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Organ Weight and Histomorphological Changes in Buck Rabbits Fed *Aspilia africana* (African Marigold) Leaves Supplemented With Zinc Gluconate

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Abstract
This work was carried out to evaluate organ weight and histological changes in 24 buck rabbits subjected to *Aspilia africana* leaves supplemented with zinc gluconate. The bucks were divided into four equal groups. Each treatment was replicated three times in a completely randomized design. Treatment group one (T_1) was fed concentrate plus conventional forages with 0.0mg zinc gluconate. Treatments 2, 3 and 4 were fed concentrate with 0.0mg zinc gluconate, 100mg zinc gluconate and 150mg zinc gluconate, respectively. *Aspilia africana* with zinc gluconate supplementation significantly increased the relative weights of the liver, lungs, heart and gastrointestinal tracts. There was no pathological change in the kidneys of bucks fed *A. africana* with or without supplementation compared to those fed conventional forages. Mature spermatid density was reduced in the testes of bucks fed *A. africana* without zinc gluconate. The density of spermatids per tubule, however, increased with the two levels of zinc gluconate. Severe portal inflammation with interface necrosis was observed in bucks fed 0.0mg zinc gluconate and 100mg zinc gluconate, respectively. The inflammation became mild with 150mg zinc gluconate without necrosis. Fresh leaves of *Aspilia africana* without zinc supplementation reduced the efficiency of spermatogenesis by lowering spermatid density. It also caused severe portal inflammation. Zinc gluconate supplementation at both levels increased the spermatid density per tubule; and at 150mg ameliorated the inflammation and eliminated necrosis of the liver cells.
Keywords: Relative organ weight, histology, Buck rabbits, Aspilia africana, and zinc

Introduction

A comprehensive evaluation of the gross and specific impacts of feeds in research animals requires more than assessing the dynamics of the blood indices. Blood cells and metabolites may be within the reference ranges in normal subjects but cannot provide all necessary information about the structural changes and functionalities of the organs such as the liver, testes, kidneys, etc., which are affected at the cellular level in feeding trials. Many herbs are being assayed for possible inclusion in the feeding of animals due to their bioactivity. It is imperative to complement blood analysis of research animals subjected to herbs with necessary studies of the potential changes that might occur in the micro-architecture of vital organs.

The twigs of *Aspilia africana* are relished by herbivores such as cattle, sheep, goats and rabbits. Fresh and wilted leaves, flowers as well as various solvent extractions of this herb have been credited with potency to improve weight gain in rams, rabbits, broilers, and growing and laying Japanese Quails (Uchewa *et al.,* 2018; Etim, 2015a; Adedeji, *et al,* 2015; Adedeji *et al*., 2014; Agiang *et al*., 2011). The leaves have also been reported to possess haematopoietic properties (Etim and Oguike 2011; Ajeigbe *et al*., 2013). The foregoing attributes of *Aspilia africana* may not be unconnected with its abundance of micro and macro elements as reported by Okwu and Josiah (2006).

However, the leaf extracts were implicated in a study in which testosterone levels were significantly reduced with varying levels of degeneration in testicular epithelium, fibrosis and abnormalities in cytoplasm (Asuquo *et al*., 2015). In Wistar rats receiving 150-300g of *Aspilia africana* flowers per kg live weight, there were dose-dependent histopathological alterations in testes. Specifically, the pathologies featured widened interstitial spaces and disrupted spermatogenic cycles (Okwuonu *et al*., 2020). It was also reported by Ezea and Ibe (2021) that fresh leaves of *Aspilia africana* fed as forage to buck rabbits without zinc gluconate

supplementation significantly compromised sperm progressive motility, live sperm percentage, sperm concentration, number of sperm per ejaculate, total viable sperm and normal sperm proportion while significantly accentuating specific sperm abnormalities.

