THE EFFECT OF ORAL ADMINISRATION OF *Pausinystalia yohimbe* ON THE HAEMATOLOGICAL PARAMETERS AND HISTOLOGY OF THE HYPOTHALAMUS OF ADULT MALE WISTAR RATS.

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SEPTEMBER, 2007.

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BY

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DEPARTMENT OF HUMAN ANATOMY
FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA.

SEPTEMBER,2007.

DECLARATION

I, MUSA, SUNDAY ABRAHAM, declare that work in the thesis entitled **"The Effect of Oral Administration of *Pausinystalia yohimbe* on the Haematological Parameters and Histology of the Hypothalamus of Adult Male Wistar Rats**" has been performed by me in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria, under the supervision of Professor J.O. Hambolu and Dr. S.S. Adebisi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

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CERTIFICATION

This thesis entitled **"The Effect of oral Administration of *Pausinyatalia yohimbe* on the Haematological Parameters and Histology of the Hypothalamus of Adult Male Wistar Rats"** by ***Musa, Sunday Abraham*** meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literacy presentation.

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DEDICATION

This work is dedicated to the glory of the LORD GOD ALMIGHTY, Who is my creator, helper, provider, sustainer, encourager and my saviour. Who is my hope and my very great reward.

And to my

Late Grandmother, **Prophetess Veronica Aina Owoniyi**. It was you whom the LORD used to bring me up when I was abandoned and rejected, at the tender age of six month old. Rest in Perfect Peace in the bosom of the LORD.

And to

My Mother Mrs. D.G. Akinwande

For Leading Her Children into intellectual Pursuit.

And to

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For your Love and Encouragement.

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ABSTRACT

The bark of the West African *Pausinystalia yohimbe* tree is a traditional aphrodisiac and the source of yohimbine, a prescription drug for impotence. It appears to be modestly effective, but it also presents numerous safety risks. The possible side effect on the haematological parameters and histology of the hypothalamus has not been considered. This study investigated experimentally using adult male Wistar rats, the changes in haematological parameters and the histology of the hypothalamus due to oral administration of the crude extract *Pausinystalia yohimbe* bark.

Thirty six (36) adult male Wistar rats of average weight of 200g, used for this experiment. The animals were grouped into four (4) groups of nine (9) animals per group. With group I serving as the control and administered distilled water. While group II, III and IV administered 30mg/kg, 50mg/kg and 100mg/kg body weight per day of the crude extract of *Pausinystalia yohimbe* bark orally respectively, for three (3) weeks.

Blood samples were collected from all the animals for the haematological studies and the brain tissues were also collected and fixed in Bouin's fluid and processed for histological studies using the method of Glee's. The photomicrographs of the tissues were taken using an Optical Eye Piece connected to a computer system. The results showed that the *Pausinystalia yohimbe* bark extract produced significant increased in the erythrocytes count values, total protein and clothing time decrease in the leukocyte count, monocyte and eosinophils differential counts. The extract also has excitatory effect on the nuclei of the hypothalamus, indicating cellular hyperplasia.

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CHAPTER ONE

1.0 INTRODUCTION

Presently, there is a growing interest in the use of natural products. Various products of plant origin are being marketed in Nigeria. They range from herbal toothpastes to various drug supplements. Some of these herbal preparations may have some inherent properties, which have contributed to their persistent use over the years, underscoring the need for validation of some or most of them. It is said that only about 2% of all the plants on earth have been subjected to pharmacological investigation. The rationale for the utilization of medicinal plants has rested largely on long term clinical experience with little or no scientific data on their efficacy and safety (Zhu *et al.,* 2002). With the upsurge in the use of herbal medicines, a thorough scientific investigation of these plants is imperative, based on the need to validate their folkloric usage (Sofowora, 1989).

There is paucity of information in the scientific literature on the toxicity profile of *Pausinystalia yohimbe*. But since sub-acute or sub-chronic toxicity data will be required to predict the safety or otherwise of long-term low dose exposure to medicinal products (McNamara, 1976), it is necessary to provide this information in order to bridge the gap in knowledge about the effect of *Pausinystalia yohimbe* on the haematological parameters and the histology of the hypothalamus. Such data may play a critical role in the continued ethano-medicinal applications of the plant and assist future development of ethno-medicine from it.

In a typical traditional African setting, satisfactory sexual performance is most often equated to masculine prowess. A man who fails to have a satisfactory sexual performance is said to have lost his manhood. Again, the issues of sex and sexual relationship is not greatly discussed in the traditional African setting, with the result that when men are plagued with erectile dysfunction, they tend to recede to the privacy of their bedrooms, ruminating over their seeming loss of manhood and willing to clench to any form of affordable and available remedy that they come across in order to restore their lost manhood. And due to the high cost of modern drugs which are used to improve or enhances sexual performance or correct erectile dysfunction, such men go for the easily affordable local herbs available in the homes of traditional medicine practitioners or readily available herbal products being sold cheaply at local markets, motor parks or in luxurious buses. Such local herbs that are purchased from these mentioned places may present with unknown side effects and numerous complications. An assessment of the therapeutic or toxic effects becomes necessary because these medicinal plants are locally available and relatively cheap to purchase by most people. When these herbal remedies have been tested and proven to be non-toxic, safe and inexpensive and culturally acceptable, then their usage can be encourage.

Many herbal combinations have been used to enhance sexual performance or to treat erectile dysfunctions. Better MAN ™ is a clinically tested Chinese's herbal supplement that improves sexual performance and prostate health. Others includes *Ginkgo biloba*, Cateaba (*Erythroxylum catuaba*), Marapuama (*Ptychopetalum olacoides),* Pygeum (*Pygeum africana*), Yohimbe (*Pausinystalia yohimbe)* (Sohn *et al.,* 1991).

.

1.2 *Pausinystalia yohimbe*

Pausinystalia yohimbe is also known as *Corynanthe yohimbe*. Another common name is Yocon.While in Hausa it is called Dankamaru.

1.2.1 Botanical information

It is an evergreen tree which grows to a height of 30m with a spread of 8m. The stem is erect and branching, the leaves are oval, acuminate and about 10cm long. The seeds are small winged silvers, almost paper thin. *Pausinystalia yohimbe* is a native of the rainforest of Nigeria, Cameroon and the Congo. It prefers rich soils in a protected part sun to shady position, and is drought and frost tender. The propagation of *Pausinystalia yohimbe* is by seed or cutting. Seeds are sown in a free draining seed mix or sphagnum moss and will need temperatures above 25^0C to germinate quickly. Seeds have a very short viability, which declines rapidly in dry and warm conditions. (Shaman Australis Botanicals, 1998)

1.2.2 Traditional Uses

Yohimbe is the only natural medicinal aphrodisiac. Its use as an aphrodisiac in its native area has been well documented and its unique effects were soon valued in many parts of the world, especially in Europe. In modern times yohimbe products have found a wide market, ranging from medically treated impotency and self administered sexual enhancement to “smart products” like “cloud 9” ™ and other herbal ecstasy formulations.

1.2.3. Pharmacology

There are several alkaloids in *Pausinystalia yohimbe,* but it is the yohimbine which is an indole alkaloid somewhat similar and related to ibogaine and mitragyine and is present in the bark of this species to between 2 and 15%.

Yohimbine is found in nature from the bark of the *Pausinystalia yohimbe* tree and is used for treatment of impotence. Yohimbine is the active constituent and acts by blocking alpha-2 adenoreceptors centrally. As a result, there is an increase in release of norepinephrine and increases in cholinergic activity. Yohimbine also inhibits monoamine oxidase that is responsible for the breakdown of norepirephrine and serotonin without increasing testosterone level (Wepser, *et al*., 1999). Yohimbe has been used for centuries in African folk medicine to treat leprosy, coughs, treat fevers, as a local anesthetic, sexual aphrodisiac, etc. African tribal men would often chew on the bark prior to going to battle to increase strength. However, its most popular use has been as an aphrodisiac and to improve erections. Yohimbine also increases blood levels of the neurotransmitter norepirnephrine. Norepinephrine stimulates the brain's sex center in the hypothalamus and is considered one of the body's natural prosexual chemicals.

1.3 STATEMENT OF THE PROBLEM

Although crude yohimbe bark preparations are available commercially, there are no commercial sources of yohimbe bark that are available to physicians or in health food stores which actually state the level of yohimbe per dosage. Without knowing the content of yohimbe, it is virtually impossible to prescribe an effective and consistent dosage. Because of the yohimbine content of yohimbe bark, the American Food and Drug Administration (FDA) classifies yohimbe as an unsafe herb. But in many developing countries, especially Nigeria, yohimbe is still being taken indiscriminately.

1.4 AIMS AND OBJECTIVES

The aims and objectives of this study are to experimentally investigate:

1. The possible changes in hematological indices as a result of oral consumption of *Pausinystalia yohimbe* in adult male Wistar rats.
2. The possible histological changes in the hypothalamus of adult male Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

Herbal drugs offer an alternative to the synthetic compounds and have been considered to be non toxic or less toxic and this has given impetus to screen herbal drugs for their ethnomedical use or ability. Ayurveda, the Indian system of medicine, dating back 5000 years, has been an integral part of Indian culture and material medicine. Ayurveda extensively uses plant-derived compound formulations for the treatment of various ailments after a careful study into the type of the disease (Sivarajan and Balachandra, 1996).

Administration of ethanolic extract of root of Vernonia amygdalina offered significant protection against pentyle-netetrazole (PT2) induced lethality. The extract also has a significant analysis activity against acetic acid induced abdominal constriction in a dose dependent fasting compared to control. It exhibited an antipyretic activity which was significant compared to control but less effective than acetyl salicylic acid. These observations suggested that the root extract possesses a central nervous system depressant action (Jude, *et al,* 2004).

Bobboi et al (2004) have reported that the chronic administration of root of Xylopia quintalsi to groups of rats orally at 5, 10, and 20 mg/kg body weight for four weeks showed a steady increase weight in the rat body weight throughout the study period. And that the level of serum protein, urea, aspartate amino transferase (AST) and the weight of the liver and kidney were not significantly affected by administration of the extract. While the level of creatinine, alanine amino transferase (ALT), Na^+, K^+ and Cl^-

were significantly raised. The increases are however not dose dependent. It has also been reported that the root aqueous extract of *xylopia quintasii* inhibit intestinal glucose absorption (Bobboi, *et al*., 2003).

