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The Effects of Ethanolic Root Extract of Rauwolfia Vomituria on the Testes of Adult Male Albino Wistar Rat

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ABSTRACT

The effect of ethanolic root extract of *Rauwolfia vomituria* on the histology of the testes of adult male wistar rat was investigated. The experiment was carried out using thirty (30) male wistar rats weighing between 180g to 220g. The animals were divided into 5 groups with 6 rats in each group. Groups A, B, C, and D served as experimental groups while group E was the control group. Group A, B, C and D animals were given 50mg/kg,100mg/kg, 200mg/kg and 400mg/kg of the ethanolic root extract of *Rauwolfia vomitoria* respectively while group E animals was given distilled water. Administration was done orally and it lasted for twenty-one (21) days and a watchout period of seven (7) days was observed to determine if the effect of the plant extract could be reversed. Rats were sacrificed at various intervals, day 8th, 15th, 22nd and finally at day 29th . Testes from various groups were carefully harvested, fixed immediately in formal saline and sent to the laboratory for tissue processing and histopathological analysis. Blood samples were also collected and sent to the laboratory for testosterone analysis. Results obtained showed behavioural changes, body weight, histopathological effects and increased testosterone level. Behavioral changes revealed generalized body weakness, drowsiness, laboured breathing, anorexia and death. The final body weight of experimental groups increased insignificantly ($P<0.05$) with the control. Histhopathology showed distortions of spermatocytes and spermatogonia of the testes in experimental groups with respect to duration, when compared to Group E which showed normal cellular architecture of the seminiferous tubules, leydig cells, sertoli cells, sperm cells and flagella.Group A and B, showed mild to moderate distortions while group C and D showed severe distortion of cells. At the watchout stage, there was a reversal effect of the damage caused by the extract. Testosterone analysis indicated a reduction in the concentration of testosterone. It is observed that the histological effect of the ethanolic root extract of *Rauwolfia vomituria* showed various grade of degenerative changes, meanwhile prolong intake at high dosage has deleterious and adverse effect on the testes of male wistar rats.

Keywords: *Rauwolfia vomituria, Histopathology, Wistar rat and Testes*

INTRODUCTION

Rauwolfia vomitoria also called poison devil's pepper, serpent wood, serpent snake and swizzle stick is a tree that grows to a height of about 15m; it has oval or oblong shiny leaves in whorls and with straight veining and a cluster of inconspicuous white or greenish flowers producing red.

It is a medicinal plant widely distributed all over the world especially in Asia and West African countries. In Nigeria it is mostly found in the forest of southern part. In Yoruba it is called asofeyeje meaning bearing fruit for the birds, in Hausa it is called ira, akata in Beni, mnoneba and utoennyin in Efik, akawa in Ibo and penpe in Ashanti [2].

Traditionally, it is used against snakebites, fever and nervous disorders. Ghanaians and Nigerians healers used the bark in high doses as powerful emetics and purgative for management of infantile convulsion, jaundice and gastrointestinal troubles[3]. It has also be found in lowering blood pressure[9], use as antimalarial[10], analgesic[11, 16], haematinic[12], antipyretic[13]. It has also been discovered to have the following effects; anti-inflammatory, anti-diabetic, anti-oxidant[14]and anti-ovarian cancer[15].

The effect of the plant has been carried out on the kidney[1], Liver[4] and Lungs[5]. It has also been found experimentally that ethanolic root extract of Rauwolfia vomitoria causes alteration or degeneration of the histological architecture of the ovary which appears to

be exacerbated with increasing dosage [4].

Sharma [6] reported that the root of *Rauwolfiavomitoria* is a sedative, hypnotics, antianxiety agent and good for reducing blood pressure, insanity and act as a stimulant to the central nervous system. Adodo[7] analysed that *Rauwolfiavomitoria* is efficacious in treating hypertension, impotence, insomnia and nervous disorders. Lambo [8] reports that *Rauwolfiavomitoria* root has effects on the brain and will restore mental activities to normal. Odugbemi[9] reported that *Rauwolfiavomitoria* is good for treatment of hypertension, insomnia, nervous disorder, jaundice, fever, diarrhoea and malaria.

The aim of this study is to determine the effect of ethanolic root extract of *Rauwolfia vomituria* on the histology of the testes of adult male albino wistar rat.

