176% and 12.068% in 3 days and 7 days respectively.

Key words : *Tephrosia purpurea*, *Channa punctatus*, Ichthyotoxic, Haematological, Aqueous extract.

Many scientists believe that the word consumption of medical plant is growing rapidly[1]. Biopesticides are more effective, less expensive bio-degradable and safe for mankind and environment then synthetic pesticides[8,13] and they are highly toxic to target organism[13]. *Tephrosia* is a genus of legume in the Fabaceae (Papilionaceae) family. Many species in the *Tephrosia* genus are poisonous, particularly to fish due to high concentration of rotenone. *Tephrosia purpurea* is a common medicinal plant widely used in traditional medicinal systems. Its toxic substance rotenone is responsible to kill fish. It has been used for fishing in lakes and streams as it paralysis fish which then float to the surface. It is also used in controlling the trash fishes in India[6].

Channa punctatus fresh water snake-headed murell is an air breathing predatory and carnivorous fish, has a wide distribution throughout plains of India upto an altitude of 600 meters.

Measurement of haematological parameters are important in diagnosing the structural and functional status of animals exposed to the toxicant because blood parameters are highly sensitive to environmental or physiological changes and health condition[15,16]. The present investigation has been formulated to understand

the efficiency of aqueous extract of *Tephrosia purpurea* on haematology in fresh water air breathing fish *Channa punctatus*.

Selection and maintenance of fishes:

The fresh water air breathing fish *Channa punctatus* ranging from 16-20 cm in length and 58-63 gm in weight were collected from local fish market, Ganga river in Narora and canals present in Aligarh. They were treated with potassium permanganate for 5 minutes to get rid of any dermal infection. Fishes were stored in glass aquaria measuring 75 cm x 37.5 cm x 37.5 cm and containing dechlorinated tap water. Experimental water condition were pH 7-7.1, dissolved oxygen 6.8-7.5 g/L, temperature 15-30^{o}C, alkalinity 630 mg/L, hardness 8.2 mg/L, and non-carbonate hardness 82 mg/L. Fishes were allowed to acclimatize to the laboratory conditions for 15 days, before commencement of experimental studies.

Collection and preparation of aqueous extract of Tephrosia purpurea :

The plant *Tephrosia purpurea* were collected from Regional Research Institute of Unani Medicine, Aligarh U.P. India and rural areas of district Aligarh. After washing, the plants were dried in shade for 3 days and then in oven at 50^{o}C for 2 days, the plants were ground mechanically soaked for 12 hrs, boiled for 30 minutes and filtered by Whatman's filter paper to prepare the experimental concentration of 0.25 mg/litre.

Haematological study :

Examination of Haematological parameters of *Channa punctatus* were performed after 3 days and 7 days exposure to sublethal concentration of aqueous extract of *Tephrosia purpurea*. Blood samples were collected into sterilized glass vials containing 1% anticoagulant dipotassium ethylene diamine tetraacetate (EDTA).

The total erythrocyte counts (TEC), total leucocyte count (TLC) were made by using the standard improved Neubaur Haemocytometer[3]. The haemoglobin concentration (HBC) of blood was estimated by the Sahli's method as outlined by Wintrobe[17]. The erythrocyte sedimentation rate was determined by the Wintrobe and Landsberg's method. The specific gravity of the blood was determined by the copper sulphate specific gravity method[11].

Statistical analysis of Data :

The data obtained from these investigations were subjected to various statistical tools. The difference in the means (±SEM), between groups were assessed using Fisher's't' test. A p-value of $P < 0.01$ was taken as highly significant (#) and $p < 0.05$ was taken as significant [*].

Sublethal concentration of aqueous extract of *Tephrosia purpurea* induced significant decrease in erythrocyte count (TEC), haemoglobin concentration (HBC) and specific gravity. While significant increase in total leucocyte count (TLC) and erythrocyte sedimentation rate (ESR) after 3 days and 7 days of exposure (Table-1).

Blood is a patho-physiological reflector of the whole body, so blood parameters are important in diagnosing the structural and

functional status of the animals exposed to the toxicant[5].

The decrease in total erythrocyte count and haemoglobin concentration in *C. punctatus* when exposed to sub-lethal concentration of aqueous extract of *Tephrosia purpurea* is similar to those reported in *Oncorhynchus mykiss* exposed to *Nasturtium nasturtium*[2] and *Oreochromis niloticus* exposed to *Nicotiana tabaccum*[9]. The decrease in erythrocytes in fishes indicate a compensation of oxygen deficit in the body to gill damage. The decreased HBC content is due to destruction of RBCs due to erythroblastosis leading to anemia.

The increase in total leucocyte count when exposed to sublethal concentration of aqueous extract of *Tephrosia purpurea* is similar to those reported in *Oncorhynchus mykiss* exposed to *Melissa officinalis*[4] and in *Catla catla* exposed to *Cynodon dactylon*[7]. The increase in WBC may have resulted from the excitation of defence mechanism of fish to counter the effect of the toxicant. The increase in erythrocyte sedimentation rate (ESR) when exposed to sublethal concentration of aqueous extract of *Tephrosia purpurea* is similar to those reported in *Channa punctatus* exposed to *Euphorbia tirucalli*[14] and in *Channa striata* exposed to *Cleistanthus collinus*[10], increase in ESR in *Channa punctatus* may be due to the infectious state of body of fish due to exposure of aqueous extract of *T. purpurea* which may lead to increase in the concentration of fibrogen which develops into fibrinogenemia *i.e.* absence of fibrinogen plasma protein in plasma. The decreased value of specific gravity when exposed to sublethal concentration of aqueous extract of *Tephrosia purpurae* is similar to those reported in *Channa punctatus* exposed to *Euphorbia tirucalli*[14]. The specific gravity of blood is primarily dependent on the ratio of plasma to red cell and indicates the presence of significant degree of anaemia. The decrease in specific gravity in fish *Channa punctatus* can be correlated with the decrease in the total erythrocyte count (TEC).

Table-1. Alteration in haematological parameters of *Channa punctatus* exposed to sublethal concentration as well as in control for aqueous extract of *Tephrosia purpurea*

Parameters	Control	Exposure Time	
		3 days	7 days
TEC (10^6/mm^3)	2.96 ± 0.09	2.64 ± 0.075**	1.7 ± 0.05***#
TLC (10^6/mm^3)	4.62 ± 0.023	4.95 ±0.011#***	5.39 ±0.010***
Hbc (gm %)	6.8 ±0.16	5.6 ±0.20**	6.8 ± 0.16**
ESR (mm/h)	1.80 ± 0.22	2.4 ±0.55	2.7 ±0.075**
Specific gravity	1.071 ±0.0010	1.066 ±0.0115**	1.064 ± 0.007***#

** - significant at P < 0.05; *** - highly significant at P < 0.05; # - significant at P < 0.01

The findings of the present study shows that sub lethal concentration of aqueous extract of *Tephrosia purpurea* affects the haematological parameters of the fish without causing mortality. It inactivates the fish so that they can be removed easily. So, it can be used as a safe and ecofriendly biopesticide. However further studies on withdrawal treatment may help to use it as a safe biopesticide.

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