Many plant species have been reported to display hypoglycemic effects, but few of them have been investigated through controlled clinical trials. The world Health Organization (WHO) has recommended further investigation in this area (WHO 1980).

The effect of the herbal product kava (kava kava 'Awa, Yoqona, Piper methysticum) on human P_{450} isoforme has been reported. Kava applied to both the plant (piper methysticum foster fx) and the mildly psychoactive beverage from which it is prepared. Kava is cultivated and used traditionally thought the south pacific for its relaxing effects and has been described as a "social lubricant" that facilitates the smooth running of daily life (Lebot *et al.,* 1997) Kava extracts and resins have been used in European phytomedicine as sedatives, tranquilizer, and muscle relaxants, and used for the treatment of menopausal symptoms, urinary tract and bladder disorders (Blumenthal et al. 2000; Lindenberg and Pitule-Schodel, 1990; Wernecke, 1991).

Zou., *et al*., 2004, reported that kava was found to be cytotoxic to human hepatocytes. Kava was also reported to be a potential inhibitor of drug metabolizing enzymes because of five of kava test compound exhibited potent inhibition of P_{450} enzymes and because kava extracts have been associated recently with fatal hepatotoxicity, (Strahl *et al.,* 1998; Escher et al. 2001; Kraft *et al*., 2001; Russmann et al 2001a; Russmann *et al*., 2001 b), possibly through the formation of reactive metabolites (Russmann *et al*., 2001 b).

Agunu, *et al.*, 2003 has reported that the stem-bark extract of *Staganotaenia araliacea* in rats causes relative toxicity in the intestine, liver and kidney. Dose of 1.0glkg and higher induced necrosis,vascular congestion and degenerative changes in a dose dependent manner.They also reported that death resulted from acute hepatotoxicity and necrotic lesions, widespread necrosis and desquamation of the epithelial cells of the intestine, pulmonary oedema and glomerulonephritis of the kidney (Agunu, *et al.*, 2003). In another study, *Staganotaenia araliacea*, was reported to be used as analgesic and anti-malaria agent and for the cure of gonorrhea, bilharzias, sore throat, lungs and liver disease in cattle and for rheumathism (Hepwood, 1971).

The rationale for the utilization of medicinal plants has rested largely on long-term clinical experience with little or no scientific data on their efficacy and safety (Zhu *et al*., 2002). In an effort to validate the scientific basis for the medicinal uses of *Berlina grandifora* (BG), Enwerem *et al.*, (2001a) observed a remarkable antihelminthic activity of the plant in rats infested with a worm, *Nippostrongylus brasilinesis*. Further evidence for the antihelminthic activity of BG was obtained by the same group of workers when one of its active constituents – betulinic acid produced remarkable activity against caenorhabditis elegans (Enwerem *et al.,* 2001 b).

Aniagu, *et al.,* (2004) has reported the toxicity profile of the aqueous metabolic extract of *Berlina granifora* (BG) stem bark studied in rats. In this work they reported that the relative weights of the heart, liver, kidney, and lungs of treated rats were unaffected but there were significant changes in the relative weights of the spleen and testes. The packed cell volume and hemoglobin concentration were slightly reduced whereas total leucocyte counts were increased remarkably. Alkaline phosphatase and

creatine kinase levels were reduced in all the groups but glutamate oxaloacetate was significantly elevated (Aniagu, *et al.*, 2004).

Anthocleista djalonensis is a plant of West African origin used in traditional medicine for the treatment of various diseases (Dalziel, 1987, Burkill, 2000). The aqueous extract of *A. djalonensis* was found to produce a rise in the blood pressure in cats and an increase in tone and amplitude of movement of rabbit duodenum preparations. The root decoction has been used in the treatment of diabetes mellitus (Amofo, 1977).

Traditionally the leaves are reputed to be used for treatment of malaria and jaundice (Iwu, 2000). The plant is known for its antipyretic, analgesic, and purgative actions. The bark of the plant is used as purgative, in small doses, as large doses are considered toxic. According to the Medico ethno medicine, when *A. djalonensis* is used as firewood, the people sitting around the fire become sick (Dalziel, 1937 and Oliver-Bever, 1986).

In a study by Onocha, *et al.*, (2003), *Anthocleista djalonensis* was found to be cytotoxic on ST-57 brain tumour transformed fibroblast. However the extracts exhibited concentration and time dependent activities. And this is consistent with the observed toxic effects of the use in large doses of *A. djalonensis* in traditional medicine.

Calotropis procera (Asclepediacae) is a xerophytic shrub distributed throughout Asia and Africa,it is locally call Arka. It has a considerable reputation in India as potent medicine in the treatment of leucoderma, leprosy, ulcers, tumours, piles, diseases of the spleen, liver and abdomen (Kirthiakar and Basu, 1935) The plant has been investigated photochemically for cardenolides, anthrocyanins, hydrocarbon and triterpenoids (Moscolo *et al*., 1988) and has been reported to show a number of pharmacological

activities such as cardiotonic, hepatoprotective, antimicrobial and anti cancer (Kamath and Rana, 2002). The alcoholic extract of the latex was evaluated for protection against isoproterenol induced myocardial infarction in albino rats. And it was found to reduce significantly the elevated levels of marker enzymes in serum and heart homogenates in isoproterenol-induced myocardial infarction. Histopathological observation revealed marked protection by the extract in myocardial necrotic damage (Mueen -Ahmed, *et al.,* 2004).

Historically, Yohimbe bark was used in Western Africa for fevers, leprosy and coughs (Duke, 1985). It has also been used to dilate pupils, for heart disease, and as a local anesthetic. It has a more recent history as an aphrodisiac and a hallucinogen. African tribal men would often chew on the bark prior to going to battle to increase their strength; however its most popular use has been as an aphrodisiac and to improve erections. (Ernst, *et al.*, 1998). There are several alkaloids in *Pausinystalia yohimbe*, but yohimbine is its primary active constituent. Yohimbine blocks alpha-2 adrenergic receptors, part of the sympathetic nervous system and it also dilates blood vessels. (Riley, 1994). Yohimbine inhibits monoamine oxidase (MAO) and therefore, theoretically is of benefits in depressive disorder. (William, 1994a). Yohimbine is contraindicated in people with high blood pressure, heart problems, anxiety, and panic attacks, all of which can be made worse with adrenergic activity. (William, 1994 b).

Yohimbine has been shown in double blind trials to help treat men with erectile dysfunction (Erns, *et al.,* 1998, Carey, *et al.,* 1996).Negative studies have also been reported. (Kunelius, *et al.,* 1997, Mann, *et al*., 1996). Standardized yohimbe products are

available, for example Yocon. A safe daily amount of yohimbine from any product is between 10-30mg (Goldberg, 1996).

Yohimbe has been associated with some side effects. Patients with kidney disease, peptic ulcer or pregnancy as well as breast feeding women, should not use yohimbe (St. Louis, 1998). Standard amounts of yohimbe may rarely cause dizziness, nausea, insomnia, anxiety, increase blood pressure and rapid heart beat (tachycardia), as reported by (Blumenthal, *et al.,* 1990), (Goldberg, 1996b). It has also been reported that using more than 40mg of yohimbe per day can cause dangerous side effects, including loss of muscle function, chills and vertigo. Yohimbe has also been reported to worsen the condition of people with post-traumatic stress disorder and panic disorders. (Bremner, *et al.,* 1997 and Charney, *et al.,* 1987).

Excessive doses have been contraindicated in hypertension, despite claims of peripheral vasodilation.Orally or intramuscular administration of Yohimbine causes sustained increases in blood pressure (DeSmet PAG, *et al.,* 1997).

According to animal screening studies, Monoamine Oxidase Inhibitors such as phenelzine increase the toxicity of yohimbine (Quinton, 1963).

Also Bagheri *et al*(1994*).,* found that 10mg of yohimbine increased both salivation and plasma noradrenaline levels for four hours in patients treated with tricyclic antidepressants but also induced a relatively large number of side effects. However, they also showed that a lower dose of 4mg of yohimbine increased salivary secretion for three hours without any side effects in patient treated with tricyclic antidepressants. It could therefore be useful in the treatment of dry mouth due to tricyclic antidepressants (Bagheri, *et al.,* 1994).

On the effect of *Pausinystalia yohimbe* in sexual dysfunction, decreased sex drive, delayed ejaculation and difficulty in achieving orgasms, which are common side effect will taking serotonin selective reuptake inhibitors (SSRI) drugs, some clinicians and researchers have suggested that yohimbine might act as a specific antidote to reverse these side effects in a limited number of cases. (Gitlin, 1994, Schmauss, 1996).

The effect of aqueous extract of *Pausinystalia yohimbe* stem bark on the histomorphology of the testis was investigated in rats, the extract was given orally at a dose of 0.1 – 0.4g/kg once for 28 days. The light microscopic study showed that the extract produced significant increase in the size of seminiferous tubules and Leydig cells showed an abundance of branding and anastomosing tubules of granular endoplasmic reticulum, while sertoli cells exhibited very large and ovoid nuclei .Numerous mitochondrial of varying sizes and several spermatids and spermatozoa with well developed acrosomal cap were also shown (Jacks, *et al2005*.,)

2.1 ERECTILE DYSFUNCTION

Erectile dysfunction has been defined at the National Institute of Health conference, December 1992 as the inability to achieve or maintain an erection sufficient for satisfactory sexual performance. Erectile dysfunction among other factors is strongly age related. As many as 30 million men may be affected in the USA (Feldman, et al, 1994). While there is an estimated prevalence across all ages of about 10% (making erectile dysfunction common), the prevalence rises to over 50% in men between 50 and 70 years of age, though it is not an inevitable consequence of normal ageing (Feldman, et al 1994, Lie, 1987).

Lakin, 1994, defined erectile dysfunction as the inability to develop and maintain and erection for satisfactory sexual intercourse or activity (in the absence of an ejaculatory disorder such as premature ejaculation). The cause of erectile dysfunction can be primarily organic, psychogenic in nature, or a mixture of the two.

