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# Haematological and biochemical changes in *Clarias gariepinus* exposed to *Trephosia vogelii* extract

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## ABSTRACT

*Biochemical and heamatological parameters in blood of Clarias gariepinus subjected to sub lethal concentrations of Tephrosia vogelii extracts for two weeks were determined. The fish species used for this study were purchased from the Ministry of Agriculture, Fisheries Division, Ogbomosho, Nigeria. The values of hematological and biochemical indices of C.gariepinus were subjected to Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT). No significant changes in hematological and biochemical indices such as Pack Cell Volume (PCV), Hemoglobin concentration (Hb), Red Blood Cell count (RBC), total protein and glucose level. The erythrocyte indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Heamoglobin (MCH) and Mean Corpuscular Heamoglobin Concentration (MCHC) did not change significantly. There was a significant ($P< 0.05$) increase in the level of White Blood Cell (WBC) and Cholesterol in the group that received the extract compared with the control. Introduction of T. vogelii in the open water during fishing activity may have negative impact on the physiology of the fish as manifested in the changes recorded.*

**Key words:** Haematological, Extracts, *Tephrosia vogelii*, Haemoglobin, Exposure.

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## INTRODUCTION

Medicinal plants are the most ancient source of drugs for curing human and animal diseases. Their use in the crude or refined form is of utmost interest in the efforts aimed at integrating herbal with orthodox medicine. Some of the secondary metabolites therein may be toxic to lower beings or Man, or indeed both (Ekpendu *et al* 1998 and Satoh *et al* 2001).

*Tephrosia vogelii* is commonly referred to as fish poison bean which has been widely used in the tropics to kill fish and in the treatment of various animal diseases. It has a potential in Western and Southern Africa for biocontrol. The research findings have revealed that *T. vogelii* is a short-lived perennial plant which may attain a height of 2 to 3 meters in a growing season of 5 to 7 months. *T. vogelii* contains four active insecticidal and pesticidal compounds collectively known as Rotenoids. These active compounds are rotenone, deguelin and tephrosin. Rotenone and deguelin are the major rotenoids compounds in *T. vogelii* and are the most toxic of the rotenoids. Researchers have shown that the differences in growing conditions, age of the plant maturity and varieties grown affect the number of rotenoids present, their concentration in different parts of the plant and their relative properties (Lambert *et al*, 1993).

The lowest concentrations of these active compounds are found in the roots. It has also been used for a long time in fishing by artisanal fishermen although it is illegal; the use of *T. vogelii* in fishing has been reported to cause either death or tranquilization of fish depending on the concentration (Mwambere, 2000).

Fukami *et al*, (1970) reported that rotenone inhibits the respiratory chain and acts by blocking the oxidation of $NADH_2$ (Nicotinamide Adenine Dinucleotide) which depletes the cells of ATP (Adenosine Triphosphate) needed to maintain mitochondrial energization when electron transport is inhibited.

A great variety of rotenoids have been reported to accumulate in the leaves of this plant, wherein the total rotenoid content can reach as much as 4% of the dry matter (Lambert *et al*, 1993). *T. vogelii* is also one of the commercial sources of rotenone listed by Gaskin and Stone (1971). It can also have deleterious impacts on aquatic ecosystems by affecting organisms other than the target species (Beal and Anderson, 1993).

Orciari (1979) reported that the 48-hour $LC_{50}$ of rotenone in golden shiners was $0.32mgL^{1.}$ Beal and Anderson, (1993) reported the eradication of grass carp from a pond at $6\mu L^{-1}$ rotenone concentration. Hegen, (1985) reported that there was a total mortality at 5.00ppm rotenone concentration, while blue crabs and brown shrimps survived at 4.00ppm and 1.80ppm concentration, respectively.

In Nigeria, fish farmers have traditionally harvested fish by persistent and indiscriminately abuse the use of ichthytoxic plants and natural piscicide particularly *T. vogelii* which is gaining popularity because of its efficacy and effectiveness in field insect pest control, treatment of various animal ailment and fishing.

*Clarias gariepinus* is a native to Africa and tropical Asia and it is the most abundant culturable fish in Nigeria apart from tilapia (Elliot, 1985). This study was undertaken primarily to evaluate the toxic effects of *Tephrosia vogelii* leaf extract on the heamatological and biochemical indices of *Clarias gariepinus.*

## MATERIALS AND METHODS

A total number of 120 *Clarias gariepinus* of mean weight and mean standard length of 450± 50g and 34 ± 5cm respectively were purchased from the Ministry of Agriculture Ogbomosho, Oyo State, Nigeria,. The fishes were acclimatized for fourteen days at ambient temperature, during the acclimation period the fishes were fed with commercial fish feed. The leaves and succulent parts of *T. vogelii* used in this study were harvested and weighed. 200g of the plant leaves were macerated with electrically powered blender and extracted for 2 hours with 1 liter distilled water. The aqueous suspension was filtered through muslin cloth and filtrate stored in plastic containers for bioassay almost immediately after extraction or stored in refrigerator till the second day for bioassay.