MATERIALS AND METHODS

Experimental Design

Collection of Plant Material: The roots of *Rauwolfia vomituria* were collected from Gokana in Ogoni Local Government Area of Rivers State and were authenticated by the Herbarium of the Department of Plant Science Biology, University of Port Harcourt, Choba Nigeria.

Extraction and Phytochemical Screening of *Rauwolfia vomituria root*:

(a) Preparation of extract: The preparation of the extract was done using rotary evaporator method. The root bark of *Rauwolfia vomitoria* was cut into pieces, oven dried at 40 ºC to a constant weight. The driedpieces was then pulverized using an electric blender and the powder obtained was then stocked in a plastic container from which varying amounts was taken and dissolved in ethanol for 72 hours (3 days) at room temperature (26 ºC–28 ºC). This was then filtered using whatman filter paper and the filtrate was evaporated to dryness using a rotary evaporator at 45 ºC.

Determination of Lethal Dose 50 (LD50)

According to Amole et al. [2] a preliminary LD50 test of *Rauwolfia vomituria* was confirmed to be 17.5g/kg.

Extract Administration: Thirty (30) male wistar rats weighing about 200g was used for this study. Rats were acclimatized for thirty (30) days, maintained on animal feed and tap water. Animals were randomly divided into five groups with six rats each in a group. Group A, B, C and D served as experimental groups while group E served as control. Animals were sacrificed at intervals of seven (7) days; Day eight (8), Day fifteen (15), Day twenty-two (22), and Day twenty-nine (29). Administration of the Ethanolic root extract of *Rauwolfia vomitoria* on the animals was done from Day one (1) to Day twenty-one (21) and from Day twenty-two (22) to Day twenty-nine (29) administration was stopped this was to check if any change caused by the Ethanolic root extract of *Rauwolfia vomitoria* could be reversed (watch out stage).

According to Aquisia et al. [4] experimental doses ranging from 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg were administered.

1. Group A was given an experimental dose of 50mg/kg for Twenty-one (21) days duration.
2. Group B was given an experimental dose of 100mg/kg for Twenty-one (21) days duration.
3. Group C was given an experimental dose of 200mg/kg for Twenty-one (21) days duration.
4. Group D was given an experimental dose of 400mg/kg for Twenty-one (21) days duration.
5. Group E which is the control group was given water throughout the period.

Tissue preparation and histological staining

Twenty four hours after the last administration, the animals were weighed and recorded. They were anaesthetized under the influence of chloroform vapour and dissected. After dissection, testis tissues were removed and immediately fixed. The tissues were trimmed down to a size of 3mm x 3mm thick. For easy study of sections under microscope, the tissues were passed through several standard laboratory processes which include; fixation, dehydration, clearing, impregnation, embedding, sectioning and staining. Blood samples were also collected and kept in lithium heparin bottles and also immediately taken to the laboratory for testosterone assay analysis.

RESULTS

Behavioural patterns of the animals by visual observation.

The most significant behavioural changes on administration of the ethanolic root extract of *Rauwolfia vomituria* on the Testes of adult male wistar rats are listed as follow;

- Anorexia (loss of appetite)
- Laboured breathing
- Weakness and drowsiness
- Death

ANOREXIA: Anorexia was seen in all experimental groups but well obvious on groups that received higher doses; 200mg/kg and 400mg/kg.

LABOURED BREATHING: Laboured breathing was observed in all experimental groups.

WEAKNESS AND DROWNSINESS: Animals treated with *Rauwolfia vomituria* were observed to be weak and drowsy.

DEATH: In the course of this study, one (1) animal died in one of the experimental group.

Body Weight

Table 1: Morphometric analysis of mean body weight in all groups

	GROUP A	GROUP B	GROUP C	GROUP D	Group E
Initial body weight before administration	203g	230g	206g	230g	245g
Final body weight after admini stration for week 1	214g	238g	228g	234g	240kg
Final body weight after administration for week 2	227g	243g	259g	239g	259kg
Final body weight after administration for week 3	221g	240g	255g	230g	268g
Final body weight after ad ministration for week 4	229g	249g	260g	239g	275kg
Weight change	26g	19g	54g	9kg	30kg

Table2: The result of the hormonal assay of testosterone

DAY 7

GROUPS	ABSORBANCES	CONCENTRATION(NG/ML)
Group A	0.360	5.4
Group B	0.551	4.0
Group C	0.629	3.5
Group D	0.694	3.0

