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EFFECT OF PHYLLNATHUS NIRURI LEAF AQUEOUS EXTRACT ON SERUM BIOCHEMISTRY AND HISTOLOGY OF WEANER PIGS.

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Abstract
A study using twenty-four (24) weaner pigs (large white hybrid) weighing an average of 9.0kg were used to investigate the effect of Phyllanthus niruri leaf aqueous extract on serum Biochemistry and histological status of weaner pigs. The pigs were randomly assigned to four treatment groups of six (6) pigs per treatment and replicated three times with two (2) pigs per treatment in a completely randomized design (CRD). The pigs were served the leaf extract at 0mls/l. 50mls/l, 100mls/l and 150 mls/l in T_1 (Control), T_2, T_3 and T_4 respectively. The animals were served water (the extract) and feed ad libitum. At the end of the twenty-four (24) weeks trial, blood samples were taken from all the replications for serum electrolytes. (Cholesterol and Conjugated Bilirubin) Similarly the heart, lungs and spleen were harvested from each replicate for histological studies. Data generated were subjected to a one-way analysis of variance (ANOVA). Results revealed significant increase in the electrolytes volume across all the treatment groups ($P<0.05$). Furthermore, the result for histological studies showed that the extract caused pockets of distortions at higher rates on the internal organs that were tested. It can be Concluded that inclusion rate in T_3 at 100mls/l as observed in this work should be sustained without any fears since it did not cause too fatal deleterious effects on the heart, spleen and lung organs.

Keywords: Phyllanthus *niruri*, Aqueous extract, Serum Biochemistry, Histological Studies, Weaner Pigs.

Introduction

Many slaughter slabs in different parts of Nigeria are tired of organ destruction by diseases and sicknesses (partial or total); and even by synthetic antibiotics occasioned by drug abuse, which may leave some residual effects on these organs. Some livestock farmers including pig farmers are screaming as a result of high cost of veterinary drugs and vaccines. Painfully, is also the high consultancy fees rural farmers pay to bring the veterinary officers to their farms. All these are factors that militates against livestock production. (Omu, 2023).

It is in view of these challenges that Phyllanthus *niruri* – the wonder herb, was being investigated because of its proximate composition and phytochemical components. Minja (2009) Fadholly, Ansari, Jayanti, Proboningrat, Kusala, Putri, Rantam, and Sudjarwo (2020), reported that Phyllanthus *niruri* possess anti-biotics, antiviral, antifungal, antibacterial, antimicrobial, anthelmintic, anti-oxidant and antispasmodic substances.

Extracts from Phyllanthus niruri have been proven to have therapeutic effects in many clinical studies. Some of the most therapeutic properties include: - anti-hepato-toxic, anti-lithic, anti-hypertensive, anti-HIV and anti-hepatitis B (Bagalkotker, Sagineedu, Saad, and Stenalas (2006). Nisar, He, Ahmed, Yang, Li, and Wan (2018).

Hakim et al (2016) and Husen et al, (2019), reported that Phyllanthus niruri has a wide range of pharmaceutical activities like anti-microbial, anti-viral, anti-inflammatory, anti-plasmodia and diuretic. Similarly, Burten et al (2003), reported that over 50 of all modern chemical drugs are of natural plant origin, and is essential in drug development programmes of the pharmaceutical industry.

Histological Studies of some internal organs such as the heart, lungs and spleen indicate that excess consumption of extracts have caused huge damages or gross lesions. Rose and Paulina, (2016).

Many person including those whose religion and/or culture do not prohibit rearing and consumption of pork do not only regard the pig as a dirty animal, but are often times of the opinion that it contains higher concentration of cholesterol than other common food animals. Asuamah, Antwi-Boateng and Owusu-Prempe(2013). Thus, the report of these same

authors show that this health concerns are among the major reasons why pork is rejected in many parts of the tropics.

Furthermore, this study intends to disabuse every one that cholesterol levels in the liver, kidneys, muscle, skin, intestine and brain of domestic animals are not the same for the pork, Today, Nigeria can now produce lean pork as against what it used to be some years ago. This is because many pig farmers in an attempt to cut down on production cost, not only compound diets using agro-industrial by-products, most of which are high in fibre but mostly maintain their animals of all age groups on restricted feed. Abonyi et al (2012).

fish and poultry. These variations could also be due to the differences in the gender or species of animals sampled. Duckett, Neel, Fontenot, and Clapbam (2009).

