Blood Glucose Response of the African Cat Fish (*Clarias gariepinus)* to Bitter Leaf (*Vernonia amygdalina*) Incorporated Diet

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Abstract: **The effect of bitter leaf (*Vernonia amygdalina)* compounded diet on the blood glucose level of *Clarias gariepinus* was investigated. This was done to gauge the possible effect of using bitter leaf in compounded feed in ponds to regulate blood glucose levels in cultured fish. Fish were exposed to 5mg, 10mg and 15mg grounded bitter leaf mixed in 50mg fish feed. The control tank had no bitter leaf in the feed. Each treatment level and control was presented in triplicates. Blood was collected by cardiac puncture two (2) hours after exposure to the bitter leaf feed using a 21' gauge needles and syringes. Blood samples were tested immediately using a Fine Test® blood tester machine. Result indicates that all exposure concentrations had higher blood glucose levels than the control population. There were however no significant difference (P>0.05; P=0.055, P=0.989) between the control and treatment groups of 5mg and 10mg bitter leaf meal but there is a significant difference (P<0.05; P=0.044) between control and 15mg bitter leaf meal. Therefore it can be concluded that bitter leaf causes elevation of blood sugar and therefore should be used with restraint as it can cause hyperglycemic health complications in fish and perhaps in humans. The assertion that bitter leaf controls hyperglycemia in diabetics in humans may be false and misleading.**

Key Words*: Bitter leaf, Blood, sugar, glucose, Clarias *gariepinus, Vernonia amygdalina

I. INTRODUCTION

Vernonia amygdalina is a 2-5m tall plant with petiolate leaves of about 6.0mm wide. It is found largely in farmlands, forest, bush fallows, and homes (gardens) in the humid parts of the tropics of many parts of Africa (Echem and Kabari, 2013). It is used mainly in Nigeria as vegetable in the preparation of soups in the Western and Southern parts of the country. It has a bitter taste which is attributed to anti-nutritional factors and phyto-chemicals such as alkaloids, saponins, tannins and glycosides (Sobukola et al., 2006).

Apart from its nutritional use in soups, the plant is touted to be medicinal having a tonic with anti-malarial, anti-tumor, anti-diabetic and anti-bacterial properties (Nascimento et al, 2000). A medicinal plant is any plant in which one or more of its organ contains substances that can be used for therapeutic purposes (WHO, 1980). Although this claim about bitter leaf is unsubstantiated, it is however an important source of energy and provides nutrients to the body organs, muscles and nervous system. The absorption, storage and production of glucose are regulated constantly by complex processes involving the small intestines, liver and pancreas (Koeslag et al, 2003).

The measurement of blood sugar or blood glucose is a classical process of diagnosis in human medicine. It is used in the diagnosis of diabetes. Blood sugar or glucose is the main sugar found in the blood. One of the key physiological indicators measured in most scientific experiment is the blood glucose concentration expressing the general health condition of organism.

The commercial culture of fish (Aquaculture) entails a lot of intrigues dealing with mortalities bothering on sugar contents in feed used in sustaining fish stocks. As high blood sugar poses a severe threat to the survival of fish, there is an acute need to regulate blood glucose levels. This study therefore investigates the alleged efficacy of bitter leaf as a food addictive that reduces and stabilizes blood glucose level in fish and perhaps in humans.

II. MATERIALS AND METHODS

2.1 Experimental fish: Procurement and Transport

Juveniles of *Clarias gariepinus* of mixed sexes with mean length 16.6±2.8cm and mean weight 65.77±1.87g were procured from a reputable Aqua-culture fish farm in Swali, Yenagoa Bayelsa State, Nigeria. The fish were transported in 50 litres kegs containing 40 litres of water in the morning to the laboratory of the Department of Biological Sciences, Niger Delta University Wilberforce Island, Amassoma, and Bayelsa State.

2.2 Acclimatization

In the laboratory, the fish were acclimatized in separate containers using borehole water. During the acclimatization process the fish were fed twice daily with 4mm pelletized multi feed to satiation. The fish were acclimatized for 4 days before the commencement of the experiment.