DAY 14

GROUPS	ABSORBANCES	CONCENTRATION(NG/ML)
Group A	0.466	4.6
Group B	0.799	2.2
Group C	0.876	1.7
Group D	1.009	0.5

DAY 21

GROUPS	ABSORBANCES	CONCENTRATION(NG/ML)
Group A	0.551	4.0
Group B	0.812	2.0
Group C	0.823	1.5
Group D	1.482	0.3

DAY 28

GROUPS	ABSORBANCES	CONCENTRATION(NG/ML)
Group A	0.390	5.2
Group B	0.506	4.3
Group C	0.765	2.5
Group D	1.258	0.7
Group E	0.227	5.9

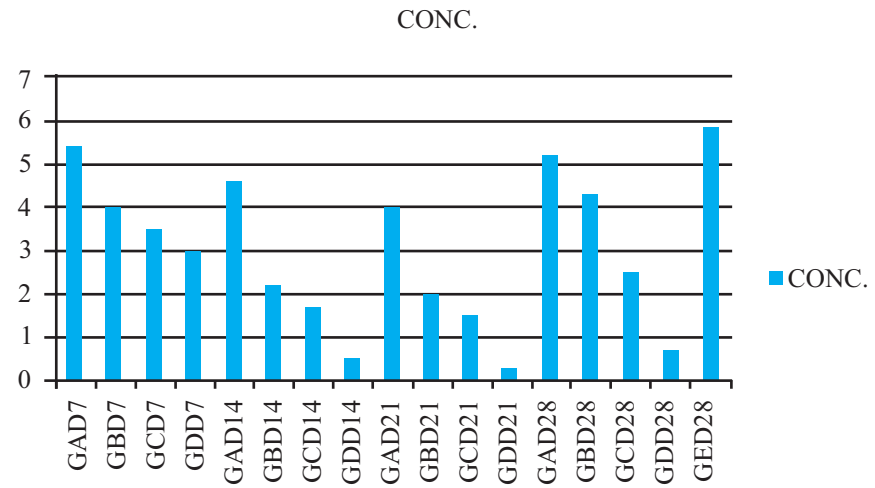


Figure 1: Bar chart showing testosterone concentration

Alphabet A, B, C, D and E represent the various groups
Numbers 7, 14, 21 and 28 represent the various weeks
G – Group
D – Day

Histopathological Effects

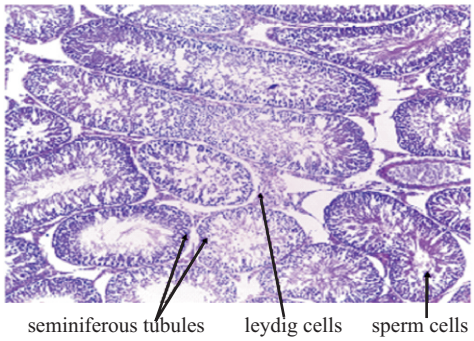
Group A: Testes treated 50mg/kg of *Rauwolfia vomituria* reveal cellular patterns within normal limit as compared to control group for the day 7. On day 14 slight areas of distorted leydig cells was seen and mild excavation of the walls of the seminiferous on day 21, while on day 28 normal cytoarchitecture of the seminiferous tubules and leydig cells was seen.

Group B: Testes treated with 100mg/kg of *Rauwolfia vomituria* reveal slight destruction of the walls of seminiferous tubules on day 7. On day 14 and day 21 distorted leydig cells and partial loss of flagella was observed while on day 28 normal cytoarchitecture of the seminiferous tubules was also seen.

Group C: Testes treated with 200mg/kg of *Rauwolfia vomituria* reveal cellular abnormalities; there was depletion of the spermatid layer on day 7 and day 14 and day 21 shows loss of flagella and early spermatid layer. On day 28 restorations of the layers of the seminiferous tubules was seen.