Blood samples were collected by severing the caudal peduncle of both the control and experimental fishes that survived the 2 weeks toxicant exposure period, by using EDTA as anticoagulant. Heamoglobin (Hb) concentrations was determined by the cyanmetheamoglobin method (Darcie and Lewis, 1984), while the packed cell volume (PCV) and the total plasma protein were determined by using the procedure described by Beal and Anderson (1993). The red and white blood cell counts were determined using neubauer counting chamber. The method used by Bamidele (2002) was used to obtain mean corpuscular volume (MCV) and mean corpuscular heamoglobin (MCH).

## RESULTS

The results obtained from the heamatological examination of the blood samples collected from the control and experimental fishes are presented in Table 1 and 2.

**Behavioral Responses**

Exposure of *Tephrosia vogelii* caused visible significant behavioural changes in *Clarias gariepinus*. After 60 minutes, their swimming activity slowed down, the test fishes felt suffocation, they tried to stay at upper water surface to gasp for air, irregular and jerky movement, loss of body equilibrium was also pronounced. Also, they settle down at the base of water, formed cluster and died. Fishes of control group were free from such behavioural changes.

Table 1, shows the results of the haematological parameters of the test organisms after exposure to sub lethal concentrations of the *Tephrosia vogelii*. These parameters are White Blood Cell count (WBC), Packed Cell Volume (PVC), Heamoglobin Concentration (Hb), Mean Cell Volume (MCV), Mean Corpuscular Heamoglobin (MCH), and the Mean Corpuscular Heamoglobin Concentration (MCHC).

The WBC of the test organisms were significantly high and ($p<0.05$) compared with the control, while the RBC, PCV, Hb and other erythrocytes indices showed insignificant differences.

There exist differences in all the biochemical indices analysed except the cholesterol but the differences were insignificant ($p>0.05$).

**Table 1: Mean values of Haematological Indices of *Clarias gariepinus* Exposed to Sub-lethal concentration of *Tephrosia vogelii* Extract**

| Parameter | PVC (%) | WBC $10^3$ (µı) | RBC $10^6$ (µı) | Hb (g/dl) | MCV (fl ) | MCH(pg) | MCHC( gdl) |
|---|---|---|---|---|---|---|---|
| Control | 49.00±48.95d | 832,000±831,999.9a | 3.500±3.48c | 15.50±14.70c | 1.400±4.40b | 4.430±4.40b | 31.630±31.56b |
| 0.00010 | 40.00±40.45c | 853,000±852998.8a | 2.950±2.86c | 12.80±12.76b | 4.340±4.22b | 4.340±4.22b | 32.00±31.94c |
| 0.00015 | 4500±44.86d | 980,000±979999.9b | 2.760±2.65bc | 14.00±13.90c | 5.070±4.92c | 5.070±4.92c | 31.110±30.96b |
| 0.00020 | 3600± 35.90b | 1,010,000±1,008,000b | 2.064±1.94a | 13.00±12.82b | 6.290±5.78d | 6.290±5.78d | 36.110±35.84d |
| 0.00025 | 40.00±40.45c | 1,250,000±1249999.6b | 3.00±2.67c | 12.50±12.49b | 4.170±4.09b | 4.170±4.09b | 31.250±30.68b |
| 0.00030 | 30.00±30.45a | 1,455,000±1,449999.2b | 2.500±2.47b | 9.00±8.88a | 3.600±3.57a | 3.600±3.57a | 30.00±29.78a |

**Table 2: Mean biochemical Parameters of *Clarias gariepinus* exposed to *T. vogelli* extract**

| Parameter | Total protein | Albumin | Globulin | Glucose | Calcium | Cholesterol |
|---|---|---|---|---|---|---|
| Control | 8.5±7.76ab | 2.7±2.86b | 5.8±4.09ab | 25±24.83a | 11.3±10.81ab | 130±129.55c |
| 0.00010 | 9.3±8.84c | 2.5±1.74a | 2.5±1.74a | 10±9.55a | 8.3±7.72b | 155±154.86e |
| 0.00015 | 9.0±8.95c | 3.0±2.95ab | 6.0±5.98b | 15±14.60c | 7.5±7.48a | 127±126.78c |
| 0.00020 | 7.6±6.9ab | 2.4±1.95ab | 5.2±5.00ab | 46±35.72c | 8.0±7.99b | 165±164.64f |
| 0.00025 | 7.1±6.9c | 2.6±1.95b | 4.5±3.96b | 25±24.77a | 9.1±9.07ab | 150±149.92a |
| 0.00030 | 8.6±7.95bc | 2.9±2.87a | 5.7±4.92ab | 35±34.92b | 7.2±6.54d | 140±139.95b |