2.1.1 PHYSICAL FACTORS

The physical factors which may induce erectile dysfunction include:

1. Systemic diseases, diabetes mellitus, syphilis etc.
2. Local disorder, prostate enlargement, congenital abnormalities and inflammatory diseases of the genitalia, prostatisis, vascular disturbances such as aortic aneurysm and atherosclerosis for example Leriche's syndrome.
3. Neurogenic disorders-multiple sclerosis, spinal cord lesions, pituitary microadenoma virus, hyperprolactinemia and cardiovascular accident drugs such as hypertensive, sedatives, prescar (finasteride), tranquilizer and amphetamines
4. Surgical procedures such as sympathectomy, prostectomy and castration which produce varying effects. Impotence is usually not induced by transurethral prostectomy, whereas it almost always occurs after perinea prostectomy.

Impotence is not inevitable with ageing, even into the 70s or 80s. While the amount of the ejaculate and thus sexual tension and the need to ejaculate are decreased, the capacity for erection is often retained. Contributing causes include a lack of exercise, smoking, excess alcohol consumption, and increased total cholesterol or reduced high-density lipid levels.

2.1.2 PSYCHOLOGICAL CAUSES

These can include abnormal fear of the vaginal, sexual guilt; fear of intimacy, or depression, problem may be situational (involving place, time or a particular partner), some perceived competitive defeat, or damage to self esteem. Psychological factors are strongly implicated if the patient has situational impotence, night or morning erection or can achieve a firm erection by stimulation.

2.1.3 MECHANISM OF ERECTION

Men get erections when sexual thoughts, originating in the brain, initiate flow of nerve signal some of which are parasympathetic, down the spinal cord to the arteries and smooth muscles in the penis. The arteries that supply the penis then dilate, and the muscles control the two rods sponge-like tissue filling the core of the penis-the corpora cavernosa and the corpus spongiosum relax, allowing the increased flow of blood through the penile arteries to fill the spongy space with blood. The increasing pressure in the penis compresses the vein that drains from the penis, preventing outflow. The more blood filled the penis the larger and harder the erection will be, because as long as blood is flowing through the arteries, the out flow remains severely restricted. The penis returns to a flaccid state when the penile arteries constrict, relaxes the pressure on the veins and allowing the blood to drain out. (Ganong, 2005, Guyton *et al,* 2000, Lue, 1987).

All these activities are under the control of nitric oxide (NO) molecules. The nerve that serves the spongy tissue and the penile arteries are rich in NO, so when are become sexually aroused, the NO rich nerves quickly convert L-arginin to NO. These NO molecules diffuse to nearby arteries and smooth muscles causing them to dilate and relax. Erection is reversed by contraction of the arteries involved, and that in turn occurs with

stimulation by other nerves (sympathetic), either with ejaculation, anxiety or other causes of inhibition. Nitric oxide (NO) can be activated by a number of common substances released from the nerves. Especially important is the neurotransmitter acetylcholine (Ganong, 2005, Guyton *et al,* 2000, Goldstein, 2000, Ignarro, 1990).

2.2 ORIGIN OF NERVOUS SYSTEM

The nervous system develops from the neural plate, which is a thickened slipper shaped area of embryonic ectoderm, as a result of differentiation of the overlying ectoderm induced by the notochord and paraxial mesoderm (Moore and Persaud, 1998). The induction is due to an embryonic protein called noggin which has been identified as a neural inducing signal in amphibian and rodent embryos (Lamb *et al*., 1993). The neural plate then undergoes shaping, a process that involves apicobasal thickening, transverse narrowing and longitudinal extension (Schoenwolf, 1985 and Schoenwolf and Sheard, 1989). There is neural plates bending immediately and is characterized by localized furrowing and folding/ rotation about median and dorsolateral hinge points which results in neural fold formation. It is the tips of the neural fold which coverage towards each other in the dorsal midline and fuse to form the neural pate (Schoenwolf, 1994, and Schoenwolf and Smith, 1990).

2.2.1 DEVELOPMENT OF THE BRAIN

The brain develops from the neural tube, cranial to the fourth pair of somites. The fusion of the neural folds in the cranial region and closure of the rostal neuropore forms three primary brain vesicles namely: Prosencepthalon (forebrain), mesencephalon (midbrain) and rhombencephalon (hindrain). (Moore and Persaud, 1998). By the 5^{th} week of intrauterine life, both forebrain and hindbrain partly derives into two secondary

vesicles each. Presencephalon divides into diencephalon and telencephalon while rhombencephalon divides into myelencephalon, metancephalon and isthmus rhombecephali and the Mesencephalon continues as the midbrain (Singh, 1999a).

2.3 THE HYPOTHALAMUS

The hypothalamus is a part of the diencephalons as its name implies it lies below the thalamus. On the medial side, it forms the wall of the third ventricle below the level of the hypothalamic sulcus. Laterally, it is in contact with the internal capsule, and (in the posterior part) with the ventral thalamus (sub thalamus). Posteriorly, the hypothalamus merges with the ventral thalamus and through it with the tegmentum of the midbrain. Interiorly, it extends up to the lamina terminals, and merges with certain olfactory structures in the region of the anterior perforated substance. Inferiorly, the hypothalamus is related to the structures in the flow of the third ventricle. These are the tuber cinerium, the infundibulum, and the mamillary bodies, which are considered as part of the hypothalamus (Singh, 1999b).

2.3.1 SUBDIVISION OF THE HYPOTHALAMUS

Hypothalamus can be subdivided into numbers of regions. These are periventricular zone, intermediate zone and lateral zone. The periventricular and intermediate zones are often described collectively as the medial zone. The column of the fornix's lies between the medial and lateral zones (Singh, 1999c).

The hypothalamus is also subdivided anteroposterirly into four (4) regions. These are as follows:

i. The preoptic region adjoins the lamina terminalis

ii. The supraoptic (or chiasmatic) region lies above the optic chiasma

iii. The tuberal (or infundibulotuberal) regions include the infundibuilum, the tuber cinerum and the region above it.

iv. The mamillary (or posterior) region consists of the mamillary body and the region consists of the mamillary body and the region above it.

The preoptic region differs from the rest of the hypothalamus in being a derivative of the telecephalon (Singh, 1999d).

2.3.2 HYPOTHALAMIC NUCLEI

The entire hypothalamus contains scattered neurons within which some aggregations can be recognized. These aggregations termed the hypothalamic nuclei: are as follows:

i. The preoptic nucleus extends through the periventricular, intermediate, and lateral zones. As the boundaries of nuclei are ill defined, different authors have allocated nu

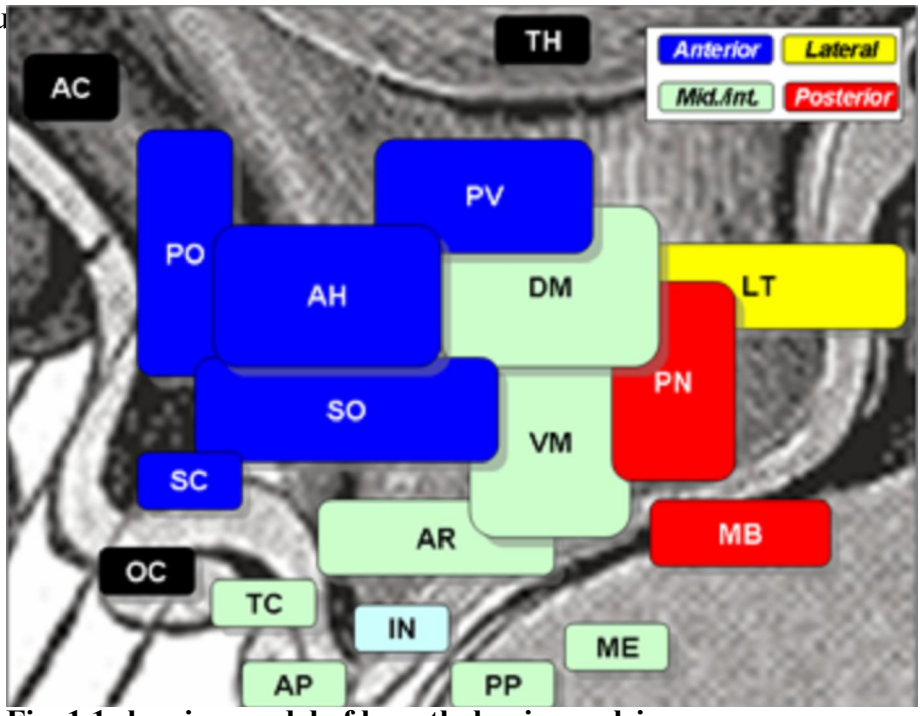


Fig. 1.1 showing model of hypothalamic nuclei

2.3.3 CONNECTIONS OF THE HYPOTHALAMUS

The hypothalamus is concerned with visceral function and is, therefore, connected to other areas having a similar function. These include the various parts of the limbic system, the reticular formation, and autonomic centres in the brainstem and spinal cord. Apart form its neural connections, the hypothalamus also acts by releasing secretions to the blood stream, and into cerebrospinal fluid (Singh, 1999f).

2.3.4 FUNCTIONS OF THE HYPOTHALAMUS

The hypothalamus plays an important role in the control of many functions that are vital for the survival of an animal. The main functions attributed to the hypothalamus are as follows:

1. Regulation of eating and drinking behavior – (Guyton and Hall, 2000a;Ganong, 2005a)
2. Influences emotional behavior – (Guyton and Hall, 2000b)
3. Regulation of sexual activities and reproduction – (Guyton and Hall, 2000c).
4. Control of autonomic activities - (Guyton and Hall, 2000d, Salah, 2000, Ganong, 2005b).
5. Control of endocrine activity .
6. Response to stress - (Guyton and Hall, 2000e).
7. Temperature regulation – (Sembuligam, *et al.,* 2001, Guyton and Hall, 2000f,)
8. Biologic clock. – (Sembuligam, 2001).

2.4 HAEMATOLOGY OF LABORATORY RATS

All types of blood cells originate from a very minor cell population, called haematopoietic stem cells (HSC) in the bone marrow. Rat leukocytes (White Blood Cell) in the peripheral blood are composed of five types of cells, similar to humans and other experimental animals (George. 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.0 COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Samples of stem bark of *Pausinystalia yohimbe* was purchased from herb sellers and verified by the Chairman of the Sokoto Traditional Medical Practitioner Association and the Traditional Medical Practitioners in the College of Health Sciences, Usman Danfodiyo University, Sokoto, Sokoto State. The plant materials were further identified and confirmed by Taxonomist in the Department of Botany, Usman Danfodiyo University, Sokoto, Sokoto State and the Taxonomist in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

3.1 PREPARATION OF STEM BARK

Samples of plant materials were kept air dried at room temperature and cut into pieces with cutlass. The pieces were grounded into powder using plant machine.