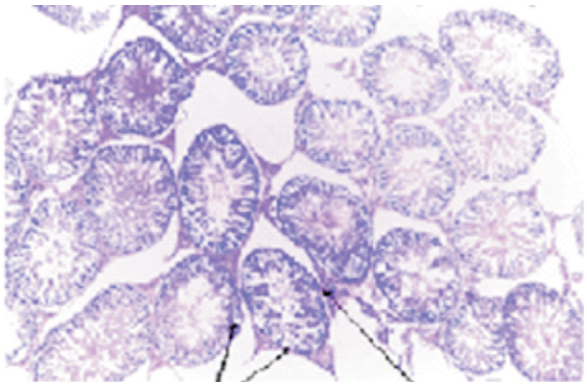
Group D: Testes treated with 400mg/kg of *Rauwolfia vomituria* shows severe cellular abnormalities, inconsistent leydig cells, destruction of seminiferous tubules, vacuolations within the spermatid layer and absent of flagella in the lumen in all stages (day 7, day 14 and day 21) but on day 28 there was a gradual regeneration of the lost layers with the leydig cells well seen.

Group E: Control testes shows normal cellular architecture of seminiferous tubules, leydig cells, sertoli cells, sperm cells, spermatid layers and flagella in the lumen in all stages (day 7, 14, 21 and 28).



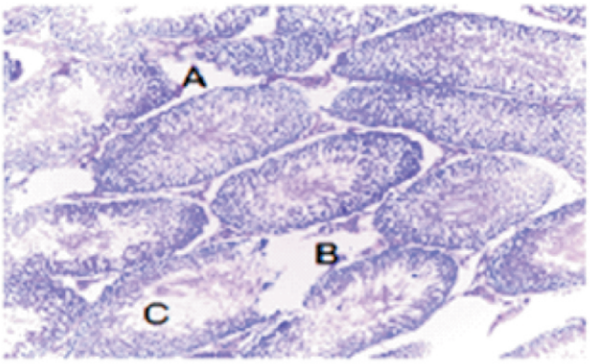
TESTIS H&E X200
GROUP E: PLATE 1 CONTROL SLIDE SHOWS NORMAL HISTOLOGY OF THE TESTES

Figure 2: Micrograph showing control slide with normal histology of the testes

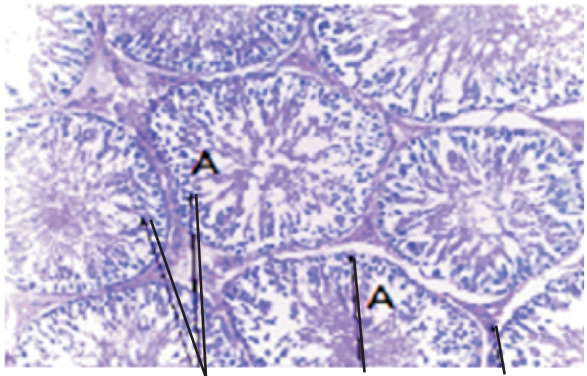


Seminiferous tubules **leydig cells**

TESTIS H&E X200
Group A Day 7
Microscopic side shows no histologic change

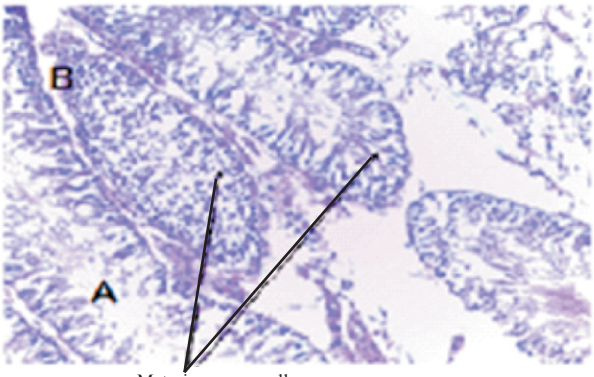


TESTIS H&E X200
Group D Day 7
A - Distorted leydig cells
B - Walls of seminiferous tubules partially destroyed
C - Partial loss of flagella



sperm cells sertoli cells leydig cells

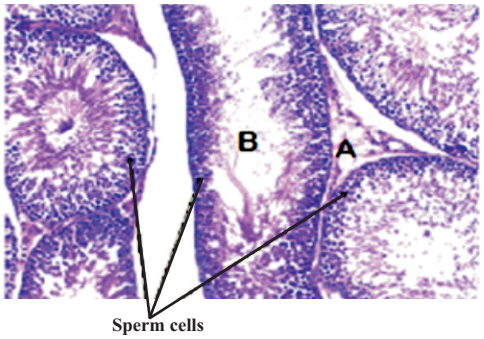
TESTIS H&E X200
Group C Day 7
A - Partial vaculation of the spermatid layer