The objective of this study was to find out the effect of Phyllanthus *niruri* leaf extract on serum Biochemistry (Cholesterol and Conjugated bilirubin) and histopathology (heart, lungs and spleen) of weaner pigs.

Materials and Methods

Experimental Site: The study was carried out at the piggery unit of the Teaching and Research Farm, Niger Delta University, Wilberforce Island, Amassoma. Amassoma is situated between latitude – 4^{o} 58^{i} 13^{ii} N and longitude 6^{o} 6^{i} 32^{ii} E of the Equator. Annual rainfall is 2400. 2900 mm Temperature is 32^{o}c and Relative Humidity is 87%. Bayelsa State Map (2006). BYSADEP (2021).

Experimental Animals and Management: Twenty-four (24) weaner pigs (large white hybrid) of either sexes were procured early this year (2023) from a reputable farm in Port-Harcourt, Rivers State. Meanwhile, the pigs were first kept in a common pool for about ten days in their new environment for acclimatization.

Experimental Design: The experimental animals were randomly allotted to four treatment groups with six (6) pigs per treatment and three replications with six pigs per replication in a completely randomized design (CRD). This study lasted for twenty four (24) weeks.

Collection and processing of the plant (Herb)

Phyllanthus *niruri* plant were collected from the University community, put into bags and taken to the animal science laboratory. They were thoroughly washed to eliminate sand, dust, mud, larvae and eggs of insects. The plants were put in a large pot and boiled for about sixty minutes at a temperature of 500^{0}c.

Inclusion Rate and Extract Administration

The extract was included in their drinking water at the levels of 0mls/l, 50mls/l, 100mls/l and 150mls/l in T_1, (Control) T_2, T_3 and T_4, respectively. The extract was served daily as their drinking water. Intake was determined as quantity served minus left over quantity the following day.

Feeding of Experimental Animals

Broiler starter and finisher concentrate feeds were bought from the open market. Cassava peels were collected and parboiled, and plantain peels (ripe and unripe) were also sourced for and generously served. The peels served as supplemental feeds. All feeds and feed materials were of equal weight.

Data Analysis

Data was subjected to a one-way analysis of variance (ANOVA) in a Completely Randomized Design (CRD) as prescribed by AOAC (2019). Significant differences

Housing of Experimental Animals

The study animals were kept and intensively managed in a well-built half wall and concrete floor with adequate ventilation.

Data Collection

At the end of the field experimentation which lasted for twenty-four (24) weeks, two weaner pigs were randomly picked from each treatment weighed, slaughtered and eviscerated. The heart, lungs and spleen were harvested for histological studies. Serum was also collected to perform serum biochemical studies.

Histological Assays

The histological studies on the heart, lungs and spleen were performed at the teaching hospital, histology department, Ibadan, Oyo State. Drying and mounting was done using Dibutyl phthalate progesterone xylene (DPX). Slides were viewed at x100 and x400 objectives. (DPX). Lesson and Lesson, (2011). Wick, (2019).

($P<0.05$) were compared using the Duncan's new multiple range test (NDMRT) as outlined by Obi (2001). SPSS (2021) statistical package for social sciences, version 27, was used to perform all the statistical calculation.

Results

Table 1: Table showing Effect of Phyllanthus *niruri* Leaf Extract on Serum Biochemistry of weaner pigs.

PARAMETER	T_1(0mls/l)	T_2 (50mls/l)	T_3 (100mls/l)	T_4 (150mls/l)	SEM
Cholesterol mg/dl	1.602	1.727	1.813	1.721	0.08
Conjugated Bilirubin mg/dl	0.035	0.042	0.057	0.046	0.19

abcd: Mean on the same rows with different superscripts are significantly (P<0.05) different
NS: Not Significant
SEM: Standard Error of Means

Results obtained from Serum Biochemistry indicate that cholesterol gave the following values: T_1 (Control) (1.602 mg /l), T_2 (1.727 mg /l), T_3 (1.813 mg /l) and T_4 (1.721 mg/l). The figures show that T_1 (control) was the least significant (P<0.05). While $T_2 – T_4$ showed some treatment effects above T_1. Also, T_3 revealed the highest significant (P<0.05) difference among all the treatment groups. Conjugated bilirubin showed T_1 (control) as the least significant (P<0.05) with the value of 0.035 mg/l. Meanwhile, the rest of the treatments $T_2 – T_4$ were significantly (P<0.05) higher: T_2 (0.042), T_3 (0.057) and T_4 (0.046) mg/dl, respectively, T_3 had the highest values of (P<0.05) values.