3.2 PREPARATION OF ALCOHOL EXTRACTS

Twenty (20)g of the powdered stem bark of the material was measured and extraction done with 200ml of 70% ethanol using a soxhlet extracting apparatus for about six hours. The extract obtained was further evaporated to dryness using a rotatory evaporator.

3.3 PHYTOCHEMICAL ANALYSIS

Phytochemical screening was carried out on the obtained alcohol extract of stem bark of *Pausinystalia yohimbe.* The following active constituents were confirmed present:

i. Alkaloids

ii. Tannins

iii. Flavonoids

iv. Cardiac glycosides

v. Anthraquinone

vi. Saponins

vii. Cyanogenic glycosides

viii. Anthcyanosides

ix. Reducing sugar

3.4 PREPARATION OF ANIMALS

Thirty six (36) male Wister rats bred in the Animal house of Department of Human Anatomy, Faculty of Medicine Ahmadu Bello University, Zaria, Nigeria, were randomly selected weighted and camped into four (4) different groups according to their bodies' weights.

A. Group 1: Control group: Having nine (9) male Wistar rats.

B. Group 2: Experimental group: Having nine (9) male Wistar rats.

C. Group 3: Experimental group: Having nine (9) male Wistar rats.

D. Group 4: Experimental group: Having nine (9) male Wistar rats.

The animals were kept in the Animal House of Department of Human Anatomy, Faculty of Medicine, A. B. U. Zaria, Nigeria, under standard environmental conditions 22± 1^0c, relative humidity of 60%, 12 hour-12 hours light dark cycle with light on. The animals were all fed with palletized growers feed obtained from vital feed, Jos, Plateau state, Nigeria. The nutritional composition of the feed as indicated on each 25kg bag is shown in Appendix V. Perspex cages with stainless steel, mesh floor and top to facilitate cross ventilation and measuring 45 x 28 x 12cm were used to house each group of nine (9) rats. Tap water was provided in plastic bottles with stainless steel nozzles while the feed was provided in plastic bowls.

3.5 ADMINISTRATION OF EXTRACT

The study was divided into three phases:

a. **Pre-administration phase**:

The animals were allowed to acclimatize for two weeks and were weighted.

b. **Administration phase**:

The phase lasted for three (3) weeks. And the extract was administered orally using syringe and rubber caped needles.

Group 1: Control group was given distilled water orally.

Group 2: Experimental group was given 30mg/kg/day of plant extract orally per day.

Group 3: Experimental group was given 50mg/kg/day of plant extract orally per day.

Group 4: Experimental group was given 100mg/kg/day of plant extract orally per day.

c. **Post Administration phase**:

After the oral administration of plant extract of *Pausinystalia yohimbe* for three (3) weeks to the animals (i.e. group, 2,3 and 4) and distilled water to group one. The following methods were carried out to study the effect of the plant extract.

3.6 HAEMATOLOGICAL STUDY

The tails of the animals were sterilized with methylated spirit. Blood samples were collected from the tails after it has been cleaned with methylated spirit and analyzed in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University Zaria, Nigeria. And Department of Microbiology and Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

i. Erythrocytes counts (Red Blood Cells)
ii. Leukocytes counts (White Blood Cells)
iii. Packed cells volume (Packed Cell Volume)
iv. Differential leukocytes counts
v. Total protein
vi. Clotting time

Check appendix VII and VIII for the materials used for the determination of haematological indices.

3.6.1 Collection of Blood

Haematological Parameters

Blood sample were collected from the tail into heparinised sample bottles using scissor sterilized with methylated spirit. Blood so collected was used for the various estimations.

3.6.2 Packed cell volume (Haematocrit)

The blood was transferred in to heparinised capillary tubes by capillary action, until the blood fills about ¾ of the tubes. The tubes were then sealed at the dry ends using flame, from a spirit lamp, rotating the tubes gently.

The sealed tubes were then placed into a centrifuge machine with the sealed ends facing forwards. They were then centrifuged for 5 minutes at a speed of 200 revelation per minute, using micro – heamatocrite method described by Mc Gown *et al*, 1955. After centrifugation, the capillary tubes were then placed on a microhaematocrite reader and the values were read and noted.

3.6.3 Total Protein

This was carried out using the plasma collected from the heparinised capillary tubes after centrifugation. A drop of the plasma was placed on the portion of the hand refractometer (Made by American Optical Corporations, Keene N.H. USA, Catalog 10400, Model 2476) and then held against light. And the reading was taken.

3.6.4 Haemoglobin Concentration

The hemoglobin content of the blood was determined from the hematocrite (PCV) values obtained as follows:

Hemoglobin level (g/dl) = PCV/3 (Sembulingam and Sembulingam, 2000)

3.6.5 White blood cell (Leucocytes) count.

White blood cell (leucocyte) count was determined by using the WBC pipette and Neubar Haemocytometer according to the method of Dacie and Lewis. 1991.

Using the WBC pipette blood was drawn from the cut tail and filled the 0.5 mark of the WBC pipette. The tip of the tube was cleaned and WBC diluting fluid (see appendix-) was draw till the 11mark of the pipette. The fluid-blood mixture was shaken and then transferred to the counting chamber (charging the chamber) taking care that the mixture does not overflow. The cells on the counting chamber was then set in the stage of light power microscope and X 10 objective was used to count the WBC's uniformly in the four(4) larger corner squares the cells counted were colleted using the formula below:

$$\frac{\text{Cell counted x blood dilution x chamber depth}}{\text{Area of chamber counted}}$$

$$= \text{Number of cells counted x } 20 \text{ x } \frac{\text{10 depH factor}}{4}$$

= number of cells counted x 50.

3.6.6 Red blood cell (erythrocyte) count

Blood was drawn to the O.5.Mark of the RBC pipette. And RBC diluting fluid (see appendix) was drawn to 101 Mark.

The mixture was shaken and then the counting chamber was charged with the mixture. The RBC's was counted using 40 X objectives in the 80 smallest squares.

RBC count =

$$\frac{\text{No of cells counted x dilution factor x depth factor}}{\text{Area counted}}$$

Where dilution is l in 200, depth is 1/10 mm

Area counted is $\frac{80}{400} = \frac{1}{5}$ sq mm

$$= \frac{\text{Number counted x 200 x 10}}{1/5}$$

= Number counted x 10,000.

3.6.7 Differential white blood cell count

A thin blood film was made by spreading a drop of blood evenly across a clean grease- free glass slide. Using a smooth edged spreader. The blood film was allowed to dry and then stained with leishman's stain (see appendix) after staining the slides were then observed under the light power microscope using the x 100 objective with immersion oil and the different cells were counted.

3.6.9 Clotting Time

Method: slide method

Materials: glass slide, applicator.

Slide method was used. A drop of blood was collected on a clean glass slide. And an applicator was dip into the blood every 15 seconds to check for clotting.

3.7 Preparation of Tissues of Examination

Materials:

Airtight sample bottles, normal saline, Bouin's fluid, graded concentrations of alcohol, chloroform, paraffin wax, egg albumen, microtome, glass slides, cover slips, oven, water bath, automatic tissue processor (Histokiottle Bench Model), Canada Balsam Oil, Microscope.

3.7.1 Methods of Tissue Preparation

The preparation of tissues was done using the standard techniques outlined by Gurr (1962), Culling (1953) and Bradbury (1977). The tissue preparation methods for histological analysis have been differentiated into a number of stages (five stages) which include:

(i) Fixation

(ii) Tissue processing (washing, dehydration and clearing)

(iii) Embedding

(iv) Sectioning

(v) Staining

(vi) Photomicrography

The cerebrum was carefully separated from the other brain components and fixed in 10% formal saline and Bouin's fluid respectively for all the four groups. These were then processed with an automatic tissue process (Histokinette Bench Model) available in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. Following dehydration in ascending grades of alcohol, and embedding, sections were cut 15-17 micro with the sliding microtone, stained with Haematoxylin and Eosin (H&E) and examined microscopically. The special technique used was the Glee's method (1954) for paraffin sections to demonstrate the nuclei of the hypothalamus.

3.7.2 Preparation of Ammoniacal Silver

To 20 cm^3 of 10% aqueous silver nitrates add 4 cm^3 of 10% aqueous potassium hydrochloride. Concentrated ammonia was added with repeated mixing until the formed precipitate is just dissolved. 10% aqueous silver nitrate was added slowly with frequent mixing, until only a faint opalescence remains. And it was diluted with an equal volume of distilled water.

This method was used for the demonstration of the nuclei of the brain (hypothalamic nuclei).

3.7.3 Glee's Method (Method of Marsland, Glees and Erikson, 1954)

1. Section was dewax in xylene
2. Flushed section with 100% alcohol
3. Celloidonized in 0.5% celloidin for 1 minute
4. Washed in 70% alcohol for 1 minute
5. Washed in tap water
6. Incubate section in 20% silver nitrate for 30 minutes at 37^0C
7. Wash in 10% formalin
8. Apply Ammonical silver solution for 30 second
9. Reduce in 10% formalin
10. Wash in tap water
11. Fix with 5% sodium thiosulphate for 5 minutes
12. Wash in tap water
13. Dehydrate in alcohol
14. clear in xylene
15. Mount

3.7.4 Photomicrography

Photomicrographs of tissue sections from the four groups was taken at different magnifications, using an Optical Eye Piece (Premiere, Digital Microscope Eye Piece, made in China, Item No. MA88, Resolution 640 X 480) connected to a computer system and the photomicrography equipment (Orthomat – Leitz Wetzlar Germany, 6391) was

available in the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria.

3.8 STATISTICAL ANALYSIS

Haematological indices from the four (4) groups were compared using their Means and Standard Error of Means (SEM). The student T-test was used in testing the level of significant and a P-value less than 0.05 ($P < 0.05$) was considered significant. One way Analysis of Variance (ANOVA) was used in comparing the mean values, All Pairwise Multiple Comparison Procedure (Turkey test) was used in assessing significant differences between the groups.