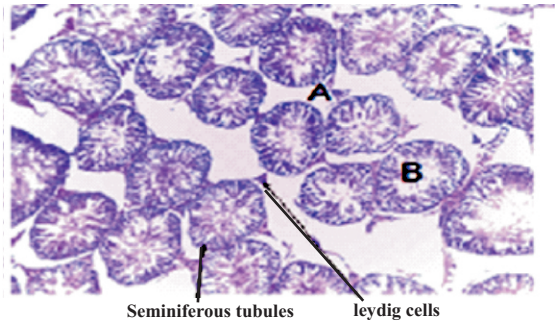
Maturing sperm cells

TESTIS H&E X200
Group B Day 7 cells shows mild distortion
A - Absent of flagella in the lumen
B - Distortion of the walls of seminiferous tubules

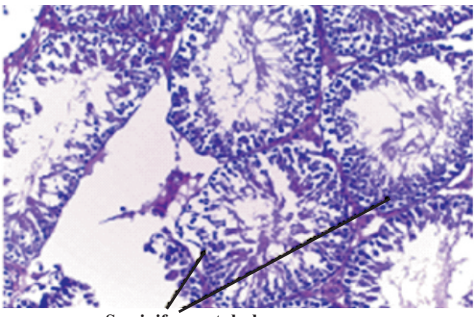
Figure 3: Micrograph showing histological features at the end of Day 7 administration of extract.



TESTIS H&E X200
Group A Day 14
A - Diatorted leydig cells
D - Partial loss flagella

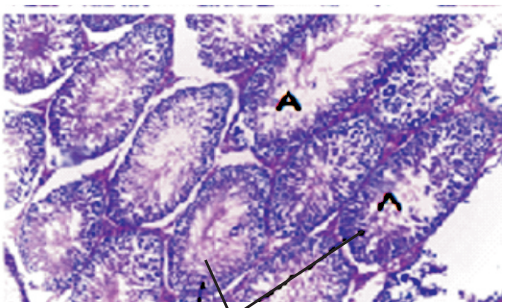


TESTIS H&E X100
Group B Day 14
A - Diatorted leydig cells
B - Incomplete loss flagella

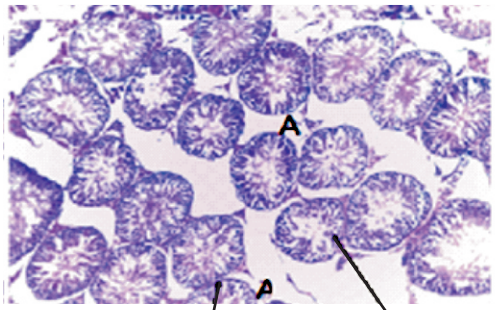


TESTIS H&E X200
Group C Day 14
- Loss of flagella and early spermatid layer

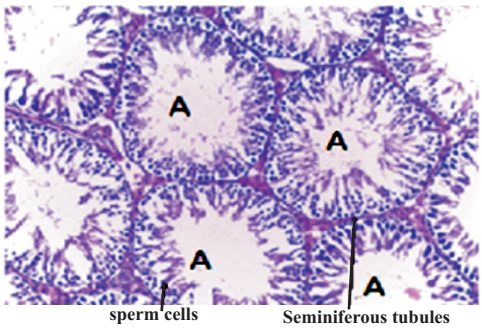
Figure 4: Micrograph showing histological features at the end of Day 14 administration of extract.