Table 2: Table showing the Effect of Phyllanthus *niruri* Leaf Extract on Histology of Waner pigs.

Parameter	T_1(0mls/l)	T_2(50mls/l)	T_3(100mls/l)	T_4(250mls/l)
Heart Muscle	-	MFLH/RHD	MCVS/DHS	MAM/MDHD
Lungs	-	MFLH	-	MIP
Spleen	-	MFLH	MCVS	MCVS

Key: Moderate Follicular Lymphoid Hyperplasia (MFLH).

Random Hepatocellular Degeneration (RHD).

Moderate Congestion of Vascular Spaces (MCVS).

Diffused Hepatocellular Swelling (DHS).

Moderate Atrophy of Myofibers (MAM)

Mild Interstitial Pneumonia (MIP).

Moderate Diffused Hepatocellular Degeneration (MDHD).

Histological investigation of the heart, lungs and spleen organs indicate that T_1 (the control) was clear of any form of gross lesions. But the treatment effects for T_2 to T_4 revealed different forms and degrees of distortions.

The Heart: T_1 No observable distortions.

T_2 Moderate Follicular Lymphoid Hyperplasia (MFLH) and Random Hepatocellular Degeneration (RHD).

T_3 Moderate Congestion of Vascular spaces (MCVS) and Diffused Hepatocellular swelling (DHS).

T_4 Moderate Atrophy of Myofibers (MAM) and Moderate Diffused Hepatocellular Degeneration (MDHD).

The lungs: T_1 No observable distortions.

T_2 Moderate Follicular Lymphoid Hyperplasia (MFLH).

T_3 No observable lesions.

T_4 Mild Interstitial Pneumonia (MIP).

The spleen: T_1 No observable distortions.

T_2 Moderate Follicular Lymphoid Hyperplasia (MFLH).

T_3 Moderate Congestion of Vascular Spaces (MCVS).

EFFECT OF PHYLLNATHUS NIRURI LEAF AQUEOUS EXTRACT ON SERUM BIOCHEMISTRY AND HISTOLOGY OF WEANER PIGS.

T_4 Moderate Congestion of Vascular Spaces (on the red pulp of the spleen) (MCVS).

Discussion

Under serum biochemistry, blood cholesterol values in the control treatment was significantly the least. It did not receive the treatment and was within the normal range. It did not pose any threat to the animals. Notably, cholesterol is biosynthesized by all animal cells and is an essential structural component of animal cell membranes (Banerjee, 2013). Moreover, it serves as a precursor (indicator) or agency for the synthesis of steroid hormones.

The membrane remains stable and durable allowing for normal rigidity and growth of the experimental animals. Results obtained from this study indicate that T_2 - T_4 had an increase in the levels of cholesterol in the blood and within normal levels of 1.00 – 2.5 mg/dl. Higher cholesterol levels suggest high risk of arteriosclerosis. Bragagnolo (2009), and Sinclair et al (2010), reported that most organ meats contain substantially more cholesterol than skeletal muscle or tissues of their respective species.

According to Williams (2007), bone marrow and brain contain much great cholesterol content that could be up to several hundred miligrams per 100g.

Measurement of Conjugated Bilirubin metabolite is of assistance in diagnosis and monitoring of the many disease states associated with raised bilirubin, (Martins 2003).

Excretion of conjugated bilirubin is impaired in a number of acquired conditions- alcoholic and viral hepatitis, biliary obstruction, cholestasis of pregnancy and in inherited disorders such as Dubin-Johnson syndrome, Roter syndrome, Benign recurrent intra-hepatic cholestasis. Guadiolar et al (2006).