Zinc plays a substantial role in physiological functions. These include but are not limited to immune modulation and anti-oxidation (Garcia-Contreras *et al*., 2011). The antioxidant capacity may be responsible for its role in cytoprotection (Lansdown *et al*., 2007). There is an information gap on the impact of supplementing fresh *Aspilia africana* leaves with zinc gluconate on organ weight and histo-architecture of testes, liver and kidneys of buck rabbits. This gap necessitated the research.

Materials and Methods

Study Location

The study was carried out at the Rabbitry Unit of the Livestock Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike, Abia State. Umudike lies at coordinates 05^0 29' N and 07^0 33' E and has an altitude of about 122m above sea level. The average annual rainfall ranges from 1700 to 2100 mm. Minimum and maximum temperatures are in the ranges of 18-23^0 C and 26-36^0 C, respectively; while relative humidity is 57-91% (NRCRI, 2023).

Experimental Rabbits and Management

A total of twenty-four (24) grower buck rabbits) of New Zealand White breed were used for this experiment. The rabbits were sourced from a private farm in Aba, Abia State; and were made to undergo acclimatization to the new environment for three (3) weeks before the study commenced. Clean water, and feed (concentrates and forages) were provided for the animals *ad-lib*. They were randomly divided into four equal-treatment groups. Each treatment group was replicated thrice, two rabbits constituting a replicate in a completely randomized design (CRD). All the bucks were fed a basal concentrate diet. Treatment one (T_1) (control) was made up of a conventional forage mixture of *Calopogonium mucunoides*, *Centrosema pubescens* and *Panicum maximum* with zero Zinc gluconate. Treatments two (T_2), three (T_3) and four (T_4) were constituted by fresh *Aspilia africana* leaves and *Panicum maximum* with Zinc gluconate supplemented at 0.00mg/kg, 100mg/kg and 150mg/kg of feed, respectively.

Procedure for Organ Histology

Histology of the liver, kidneys and testes was carried out according to Clayden (1967) and John and Alan (1977). A slice of each organ was fixed in a 10 % formal saline for 48 hours. Specimens were processed by placing them in ascending grades of alcohol in this order: 50% alcohol for 1 hour, 70% alcohol for 1 hour, 95% alcohol for 1 hour (first), 95% alcohol (second) for 1 hour 15 minutes, absolute alcohol for 1½ hours (first), and absolute alcohol (second) for 2 hours, to ensure proper dehydration of the tissues. The dehydrated tissues were transferred to a mixture of equal volumes of alcohol and xylene and left overnight; and were cleared with two changes of xylene for 1 hour each. They were thereafter infiltrated twice for 1 hour each with molten paraffin wax in the oven at 60^0C. They were then embedded in paraffin wax, trimmed and mounted on a wooden chuck and taken to a microtome for sectioning at 5µm thickness. The sections were floated in a floating-out bath from where they were picked with clean albumenized slides. The slides were placed in a staining dish. Excess wax was removed by two changes of xylene. The specimens were hydrated by descending grades of alcohol in this order: absolute alcohol, 95% alcohol and 70% alcohol, for 2 minutes each. The slides were then stained by infiltrated Ehrlich haematoxylin for 15 minutes, washed in water for 5 minutes, differentiated in 10% acid alcohol and blued in a running tap for 10 minutes. They were counter-stained with filtered eosin for 2 minutes. Excess eosin was removed in ascending grades of alcohol in this order: 75% alcohol, 95% alcohol and absolute alcohol for 2 minutes each, before clearing in two changes of xylene and each cover slipped with duplex mountant. The slides were viewed under a light microscope. Selected images were captured using a Motican 2.0 digital camera attached to a computer.