TESTIS H&E X200
Group A Day 21
A - Palehes diatorted sperm cells

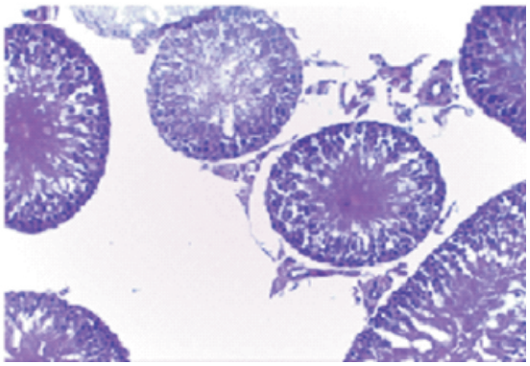


TESTIS H&E X200
Group B Day 21
A - Loss of leydig cells

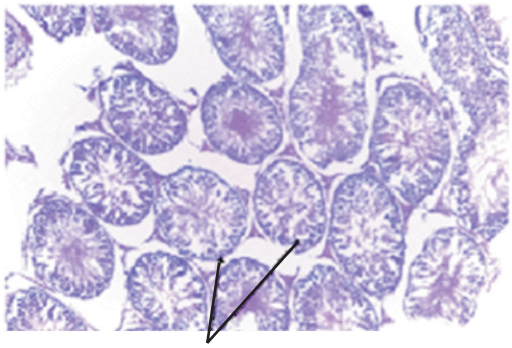


TESTIS H&E X200
Group C Day 21
A - Loss of flagella

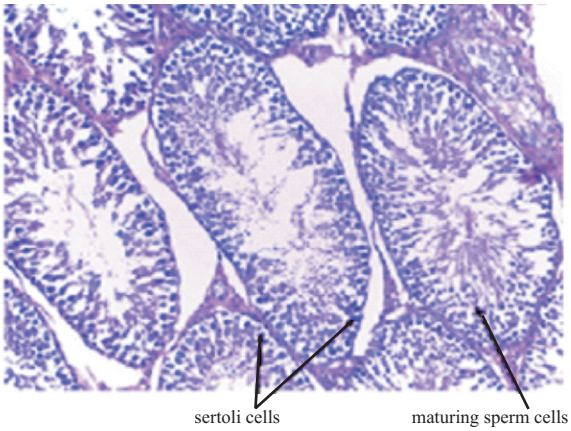
Figure 5: Micrograph showing histological features at the end of Day 21 administration of extract.



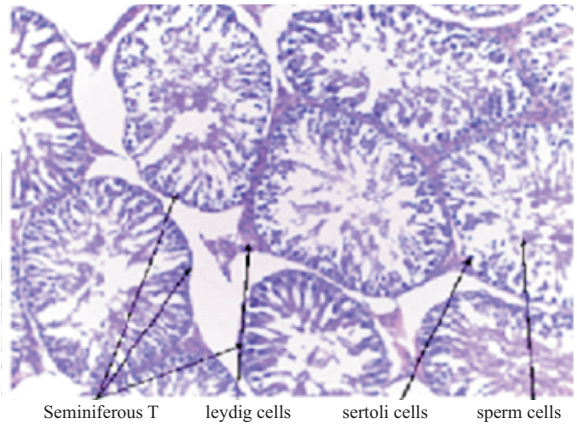
TESTIS H&E X200
Group A Day 28
Restoration of the normal cytoarchitecture of the seminiferous tubule



TESTIS H&E X400
Group B Day 28
More appearances of leydig cells



TESTIS H&E X400
Group C Day 28 slide shows regeneration of flagella



TESTIS H&E X400
Group D Day 28 slide shows cells gradually returning from distorted stats

Figure 6: Micrograph showing histological features restore during the reversal period (on day 28).

DISCUSSION

An extensive literature search did not reveal any study on the effect of ethanolic root extract of *Rauwolfia vomituria* on the testes and this study appears to be a pioneering effort with regard to the effect of the herb on the testes. The various behavioural patterns and signs observed in the experimental rats were good indicators of the activeness of the plant extract (*Rauwolfia vomituria*).

Animals treated with *Rauwolfia vomituria* were observed to be weak and drowsy. The drowsiness could be attributed to the sedative and hyponotic effect of *Rauwolfia vomituria*[6] .

Laboured breathing observed in the groups treated with the plant extract was attributed to respiratory insufficiency proposed by Luiz and Joe [7]. Respiratory passage of animals treated with *Rauwolfia vomituria* was found to have enlarged air spaces distal to the bronchioles with destruction of the interalveolar walls.

Loss of appetite is due to the presence of various alkaloids in the root. *Rauwolfia vomituria* contain reserpine as its major alkaloid and reserpine impairs mucosal quality by increasing cholinergic activity in gastric tissue which reduces nutrients absorption and loss of appetite[8]. Death could not have resulted from the effect of the plant extract on the animal rather as a result of mishandling of the animal. Body weight data among the groups with different dosage of the plant extract was analysed by one way analysis of variance (ANOVA). Data were presented as mean +- SEM (standard error of mean), P value less than 0.05 ($P<0.05$) were considered statically significant. Results showed that body weights were seen to be insignificant. Some of the herbal remedies have been linked with haematological toxic effect, neurotoxic, nephrotoxic, carcinogenic effect and allergic reaction thus the belief that anything natural is safe is incorrect. However, the result obtained from this study has shown that the ethanolic root extract of *Rauwolfia vomituria* has detrimental effect on testosterone concentration especially with respect to experimental groups that

receives higher doses in a longer duration.