Results from data generated on conjugated bilirubin shows a yellow cast on the skin and the whites of the eyes. High bilirubin in the blood can also leak out in the urine, making it darker for all domestic animals and man. Banerjee (2013).

From the histopathological findings in this present work, the heart revealed so many gross lesions such as moderate follicular Hyperplasia, and random hepatocellular degeneration; moderate Congestion of vascular spaces and diffused Hepatocellular swelling; moderate Atrophy of myofibrils and moderate diffused hepatocellular degeneration in T_2, T_3 and T_4, respectively. T_1 (the control) was free of any distortions as it did not receive the extract

Contrary to the negative findings from this work, Bernatoniene et al (2014) reported that Leonuru cardiaca (Motherwort) herb extract and some of its flavonoids on the mitochondrial oxidative phosphorylation in the heart was used as a complementary circulation. The same authors posited that the extract can be effective in treating heart disease.

The heart is a critical organ that keeps blood moving throughout the body. Blood is an important medium that not only carries nutrients and oxygen throughout the body, but also collects waste products and returns them to the liver and kidney for further processing and excretion. The heart is able to achieve all of this because of its autonomy based on its histological make-up. Lerenzo (2023), Zhou, Chang, Zhu, Nasser, Zhang and Zhao (2020).

The spleen is the largest lymphatic organ in the body. It is surrounded by a connective tissue capsules which extends inwards to divide the organ with lobules. The spleen consists of two types of tissues called white pulp and red pulp. The red pulp is a blood filter that removes foreign materials, damaged, and effete erythrocytes. It is also a storage site for iron, erythrocytes and platelets.

Results of the present study on spleen indicate that the leaf extract at increasing levels caused some degrees of distortions including Moderate Follicular Lymphoid Hyperplasia (MFLH) and Mild Interstitial Pneumonia (MIP) in T_2 and T_4: except T_1 (control), without any observable lesions.

El-Tazi et al (2014), and Pond (2009), reported that excess consumption (overdose) of extracts from seeds, leaves and roots have been indicated in severe damages of internal organs. In a related work, Safa and El-Tazi (2011), stated that extracts when consumed over a long period of time in large quantities and high concentration are capable of causing deleterious effects on internal organs. The same authors advised that caution should always be taken when extracts are to be served.

Journal of Agriculture, Environmental Resources & Management

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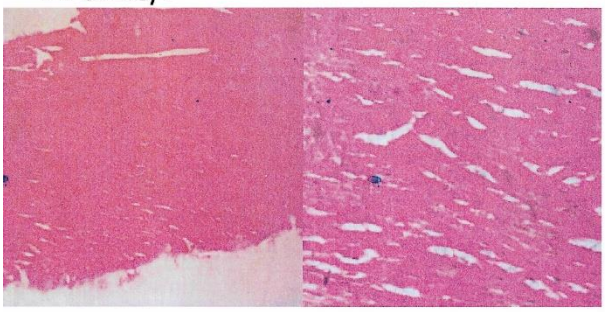
HISTOPATHOLOGICAL STATUS OF THE HEART MUSCLE, LUNGS AND SPLEEN.