Statistical Analysis

Data collected on the different parameters were subjected to analysis of variance (ANOVA), following the methods of Steel and Torrie (1980). ANOVA procedure was used to generate the means and standard errors of the means. Significant means were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

The relative organ weight of buck rabbits fed *Aspilia africana* leaves supplemented with zinc gluconate is presented in Table 2. There were significant differences in the organ proportions (absolute weight of organ expressed as a percentage of the live weight) of bucks in the various treatment groups, except the kidneys and spleen. The liver, lungs and gastrointestinal organs seemed to have constituted significantly greater proportions of the weight of the live bucks with zinc supplementation. The quantities of zinc gluconate administered to the bucks might have more or less unsettled the mineral homeostasis. The liver as a principal organ involved in metabolism might have reacted to the increase in size of the hepatocytes to contain the challenge. An increase in zinc supplementation resulted in a greater proportion of the buck's live weight being occupied by the gastrointestinal tracts. Zinc might have evolved to increase the growth of the smooth muscle of the GIT. This may not be in the favour of a rabbit farmer who is producing rabbits for meat. The photomicrographs of the kidneys of Buck rabbits fed *Aspilia africana* leaves supplemented with Zinc gluconate are presented in plates 1a-1d.

Plates 1a-1d: Histomorphological changes in the kidneys of rabbit bucks fed *Aspilia africana* supplemented with Zinc gluconate.

Plate 1a: Photomicrographs of kidneys showed evenly distributed glomeruli (G), of uniform size, with normal

cellularity of the mesangium (M). There were numerous open capillaries of the glomeruli; the endothelium was normal. There was a normal density of the tubules (T). The tubular epithelium was viable.

Plate 1b: In comparison with 1a, there was no pathological lesion.

Plate 1c: In comparison with, 1a there was no pathological lesion

Plate 1d: In comparison with, 1a there was no pathological lesion

There was no significant difference in the photomicrographs of the kidneys of all the bucks.

The photomicrographs of testes of buck rabbits fed *Aspilia africana* supplemented with Zinc gluconate are presented in plates 2a-2d

Plates 2a-2d: Histomorphological changes in the testes of rabbit bucks fed *Aspilia africana* supplemented with Zinc gluconate.

Plate 2a: Photomicrographs of testes showed unaltered seminiferous tubules and interstitium. There were spermatozoa at different stages of maturation around the tubules. The mature spermatid density was about 420 per tubule

Plate 2b: Relative to 2a, there were spermatozoa at different stages of maturation around the tubules with the mature spermatid density averaging 240 per tubule.

Plate 2c: In comparison with 2a, there were spermatozoa at different stages of maturation around the tubules. Mature spermatid density was about 280 per tubule.

Plate 2d: Compared to 2a, there were spermatozoa at different stages of maturation around the seminiferous tubules with the density of mature spermatid being about 400 per tubule.

The photomicrographs of livers of buck rabbits fed *Aspilia africana* leaves supplemented with Zinc gluconate are presented in plates 3a-3d

Plates 3a-d: Histomorphological changes in the liver of buck rabbits fed *Aspilia africana* leaves supplemented with Zinc glucona

Plate 3a: Photomicrographs showed well-preserved micro-architecture of the livers. The portal triads were evenly spaced around a central vein. There was mild portal inflammation devoid of interface/piecemeal necrosis or fibrosis. There was no fat deposit (steatosis).

Plate 3b: In relation to 3a, there was severe portal inflammation and interface/piecemeal necrosis without fibrosis or steatosis

Plate 3c: Compared to 3a, there was severe portal inflammation with interface (piecemeal) necrosis without fibrosis or steatosis.

Plate 3d: In comparison with 3a, there was mild portal inflammation without interface necrosis or fibrosis. There was no fat deposit (steatosis).

Products of *Aspilia africana* leaves have been implicated in various abnormal histology of organs such as testes, liver, ovaries and uterus; and associated bio-functions. It is worthy of note that the same phyto-components that mediate some essential bioactivities may also be responsible for some unpalatable observations in the organs of animals. Implicated in the abnormal physiology of organs are phytoestrogens (saponins, essential oils). Phyto-estrogenic principles possess both estrogenic and counter-estrogenic effects on reproductive systems. Application of *Aspilia africana* for a long period could cause reduced fertility e.g. reduced spermatid density. Oguike *et al*. (2019) reported a reduction in the weight of testes, epididymides, seminal vesicles and serum testosterone in buck rabbits fed *Aspilia africana*. According to Dimo *et al*. (2002), methylene extracts of the leaf caused increased *in vitro* vascular smooth muscle contraction in preparations made from rings of the aorta of rats. There was atrophy of smooth muscles of blood vessels and myometrium with dose increase. Oyesola *et al*. (2010), reported significantly reduced weight gain, and ovulation rate, inflamed oviducts, degenerated ovarian cortex and disrupted uterine endometria in rats subjected to 500-1000mgkg^{-1} body weight. Flavonoids in the leaves of *Aspilia africana* were implicated in the inhibition of cyclo-oxygenase activity and prostaglandin synthesis. The increasing spermatid density and improvement in liver micro-architecture with an increase in Zinc gluconate could be a pointer to the ameliorative role of zinc in biological systems under induced stress (Bayram *et al*., 2022, El-Kossi *et al*., 2024).