From Day 1 - Day 21 where the plant extract was administered, there was a general increased in the rate of absorbance leading to a corresponding decreased in the testosterone concentration while from Day 22 - Day 29 which is the watch out stage where administration of the plant extract was stopped, the reversed was the seen as absorbance rate decreases with respect to increase in concentration of testosterone.

The result of Histological study reveals that consumption of ethanolic root extract of *Rauwolfia vomituria* at different doses and duration is toxic on the testes of adult male albino wistar rats. Animals in group A – D which received experimental dose of the extract of *Rauwolfia vomituria* 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg showed cellular abnormality in the testes. The seminiferous tubules, leydig cells, sertoli cells and sperm cells were all affected at different stages (day 7, 21 and 21). Normal cellular architecture was also observed in animals in group E which were treated with distilled water (control group). From past researches carried out, animals treated with the higher doses (200mg/kg and above) of the ethanolic root extract of *Rauwolfia vomituria* were found to have some distortions in there tissue cytoarchitecture since the root of the plant have been found to be the most toxic part of the plant. Animals in group A and B which were treated with 50mg/kg and 100mg/kg respectively slight deviation from the normal cellular architecture of the seen and this changes were observed from testes treated in a longer duration. Animals in group C and D which were treated with 200mg/kg and 400mg/kg respectively shows severe cellular abnormalities of testes in all stages (day 7, 14 and 21). The watchout stage which was a period of seven (7) days shows complete restoration of testes cytoarchitecture in group A and B which receive a lesser dose while in group C and D where higher doses were administered there was a gradual restoration of the cytoarchitecture of the testes.

The abnormalities in the testes include reduction of leydig cells, eruption of the walls of the seminiferous tubules, loss of sertoli cells and depletion in the spermatozoa, spermatid and flagella layer. The reduction of leydig cells shows a corresponding decrease in the amount of testosterone produce since leydig cells are responsible for the about 95% of testosterone produced in the body. The primary function of testosterone produced in the Leydig cells is to stimulate spermatogenesis (sperm production) and support the development of immature spermatozoa (sperm). Loss of leydig cells will also results to low regulation of central nervous system functions which influence libido and sexual behaviour, reduced stimulation of the metabolism, particularly those functions involved in protein synthesis and muscle growth, inability to maintain glands and organs in male reproductive system, underdevelopment and inability to maintain male secondary sexual characteristics.

Widened interstitial spaces result in the leydig cells clustering together and also creating a wide distance to the seminiferous tubules thus allowing just small amount of androgen and testosterone into the seminiferous tubules. Partial loss of sertoli cells results in non-regulation of spermatogenesis and spermiogenesis, thus resulting in abnormal production of spermatozoa. Depleted flagellum results in failure of the sperm cells to move freely and fast. Depleted spermatogonia could also results in infertility because depleted spermatogonia will results in the production of fewer spermatozoa in a condition known as low sperm count. These above mentioned effects are regarded as being toxic because it leads to infertility. Infertility could be as a result of decreased motility of spermatozoa, or failure of the leydig cells to produce the required amount of the testosterone in order to initiate spermatogenesis or sertoli cells not being able to regulate spermatogenesis and spermiogenesis, thus resulting in abnormal production of spermatozoa.

CONCLUSION

A detailed investigation of the effect of the ethanolic root extract of *Rauwolfia vomituria* on the testes of adult male albino wistar rat revealed the relative toxicity potential of the plant extract.

It has been observed that administration of *Rauwolfia vomituria* causes alteration or degeneration of the histological architecture of the testes which appears to be exacerbated with increasing dosage and duration. There was also a recorded effect on the level of the testosterone concentration which could be harmful to male at a given dosage and duration thereby leading to infertility. It is important to note that there is a reversal effect of the ethanolic root extract of *Rauwolfia vomituria* on the testes of adult male albino wistar rat.

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