2.3 Collection of Vernonia amygdalina (bitter leaf)

Fresh leaves of *Vernonia amygdalina* (bitter leaf) were bought from a local market in Azikoro, yenagoa, Bayelsa state Nigeria. The leaves were washed thoroughly without squeezing with clean tap water to remove dirt (sand and dust). Washed leaves were sun-dried for 5 days and the dried leaves pulverized with a manual blender and stored in an air tight plastic container.

2.4 Definitive Test (Experimental Tank)

The experimental tanks consist of four (4) plastic tanks labelled A, B, C and D. Five fishes were placed randomly in each of the four plastic aquaria tanks with 40 litres of water respectively. The tank labelled A was used as control. Tanks B, C and D contained the powdered leaves of *Vernonia amygdalina* of 5mg, 10mg and 20mg mixed properly and incorporated with 50mg of fish feed respectively. The control tank had no inclusion of bitter leaf (0mg).

2.5 Blood collection and Analysis

The fish were taken out individually after two (2) hours post feeding using a small hand net and placed belly upward on a table. Blood samples were obtained from the fish by cardiac puncture with physical restrain with the aid of a 21" gauge hypodermic needles and 2ml plastic syringes. Collected blood samples were analysed immediately using a Fine Test® blood tester machine. The result was recorded in millimoles per liter (mmol/l) for each of the test dose of bitter leaf inclusion and control.

2.6 Statistical Analysis

Data was analysed for means and standard deviations. Analysis of variance (ANNOVA) was carried out to determine the similarity and variability of blood sugar levels in the different treatment doses of bitter leaf and control at the 95% confidence limit. Turkey HSD Post Hoc Test was used to separate means where differences were found. Analysis was aided by the use of the SPSS® 20.0 tool kit.

III. RESULT AND DISCUSSION

3.1 Result

The result from the study is presented in Table 1 and Figure 1. All blood sugar levels were higher in the treatment groups than in the control group. There was no significant difference ($P>0.05$; $P=0.055$, $P=0.989$) between the control and treatment groups of 5mg and 10mg bitter leaf meal but there is a significant difference ($P<0.05$; $P=0.044$) between control and 15mg bitter leaf meal.

Table 1: Mean blood glucose response of *Clarias gariepinus* to Bitterleaf meal

Dose of Bitter leaf (mg)	Mean Blood Glucose (mmol/l)
0	*1.2±0.173[a]
5	6.06 ±3.68[ab]
10	2.26 ±0.77[ab]
15	6.30 ± 0.458[b]

*Mean ± Standard Deviation. Means with the same letter superscript on the same column are not significantly different ($P<0.05$).

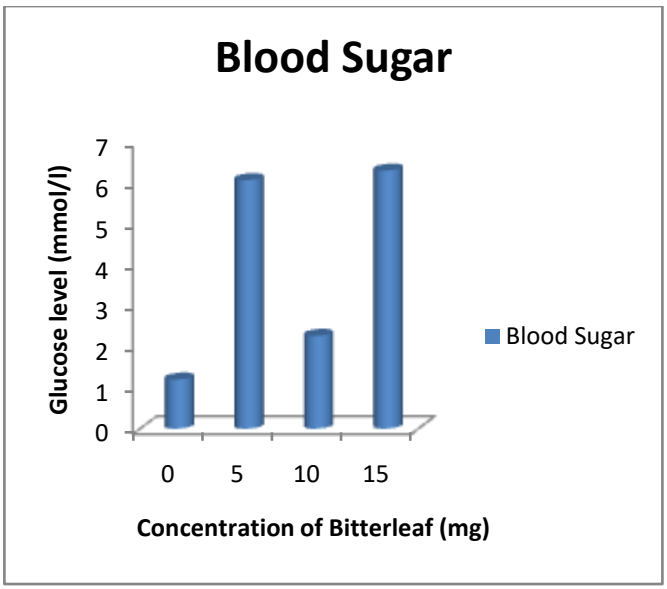

Figure 1: Mean blood glucose response of *Clarias gariepinus* to Bitterleaf meal

IV. DISCUSSION

The study recorded a rapid spike in blood sugar from control (0mg) to 5mg bitter leaf inclusion in feed, rising from a mean of 1.2mmol/l to 6.06mmol/l before dropping to a blood sugar level of 2.26mmol/l in the 10mg bitter leaf inclusion group before rising to 6.30mmol/l in the 15mg bitter leaf inclusion group.