Conclusion

Aspilia africana with zinc gluconate supplementation caused a significant increase in relative weights of the liver, lungs, heart and GIT. There was no pathological change in the kidneys of bucks fed *A. africana* with or without ZG supplementation, compared to those fed CFM. There was reduced mature spermatid density in the testes of bucks fed *A. africana* without ZG. The density increased with levels of ZG supplementation. Severe portal inflammation with interface necrosis was observed in bucks-fed *A. africana* with zero ZG and 100mgZG, respectively. The inflammation became mild with 150mgZG with a complete absence of necrosis. The twigs of *Aspilia africana* should not be fed alone regularly to breed rabbit bucks for a long period. Breeding rabbit bucks that are fed fresh leaves of *Aspilia africana* should be supplemented with up to 150mg of Zinc gluconate/kg of basal diet for enhanced reproductive and hepatic health.

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Ingredients Composition of Grower Rabbit Diet

Table 1: Composition (%) of concentrate ration for the buck rabbits

	Treatment			
Ingredient	**CFM**	**Asp+ 0.0mgZG**	**Asp+ 100mgZG**	**Asp+ 150mgZG**
Maize	44.94	44.94	44.94	44.94
Soya bean meal	17.31	17.31	17.31	17.31
Rice husk	32.00	32.00	32.00	32.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00
Vitamin/mineral premix	0.25	0.25	0.25	0.25
Common Salt	0.50	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**
Zinc gluconate	0.00	0.00	100	150
Crude protein	17.00	17.00	17.00	17.00
Metabolizable energy	2505.42	2505.42	2505.42	2505.42

CFM = Conventional forage mixture, Asp = *Aspilia africana*, ZG = Zinc gluconate

Table 2: Relative organ weight (%) of buck rabbits fed *Aspilia africana* leaves supplemented with Zinc gluconate

Parameters	CFM	Asp+ 0.0mgZG	Asp+ 100mgZG	Asp+ 150mgZG	SEM
Live weight (g)	2055.00[a]	1877.00[a]	1520.00[b]	1906.00[a]	72.81
Liver	2.17[c]	2.29[c]	2.67[b]	3.08[a]	0.116
Lungs	0.51[b]	0.35[c]	0.52[a]	0.60[a]	0.03
Kidney	0.35	0.53	0.51	0.60	0.45
Heart	0.26[a]	0.21[b]	0.28[a]	0.20[b]	0.01
G.I.T	9.10[d]	11.61[c]	12.51[b]	15.14[a]	0.66
Spleen	0.04	0.02	0.38	0.45	0.08

[a-d] Means along the same row with different superscripts are significantly ($p<0.05$) different. ZG = Zinc gluconate

SEM= Standard Error of Mean. G.I.T = Gastrointestinal tract, CFM = Conventional forage mixture, Asp = *Aspilia africana*

Plates 1a-1d: Histomorphological changes in the kidneys of rabbit bucks fed *Aspilia africana* leaves supplemented with Zinc gluconate