CHAPTER FOUR

4.0 RESULTS

4.0.1 WEIGHT AND HAEMATOLOGICAL INDICES

The results of the study of the effect of oral administration of *Pausinystalia yohimbe* on the weight and haematologicial indices of male Wistar rats are represented in Table 4.1 to 4.11 and Figure 4.1 to 4.11.

4.1 WEIGHT OF ADULT MALE WISTAR RATS

The mean weight of the adult male wistar rat was affected by the oral administration of the crude extract of the stem bark of *Pausinystalia yohimbe.* The result showed decreased in weight when the treated groups were compared with the control group. There was statistically significant difference at P<.001.

Table 4.1 Mean Weight of adult male Wistar rats following oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean wt(g) ± SEM	F-value	P- value
Group 1 (control)	Control	5	147.200 ± 9.739	25.711	<0.001
Group II	30	5	100.00 ± 0.000		
Group III	50	5	128.200 ± 1.200		
Group IV	100	5	165.800± 5.152		

4.2 ERYTHROCYTE (RED BLOOD CELL) COUNT

The mean erythrocyte (Red Blood Cell) count of the adult male Wistar Rats administered with the crude extract of *Pausinystalia yohimbe* bark are shown in Table 4.2 and Figure 4.2. The mean erythrocyte count value in the treated animals were significantly i.e. group II (30mg/kg/day) was 5.144 ± 0.223, group III (50mg/kg/day) was 5.532 ± 0.235 and group IV (100mg/kg/day) was 5.778 ± 0.215, increased at the end of the administration period as compared to the control group I which had 3.904 ± 0.553, which was administered distiled water orally. There was difference between the control and the treated groups. Using the all Pairwise Multiple Comparison Procedures (Turkey Test), there was significant difference between group IV and I and group III and I at $P<0.05$.

Table 4.2 Mean and Standard Error of Mean (SEM) of the Erythrocytes (Red Blood cell) counts of adult male Wistar rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F-value	P-value
Group 1 (control)	Control	5	3.904 ± 0.553	6.069	0.006
Group II	30	5	5.114 ± 0.223		
Group III	50	5	5.532 ± 0.235*		
Group IV	100	5	5.778 ± 0.215*		

***Mean erythrocyte count significantly higher than the control at P <0.05**

4.3 PACKED C ELL VOLUME (HEMATOCRITE)

The mean packed cell volume (PCV) values of the adult male Wistar Rats administered with the crude extract of *Pausinystalia yohimbe* are shown in Table 4.3 and Figure 4.3. The mean PCV values in the treated animals were not significantly (i.e.) group II (30mg/kg/day) was 48.600 ± 0.872 and group IV (100mg/kg/day) was 49.200 ± 1.655, increased at the end of the period of administration when compared with the mean value of the control group I, has 45.200 ± 1.855, which was administered distill water orally. There was no statistical significant difference between the control and the treated group.

Table 4.3 Mean and Standard Error of Mean (SEM) of the Packed Cell Volume (PCV) values of adult male Wistar Rats after the administration of the crude extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F- value	P-value
Group 1 (control)	Control	5	45.200 ±1.855	1.135	0.365
Group II	30	5	48.200 ± 2.059		
Group III	50	5	48.600 ± 0.872		
Group IV	100	5	49.200 ± 1.655		

4.4 HAEMOGLOBIN CONCENTRATION (HB)

The values of the mean haemoglobin concentration of the adult male Wistar Rats administered with the crude extract of *Pausinystalia yohimbe* are shown in Table 4.4 and Figure 4.4. The mean haemoglobin concentration values in the treated animals were not significantly different ($p>0.05$) from the control value, at the end of the administration period. Group II (30mg/kg/day) had 16.080 ± 0.697, Group III (50mg/kg/day) had 16.220 ± 0.301 and Group IV (100mg/kg/day) had 16.400 ± 0.556. While Group I which served as control had the mean value of 15.060 ± 0.627. This means that the administration of the crude extract of *Pausinystalia yohimbe* to adult male Wistar Rats has no effects on the haemoglobin concentration for the period of administration in these experiment.

Table 4.4 Showing Mean and Standard Error of Mean (SEM) of the Haemoglobin concentration (Hb) values of adult male Wistar Rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F- value	P- value
Group 1 (control)	Control	5	15.060 ± 0.627	1.131	0.366
Group II	30	5	16.080 ± 0.697		
Group III	50	5	16.220 ± 0.301		
Group IV	100	5	16.400 ± 0.556		

4.5 LEUCOCYTES (WHITE BLOOD CELL) COUNT

The mean leucocytes (white blood cell) count of the adults male Wistar Rats administered with the crude extract of *Pausinystalia yohimbe* bark are shown in table 4.5 and figure 4.5. The mean leucocyte counts values in the treated animals were not statistically different ($P>0.05$) from the control group ($P<0.05$). the treated animals, Group II (30mg/kg/day) had 11.700 ± 1.247, Group III (50mg/kg/day) had 9.380 ± 1.238, Group IV 100mg/kg/day had 7.240 ± 1.061, while the control group which was administered distill water had: 7.57 ± Although, Group II (30mg/kg/day) showed an increased in mean (11.700 ± 1.247) when compared with the control Group had 7.57 ± 1.229, but the increased was not statistically significant ($P<0.05$). So it can be said that, considering the period of administration of the crude extract of *Pausinystalia yohimbe* bark it has no effect on the rats.

Tale 4.5 Showing Mean and Standard Error of Mean (SEM) of the Leucocytes (white blood cell) counts values of adult male Wistar Rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F-value	P-value
Group 1 (control)	Control	5	7.57 ± 1.229	3.541	0.062
Group II	30	5	11.700 ± 1.247		
Group III	50	5	9.380 ± 1.238		
Group IV	100	5	7.240 ± 1.061		

4.6 LYMPHOCYTES

The mean lymphocytes value of the adult male Wistar rat administered with the crude extract of *Pausinystalia yohimbe* bark are shown in Table 4.6 and Figure 4.6. The mean lymphocytes values in the three groups of treated animals were significantly (P<0.00I) reduced at the end of the administration period as compared with the control group. The treated animals, Group II (30mg/kg/day) had a mean value of 54.400 ± 1.887, Group III (50m/kg/day) had, 47.000 ± 1.122, and Group IV (100mg.kg/day) had 51. 400 ± 1.030. While the control Group I had 57.800 ± 1.158.

All the treated Groups II, III and IV, demonstrated decrease in value when compared to the control group I. and this decrease was statically significant (P<0.05). This result showed that extracts produced effect in the animals.

Tale 4.6 Showing Mean and Standard Error of Mean (SEM) of the Lymphocyte values of adult male Wistar Rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F-value	P-value
Group 1 (control)	Control	5	57.800 ± 1.158	10.364	< 0.001
Group II	30	5	53.400 ± 1.887***		
Group III	50	5	47.400 ± 1.122***		
Group IV	100	5	51.400 ± 1.030***		

***** Mean values significantly lower than the control value at p>0.001**

4.7 NEUTROPHILS

The mean neutrophil values of the adult male Wistar Rats administered with the crude extracts of *Pausinystalia yohimbe* bark are shown in Table 4.7 and Figure 4.7. The mean neutrophils values in the treated animals were significantly ($P<0.001$) increased, when compared with the control group. The treated animals all showed increased in neutrophil values. Group II (30mg/kg/day) had 39.000 ± 0.632, Group III (50mg/kg/day) had 44.600 ± 1.631 and Group IV (100mg/kg/day) had 42.000 ± 1.049. While the control Group I which was administered distil water has a mean value of 32.200 ± 1.158. These were increase in all the treated groups. Thus, showing that the extract produced effect in the neutreophils of the adult male Wistar Rats.

Tale 4.7 Showing Mean and Standard Error of Mean (SEM) of the Neutrophils values of adult male Wistar Rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* back.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F-value	P-value
Group 1 (control)	Control	5	32.200 ± 1.158	20.798	< 0.001
Group II	30	5	39.000 ± 0.632***		
Group III	50	5	44.600 ± 1.631***		
Group IV	100	5	42.000 ± 1.049***		

*** Mean values were significantly higher than the control values at p< 0.001

4.8 MONOCYTES

The mean monocyte values of the adult male Wistar Rats administered with the crude extract of *Pausinystalia yohimbe* bark are shown in Table 4.8 and Figure 4.8. The mean monocyte values in all treated animals groups were significantly ($P<0.05$) reduced, when compared with the control group. The treated animals all showed decreased monocytes values. Group II (30mg/kg/day) had a mean value of 6.600 ± 0.872, Group III (50mg/kg/day) had 5.800 ± 0.0490, Group IV (100mg/kg/day) had 6.000 ± 0.8373. While the control, Group I administered distil water orally, had a mean value of 8.00 ± 0.374. Using all Pairswise Multiple Comparison Procedures (Turket Test) at $P<0.05$, there was significant difference between Group I and II, and also between group I and IV. Thus, the result showed that the extract produced effect in the animals.

Tale 4.8 Showing Mean and Standard Error of Mean (SEM) of the Monocytes values of adult male Wistar Rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F-value	P-value
Group 1 (control)	Control	5	8.800 ± 0.374	4.116	0.024
Group II	30	5	6.600 ± 0.872		
Group III	50	5	5.800 ± 0.490		
Group IV	100	5	6.000 ± 1.837		

4.9 EOSINOPHILS

The mean eosinophils values of the male Wistar rats administered with the crude extract of *Pausinystalia yohimbe* bark are shown in Table 4.9 and Figure 4.9. The mean eosinophil values in the crude extract. Group II (30mg/kg/day), had a mean value of 3.600 ± 0.245, Group III (50mg/kg/day) had 2.667 ± 0.333 and Group IV (100mg/kg/day) had 1.667 ± 0.333. While the control Group I had a mean value of 2.000 ± 0.755. Using all Pairwise Multiple Comparison Procedures (Turkey Test), there was significantly ($P<0.05$) decreased in Group IV (1.667 ± 0.333) when compared with Group II (3.600 ± 0.245). Also between group (3.600 ± 0.245) and Group I (2.000 ± 0.577) there was a significantly ($P<0.05$) increase. Thus, the result showed that the extract has effect on the monocytes values of the animals.