The findings of this study are in disagreement with the works of a few scholars who observed reduction of blood sugar levels with the addition of phytochemicals. Malonyl ginsenosides, from the roots of *Panax ginseng* showed significantly lower fasting blood glucose level, improvement of insulin sensitivity and improvement of lipid profile in diabetic rats (Liu et al., 2013). In another study, two new flavones isolated from *Callistemon lanceolatus* DC (Myrtaceae) characterized as 5,7-dihydroxy-6,8-dimethyl- 4′ -methoxy flavone and 8-(2-hydroxypropan-2-yl)-5-hydroxy-7-methoxy-6-methyl-4′-methoxy flavones exhibited blood glucose lowering effect in streptozotocin induced diabetic rats (Syed et al., 2012).

The increase in blood sugar in this study may be as a result of mechanisms involved in medication-induced hyperglycemia includes β cell destruction, decreased insulin secretion and/or sensitivity, and excessive glucose influx. The majority of drugs associated with hyperglycemia affect insulin production, secretion, or action, leading to an imbalance in insulin and glucose homeostasis (Tosur et al, 2020).

On the other hand, the spike in blood sugar from 1.2mmol to 6.06mmol/l and the sudden drop to 2.26mmol/l from the control to 10mg and 15mg inclusion groups respectively is difficult to explain. However, hyperglycemia does not occur

in all individuals exposed to diabetogenic drugs but it is more common when several factors are involved, including: (1) Host-specific factors such as obesity, insulin resistance or β cell autoimmunity. (2) High doses of diabetogenic medications or multiple medications that affect glucose metabolism (ie, additive effect). (3) Environmental influences (eg, diet, stress, illness, lack of physical activity) (Tosur et al, 2020). This is the cardinal principle of multihit hypothesis and may be due to the predominance of either males or females in a particular group or ailing or stressed individuals as experienced in this study. Sex has been known to play a pivotal role in blood parameter classification, as males and females do not have same baseline blood ranges even for healthy subjects.

In this study, sex selection was not put into consideration. James *et al* (2017) studied growth and sugar response of *Clarias gariepinnus* brood stock fed diets enriched with bitter leaf meal (*Vernonia amygbalina*). Intersexual variation was observed with males exhibiting significantly different ($P < 0.05$) and higher haemoglobin, packed cell volume and total white blood cell values than females. The blood reference values obtained for the brood fish were observed to be higher than those recorded for healthy European and African catfish species.

The result from this study shows that bitter leaf causes hyperglycemia in fishes. Therefore, care should be taken in its administration as feed, food or medicine both for fish and humans.

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APPENDICES

Appendix I: mean blood sugar of C. gariepinus			
bitterleaf	Mean	N	Std. Deviation
0mg	1.2000	3	.17321
5mg	6.0667	3	3.68284
10mg	2.2667	3	.77675
15mg	6.3000	3	.45826
Total	3.9583	12	2.86053

APPENDIX II: ANOVA of blood sugar of C. gariepinus							
			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		61.196	3	20.399	5.664	.022
	Linear Term	Contrast	19.838	1	19.838	5.508	.047
		Deviation	41.358	2	20.679	5.742	.028
Within Groups			28.813	8	3.602		
Total			90.009	11			

Appendix III: Tukey HSD Post Hoc Test

(I) bitterleaf	(J) bitterleaf	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0mg	5mg	-4.86667	1.54955	.055	-9.8289	.0955
	10mg	-1.06667	1.54955	.899	-6.0289	3.8955
	15mg	-5.10000*	1.54955	.044	-10.0622	-.1378
5mg	0mg	4.86667	1.54955	.055	-.0955	9.8289
	10mg	3.80000	1.54955	.144	-1.1622	8.7622
	15mg	-.23333	1.54955	.999	-5.1955	4.7289
10mg	0mg	1.06667	1.54955	.899	-3.8955	6.0289
	5mg	-3.80000	1.54955	.144	-8.7622	1.1622
	15mg	-4.03333	1.54955	.116	-8.9955	.9289
15mg	0mg	5.10000*	1.54955	.044	.1378	10.0622
	5mg	.23333	1.54955	.999	-4.7289	5.1955
	10mg	4.03333	1.54955	.116	-.9289	8.9955

*. The mean difference is significant at the 0.05 level.