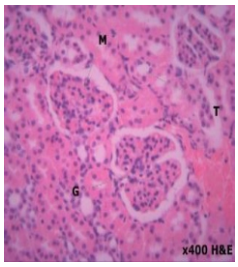

Plate 1a: Kidneys of bucks fed CFM/0.00mgZG

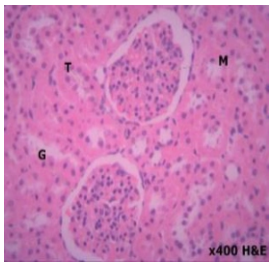

Plate 1b: Kidneys of bucks fed A. *africana*/0.00mgZG

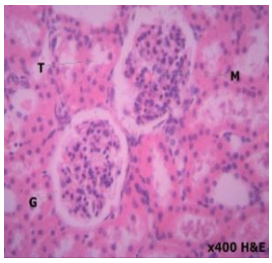

Plate 1c: Kideys of bucks fed *A. africana*/100mgZG

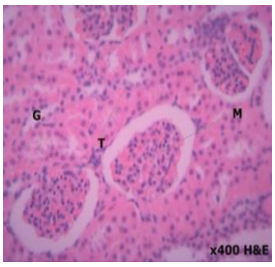

Plate 1d: Kidneys of bucks fed *A. africana*/150mgZG

Keys: G = Glomerulus, M =Mesangium, T =Tubule, CFM = conventional forage mixture, ZG = zinc gluconate

Plates 2a-2d: Histomorphological changes in the testes of rabbit bucks fed *Aspilia africana* supplemented with Zinc gluconate

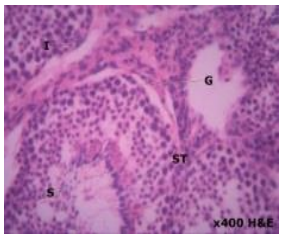

Plate 2a: Testes of bucks fed CFM/0.00mgZG

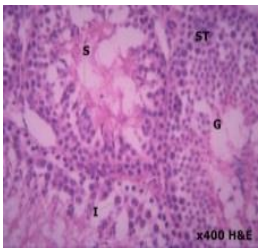

Plate 2b: Testes of bucks fed *A. africana*/0.00mgZG

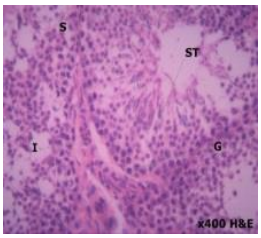

Plate 2c: Testes of bucks fed *A. africana* /100mgZG

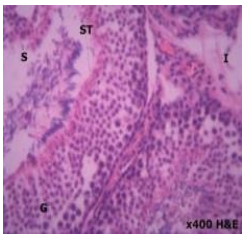

Plate 2d: Testes of bucks fed *A. africana* /150mgZG

Key: G = Germinative cells (spermatogonia), I = Interstitial cells of Leydig. ST = Sertoli's cell. S = mature spermatid, CFM = conventional forage mixture, ZG = zinc gluconate

Plates 3a-d: Histomorphological changes in the liver of buck rabbits fed *Aspilia africana* leaves supplemented with Zinc gluconate

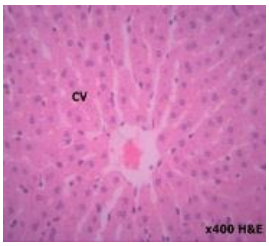

Plate 3a: Liver of bucks fed CFM/0.00mgZG

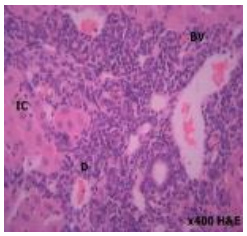

Plate 3b: Liver of bucks fed *A. africana*/0.00mgZG

Plate 3c: Liver of bucks fed *A. africana*/100mgZG

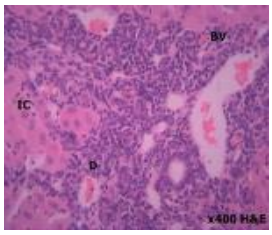

Plate 3d: Liver of bucks fed *A.africana*/150mgZG

Keys: IC = Inflammatory cells, BV = Blood vessel, D = Hepatic ductile, CV = Central vein, ZG = zinc gluconate, CFM = conventional forage mixture