Tale 4.9 Showing Mean and Standard Error of Mean (SEM) of the Eosinophils values of adult male Wistar Rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F-value	P-value
Group 1 (control)	Control	5	2.000 ± 0.577	6.383	0.011
Group II	30	5	3.600 ± 0.245		
Group III	50	5	2.667 ± 0.333		
Group IV	100	5	1.667 ± 0.333		

4.10 TOTAL PROTEIN

The mean total protein values of the adult Wistar rats administered with the crude ethanolic extract of *Pausinystalia yohimbe* bark are shown in table 4.10 and figure 4.10. The mean total protein values in the treated animals were significantly ($P<0.05$) increased when compared with the control group. The treated animals in Group II (30mg/kg/day) had a mean value of 7.470 ± 0.169, Group III (50mg/kg/day) had a mean value of 7.120 ± 0.256, and Group IV (100mg/kg/day) had a mean value of 7.040 ± 0.186. While the control Group I which was only administered distils water had a mean total protein value of 6.420 ± 0.123. The difference in mean values were statistically significant at $P<0.05$. Also, using the All Pairwise Multiple Comparison Procedures (Turkey Test), there was a statistical significance difference between Group II and Group I. thus, showing that the extract produced effects in the total protein values of the animals.

Tale 4.10 Showing Mean and Standard Error of Mean (SEM) of the Total Protein values of adult male Wistar Rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F-value	P-value
Group 1 (control)	Control	5	6.420 ± 0.123	5.319	0.010
Group II	30	5	7.470 ± 0.169		
Group III	50	5	7.120 ± 0.256		
Group IV	100	5	7.040 ± 0.186		

4.11 CLOTTING TIME

The mean clotting time values of the adult male Wistar rats administered with the crude ethanolic extract of *Pausinystalia yohimbe* bark are shown in Table 4.11 and Figure 4.11. The mean clothing time in treated animals were significantly ($P<0.05$) affected when compared with the control group. The treated animals had mean values of Group II (30mg/kg/day) had 116.00 ± 19.391, Group III (50mg/kg/day) had 149.200 ± 7.755 and Group IV (100mg/kg/day) had 51.400 ± 1.030. While the control Group I which was administered distil water had a clothing time mean value of 57.800 ± 1.158. The result showed a statistical significant difference at $P<0.05$. There was an initial increase in mean value in Groups II and III, i.e. 116.000 ± 19.391 and 149.200 ± 7.755 respectively. Using the All Pairwise Multiple Comparison Procedures (Turkey Test), there was a statically significant difference between Group III and IV, Groups III and I, Groups II and IV and Group II and I at $P<0.05$.

Tale 4.11 Showing Mean and Standard Error of Mean (SEM) of the Clotting Time values of adult male Wistar Rats after oral administration of the crude ethanolic extract of *Pausinystalia yohimbe* bark.

Groups	Dose (mg/kg/day)	N	Mean ± SEM	F-value	P-value
Group 1 (control)	Control	5	57.800 ± 1.158	20.236	0.001
Group II	30	5	116.000 ± 19.391		
Group III	50	5	149.200 ± 7.755		
Group IV	100	5	51.400 ± 1.030		

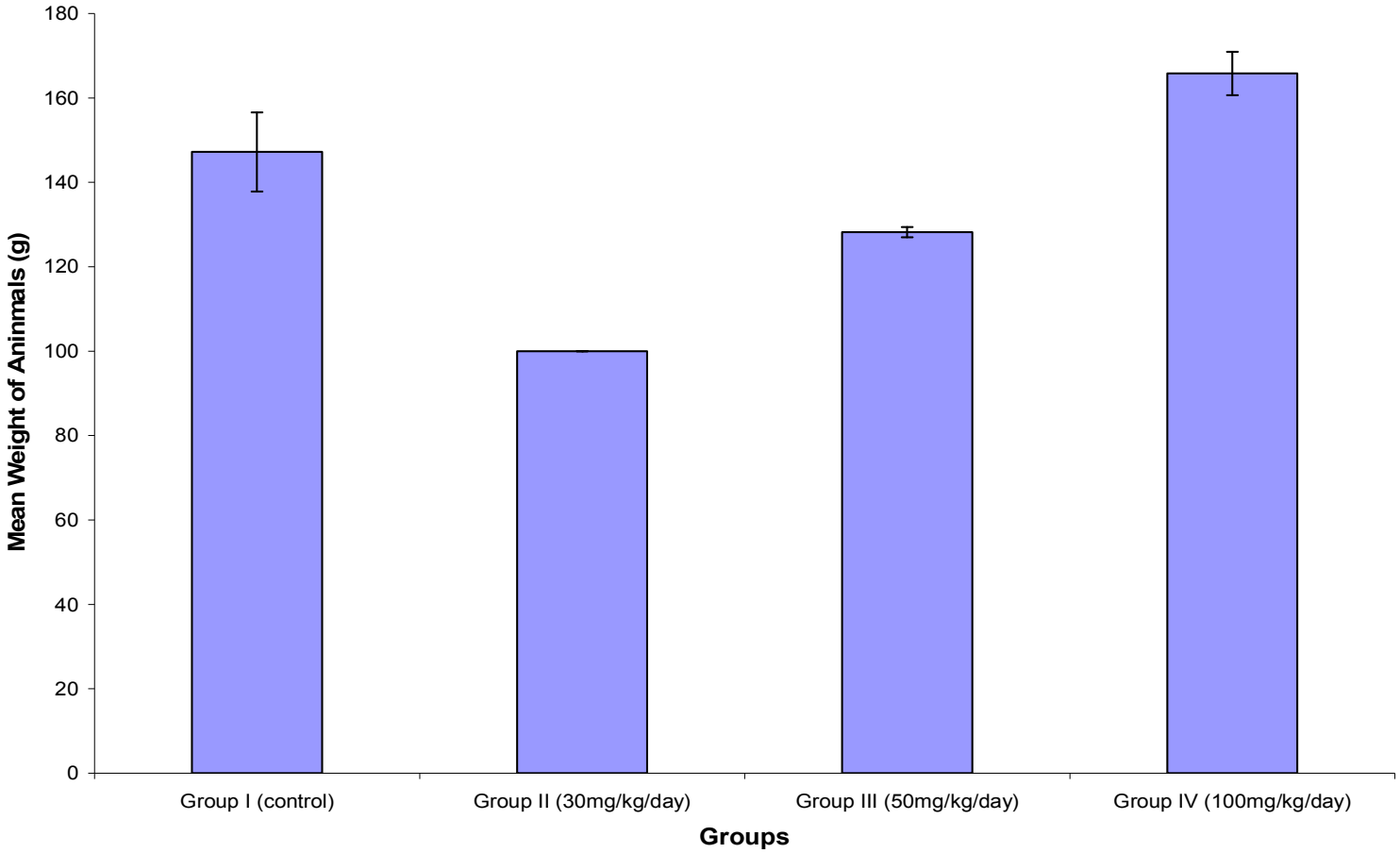


Fig. 4.1: Showing Mean and Standard Error of Mean of weight of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe*

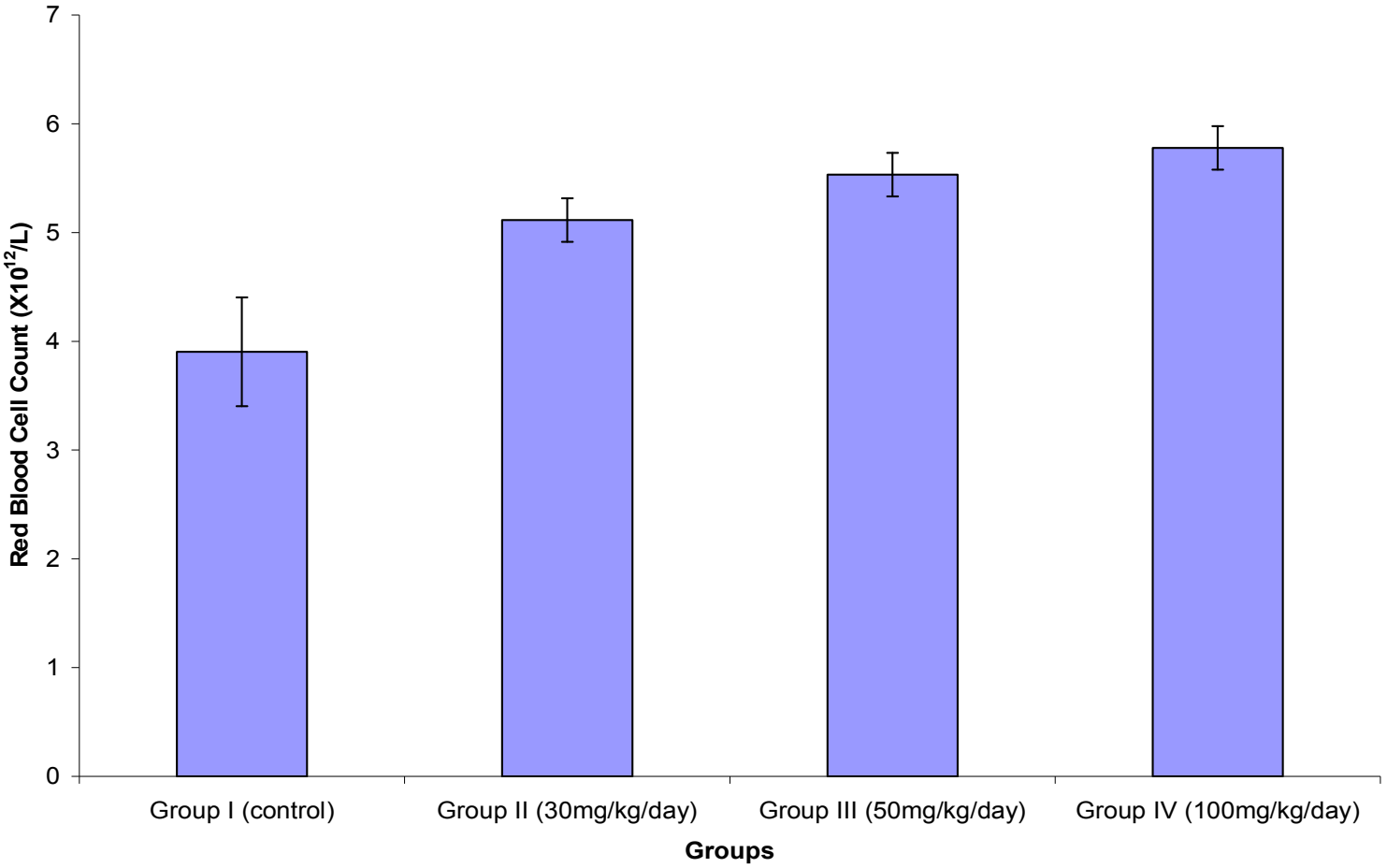


Fig. 4.2: Showing Mean and Standard Error of Mean of Red Blood Cell (RBC) of Male Wistar Rats after Oral Administration of Extract of *Pausinystalia yohimbe*