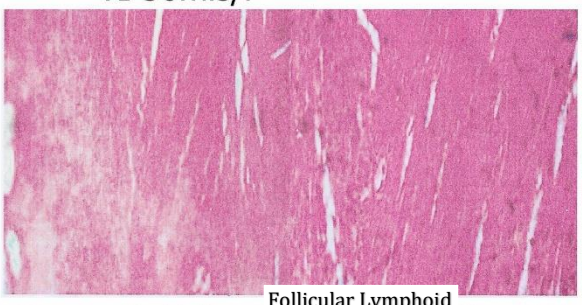
Picture 1: Heart Muscle

T_1 0mls/l



Heart Muscle- No Observable Distortion HE x100,x400

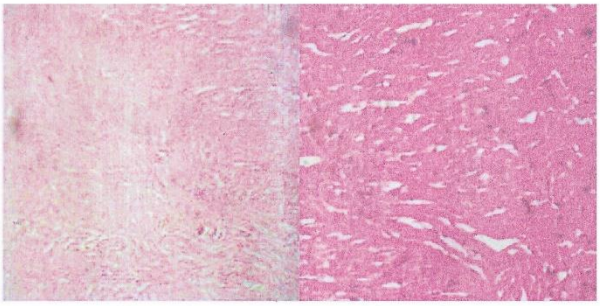
T_2 50mls/l



Follicular Lymphoid

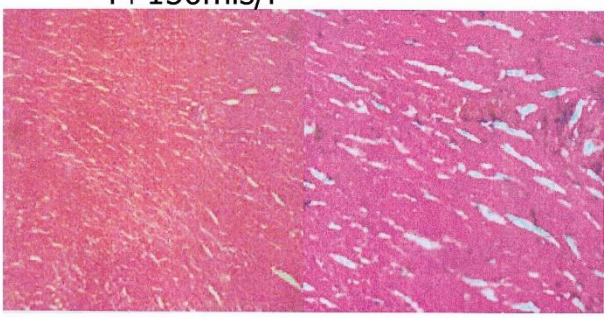
Heart muscle- Moderate Fellicular Hymhoid Hyperplasia, Random Hepatocellular Degeneration HE x100,x400

T_3 100mls/l



Heart muscle- Moderate Congestion of Vascular Spaces Diffused Hepatocellular Swelling HE x100,x400

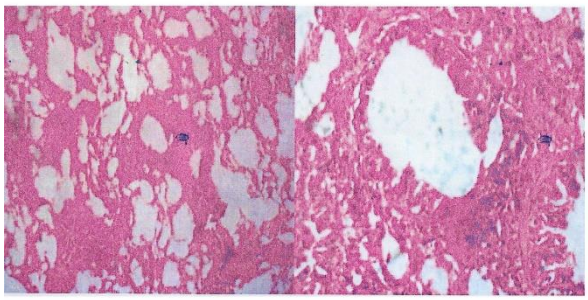
T_4 150mls/l



Heart muscle- Moderate Atrophy of my Fibres, Moderate Diffused Hepatocellular Degeneration. HE x100,x400

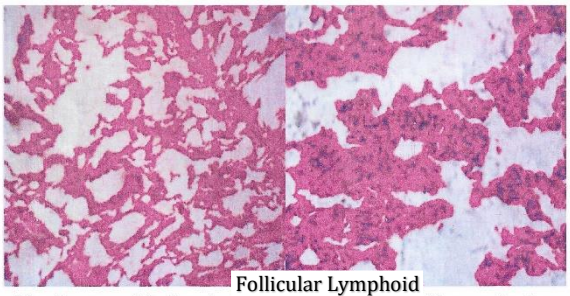
Picture 2: The Lungs

T_1 0mls/l



The Lungs - No Observable Distortion HE x100, x400

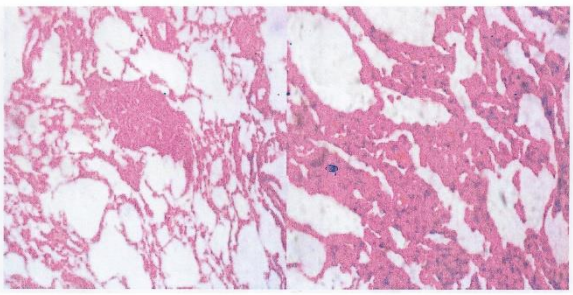
T_2 50mls/l



Follicular Lymphoid

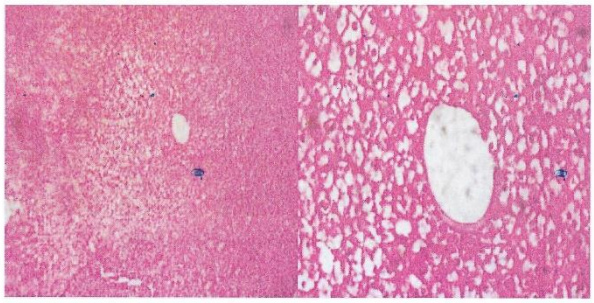
The Lungs - Moderate Fellicular Hymhoid Hyperplasia, HE x100, x400