*Means having the same alphabet are not significantly different p<0.0005*

## DISCUSSION AND CONCLUSION

According to Barton (2002), stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintenance of its homeostatic response. If severe or long lasting, the response then becomes mal-adaptive and threatens the fish health and wellbeing. Therefore, in the presence of stressors, blood parameters can be employed as standard laboratory test to determine diseased conditions and metabolic disturbances in fish (Celic, 2004).

The decreased in heamoglobin concentration (Hb) with increase in the concentration of the plant extract is similar to those reported in *C gariepinus* exposed to cassava effluents and tobacco leaf extracts ( Omoniyi *et al* 2002; Adewoye, 2005; Adeyemo, 2005). This pattern of response may be attributed to heterolysis which results in haemodilution, a means of diluting haemoconcentration of this extract, this reduces the effect of the toxicant in the fish system (Smith *et al,* 1979). Besides, it may results from either an increase in the rate of haemoglobin destruction or decrease in its productivity or synthesis (Reddy and Bashamo, 1989). Prolong reduction also leads to blood dyscresis and degeneration of the erythrocyte (Buckley *et al;* 1976).

Adeyemo, (2005) and Gabriel *et al,* (2007) recorded significant changes in the WBC of *C. gariepinus* exposed to petroleum oil and cassava mill effluent respectively similar changes was also reported by Shakoori *et al,* (1996) in *Cyprinus ideela* exposed to fenvalerate, pyrethriod pesticide.

The increase in WBC counts recorded in this research when compared with other parameters could be due to the attempt of the fish to fight against the antigens (pollutant) and this augmented the production of more WBC to improve the health status of the fishes which however, agreed with the reports of Adeyemo (2005) and Gabriel *et al*, (2007) .The level of biochemical accumulation of total protein value obtained for the test organisms in 0.001mg/l, 0.00015mg/l and 0.0003mg/l were found to be higher than the control, While fluctuating values were obtained in 0.00025mg/l, this corroborated the submission of Vermer *et al*, (1979), and Kori esiakpere (1995) where they attributed depression in total protein to breakdown of amino acid and further into nitrogen and other elementary values. The notable decrease in plasma glucose of test organisms recorded in this study is in line with the report of Siwicki and Anderson (1993) that glucose reduction might be due to absorption of soluble glucose from intestine or the breaking down of lining cells by toxicant action.

Sudansu *et al*, (2008) also reported that biochemical parameters such as glucose, total protein and free amino acid are sensitive to pollutant in exposed organisms, depletion in glucose may be due to direct utilization of energy generation, while the decrease in protein value observed in some concentration was attributed to higher energy demand for metabolic purposes.The observed high of cholesterol in the exposed *C.gariepinus* could be linked to inhibitory potency of rotenone (the active ingredient in *T. vogelii*) which may impair the electron transport activity of the fish thus causing a deposit of which in consonance with the submission of Fukanmi 1970 and 1976 that rotenone act as general inhibitor of mitochondrial electron transport system.

In this study the decreased in protein could be linked to hypertrophy changes in liver, alternatively the increase in protein could as well indicate mild dehydration that is constant with relative water balance observed in the rate at which the test organisms respond in the polluted environment. Also, non-uniformity level of calcium, globulin and albumin recorded in this study may be traced to kidney clearance and liver malfunctioning; the same result was obtained by Fucci *et al*, (1983). He submitted that abnormal increase and decrease in this parameter may be connected to kidney and liver malfunctioning.

In conclusion, this research work revealed that exposure of *C. gariepinus* to *T vogelli* under laboratory conditions moderately affected some aspects of its physiology, a condition which may become aggravated in field application where lethal doses of the plant materials are employed in harvesting fish in many parts of Nigeria. The results obtained from this study implicated *T. vogelii* to be a poisonous plant hence the continuous use of the plant (*T.vogelii*) as fish bait by artisanal fishermen should be campaign against, as a result of its resultant negative effects on the quality of the water and the impairment of the body chemistry of the fishes due to bioaccumulation.

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