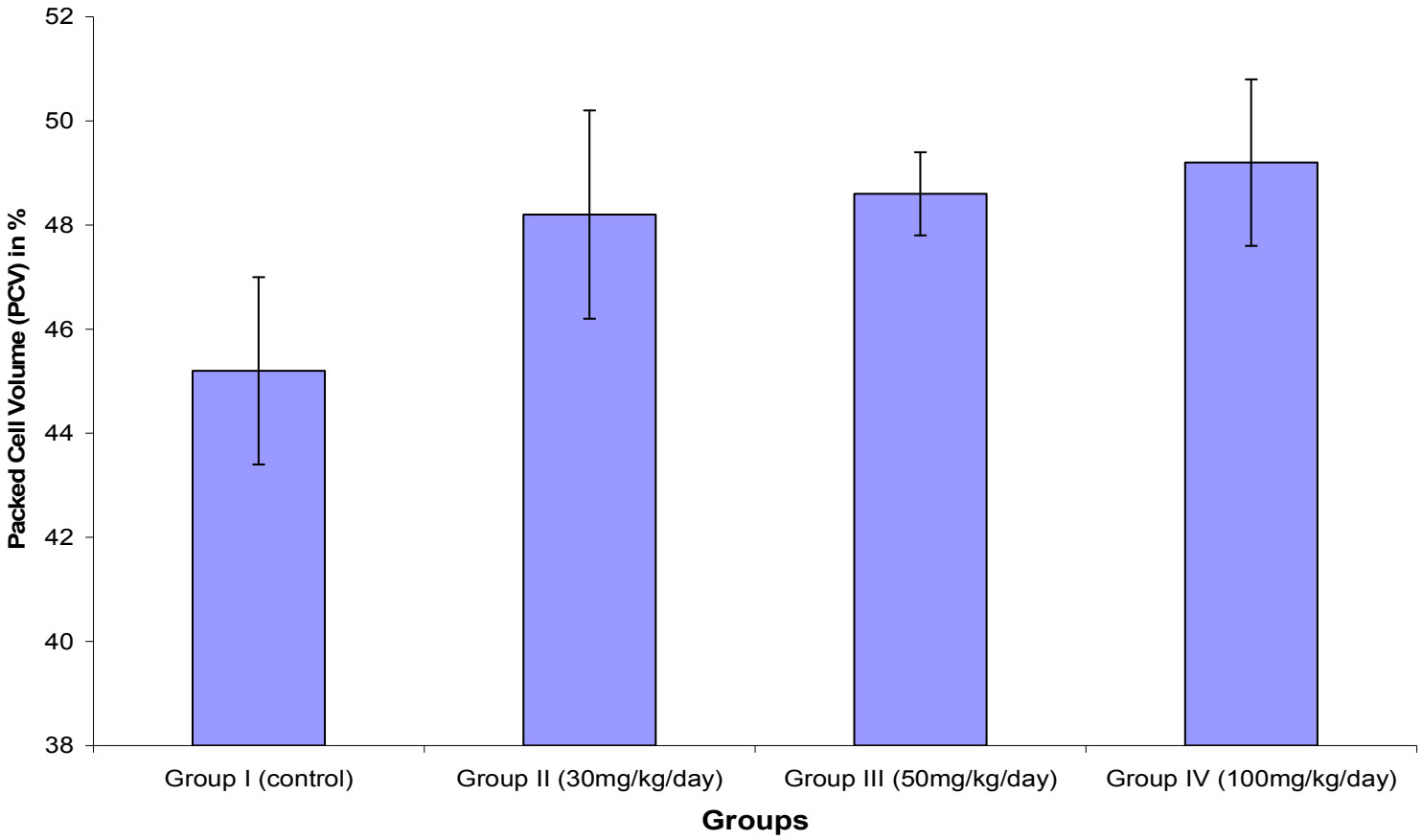


Fig. 4.3: Showing Mean and Standard Error of Mean of Packed Cell Volume (PCV) of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe*

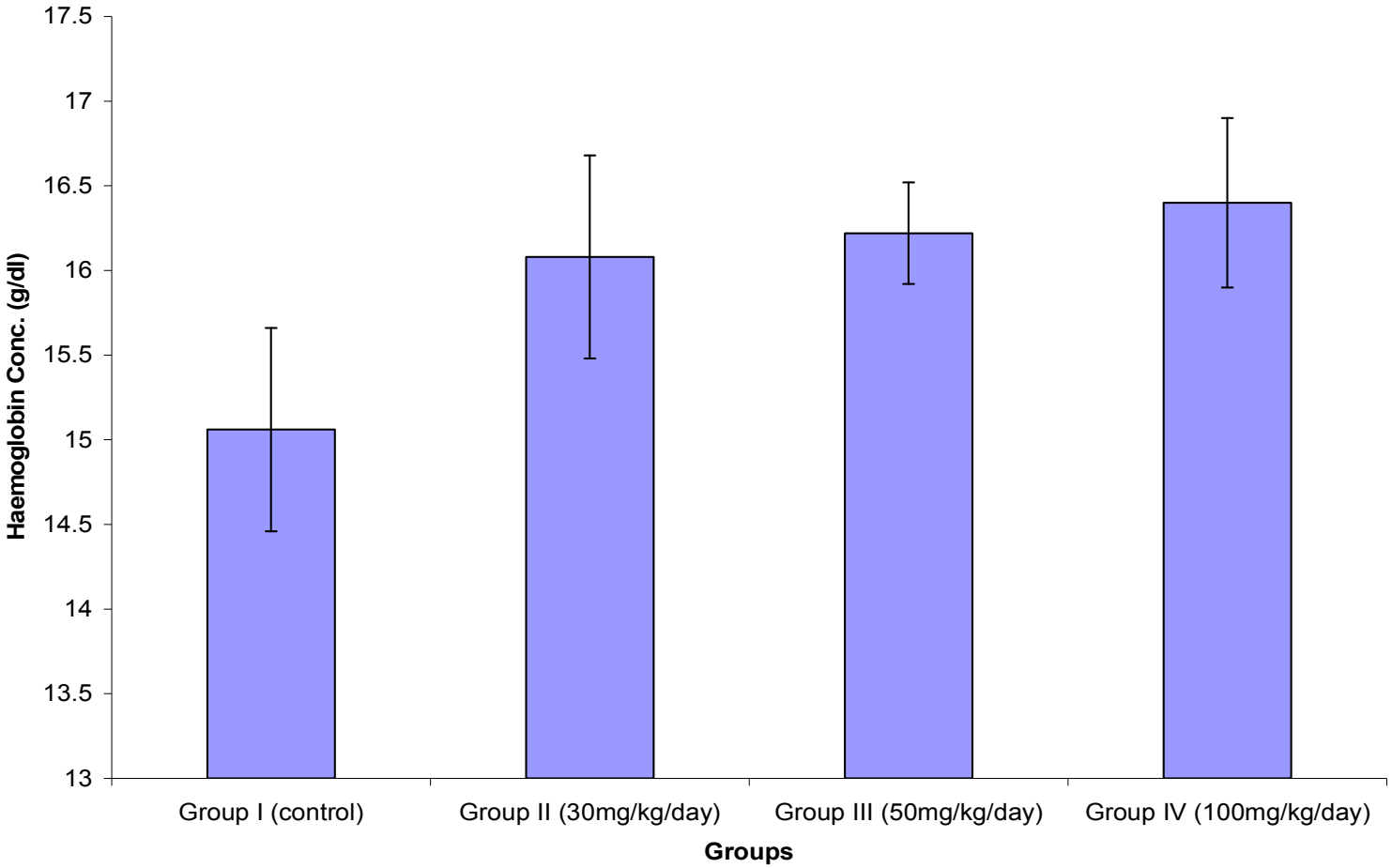


Fig. 4.4: **Showing Mean and Standard Error of Mean of Haemoglobin Concentration (Hb) of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe***