T_3 100mls/l



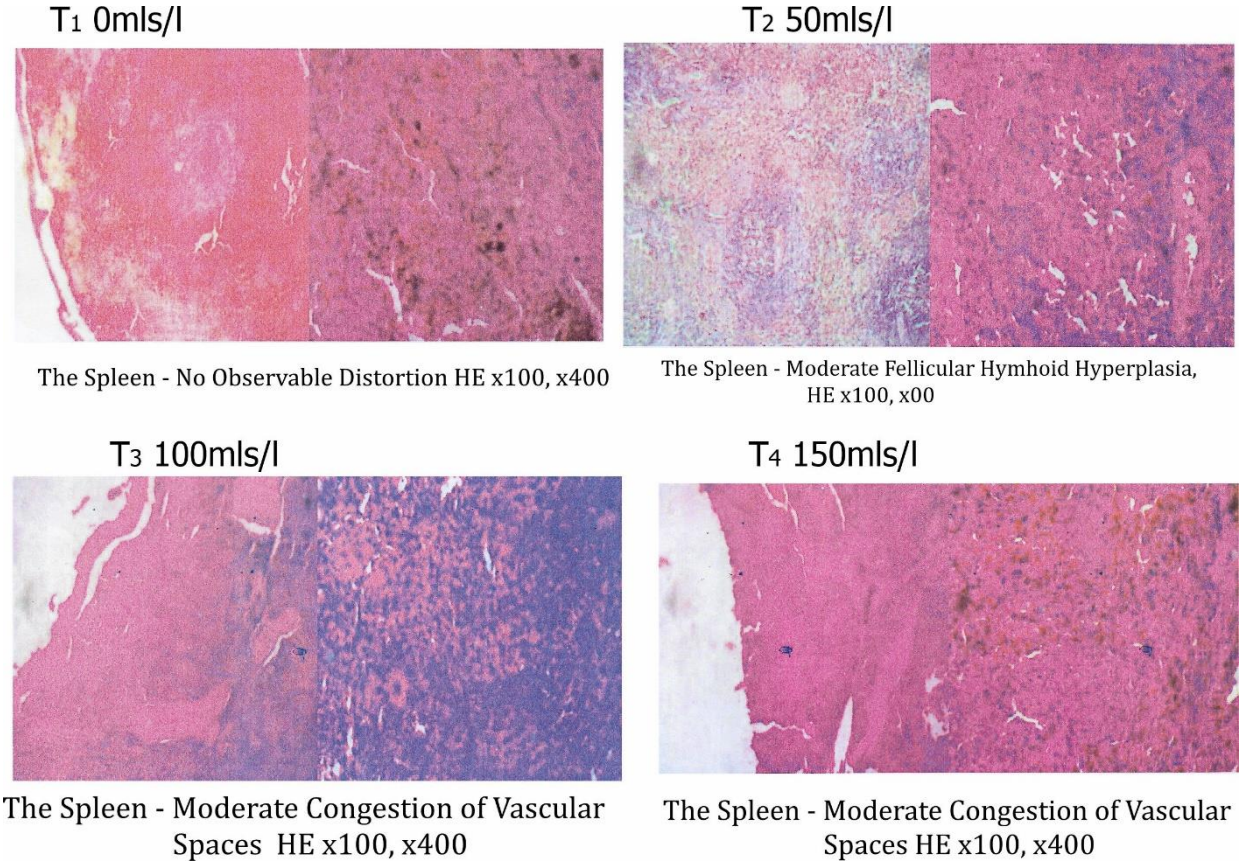
The Lungs - No Observable Lesion HE x100, x400

T_4 150mls/l



The Lungs - Mild Interstitial Pneumonia HE x100, x400

Picture 3: The Spleen



Conclusion and Recommendations

Duckett et al (2009) reported that multiple factors including gender, age, degree of marbling, subcutaneous fat thickness, animal breed, diet, feeding treatments (restricted or ad libitum), and muscle location/type of cut affect the cholesterol content in meat. Conjugated (direct) bilirubin is the form of bilirubin which has been juxtaposed with glucuronic acid and is excreted in the bile from the histological studies of the heart, lungs and spleen, our findings indicate that the leaf extract created gross distortions in these organs at moderate degrees.

It is hereby recommended that pig farmers should go for the production of lean pork. Caution should be taken not to exceed the recommended dose in this work.

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