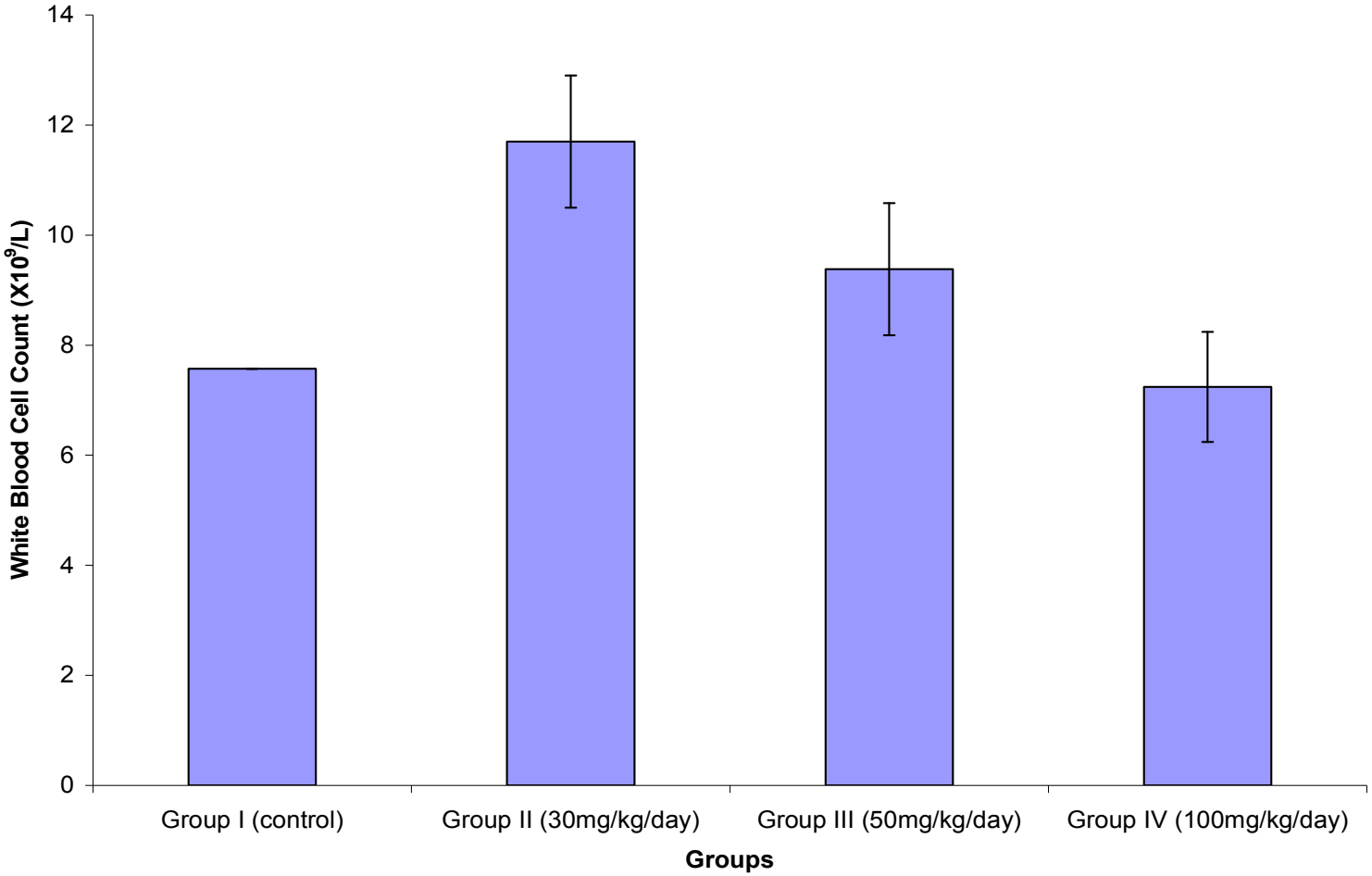


Fig. 4.5 Showing Mean and Standard Error of Mean of White Blood Cells (WBC) of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe*

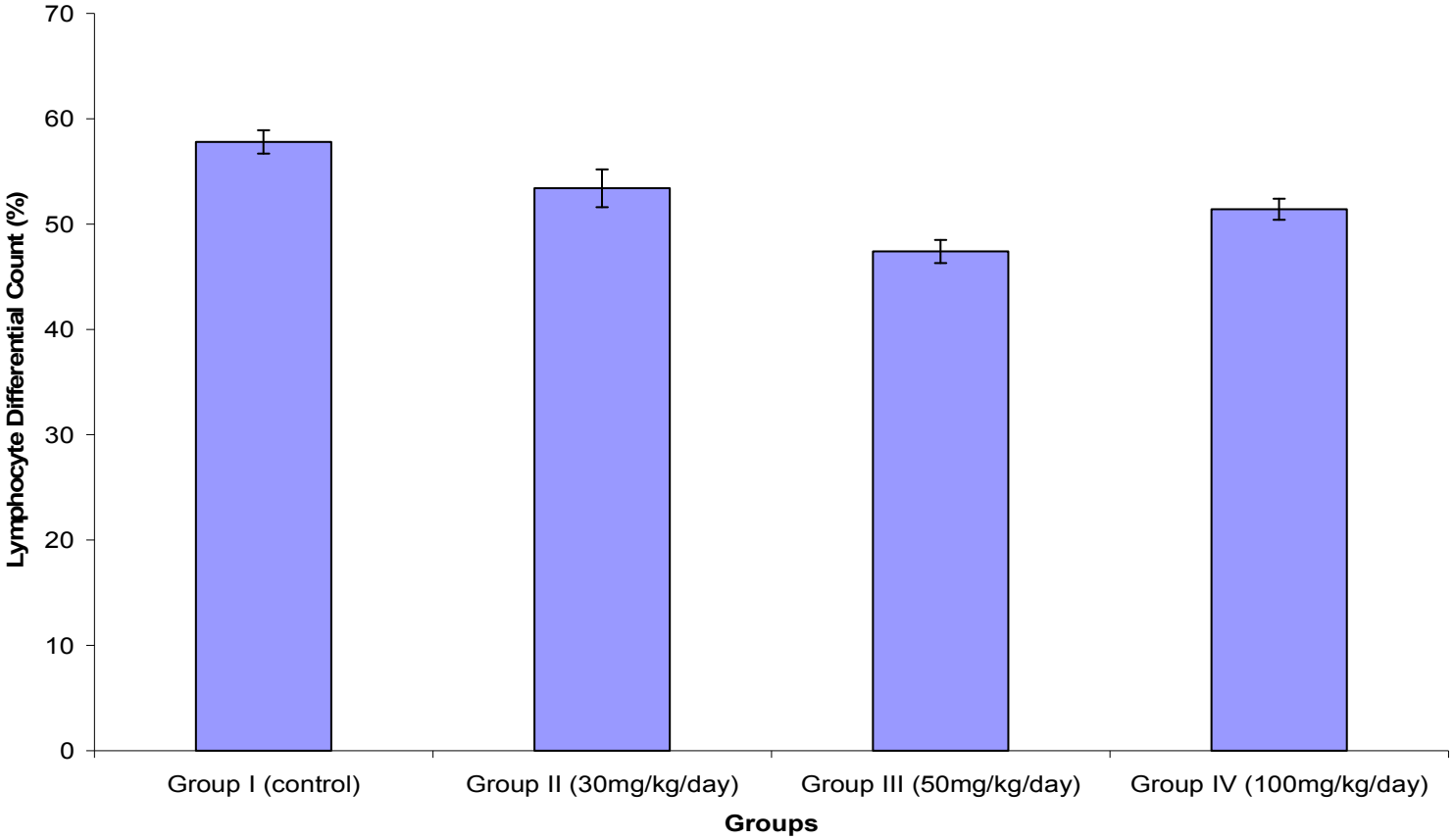


Fig. 4.6 Showing Mean and Standard Error of Mean of Lymphocytes of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe*

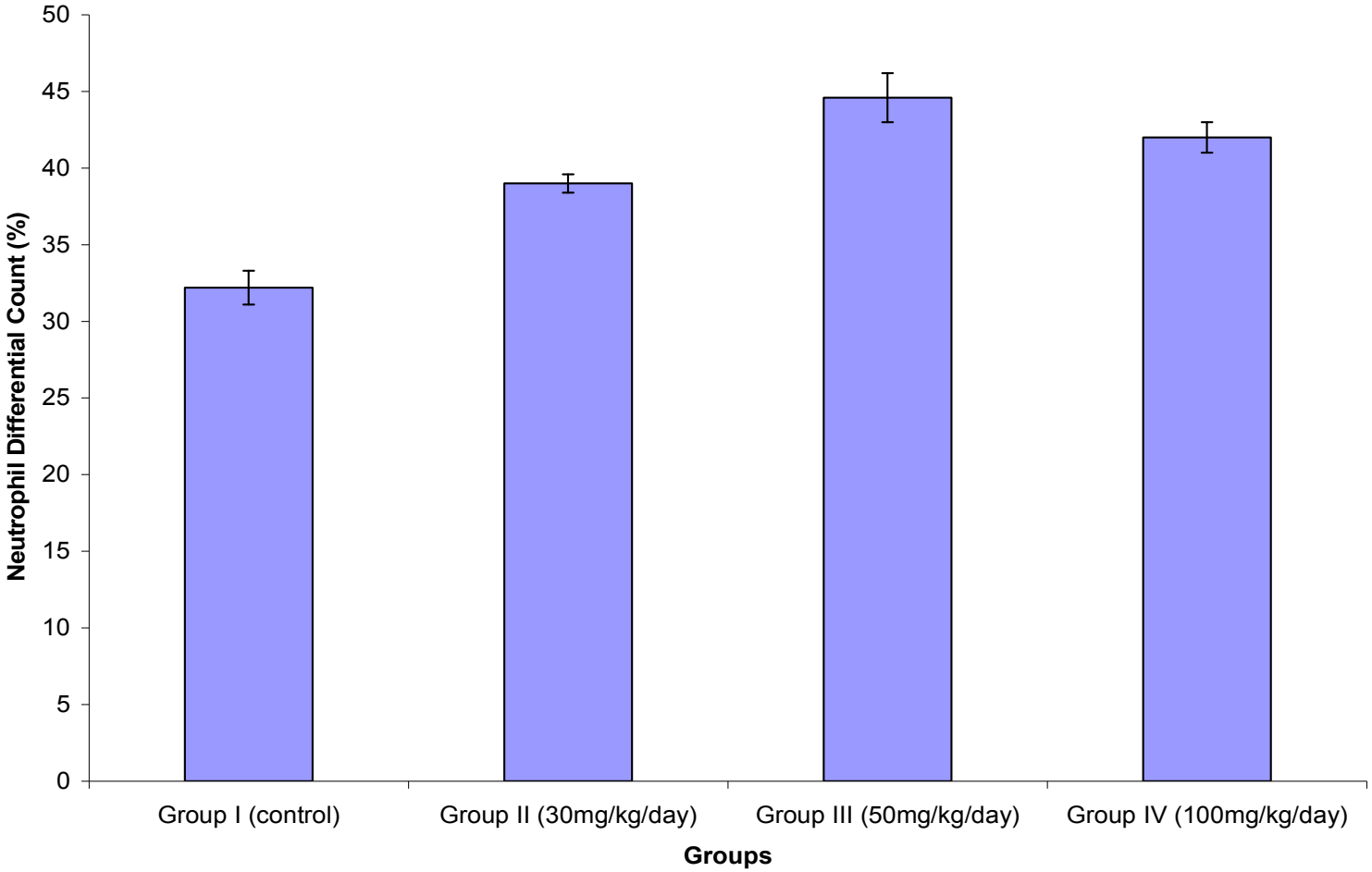


Fig. 4.7 Showing Mean and Standard Error of Mean of Neutrophils of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe*

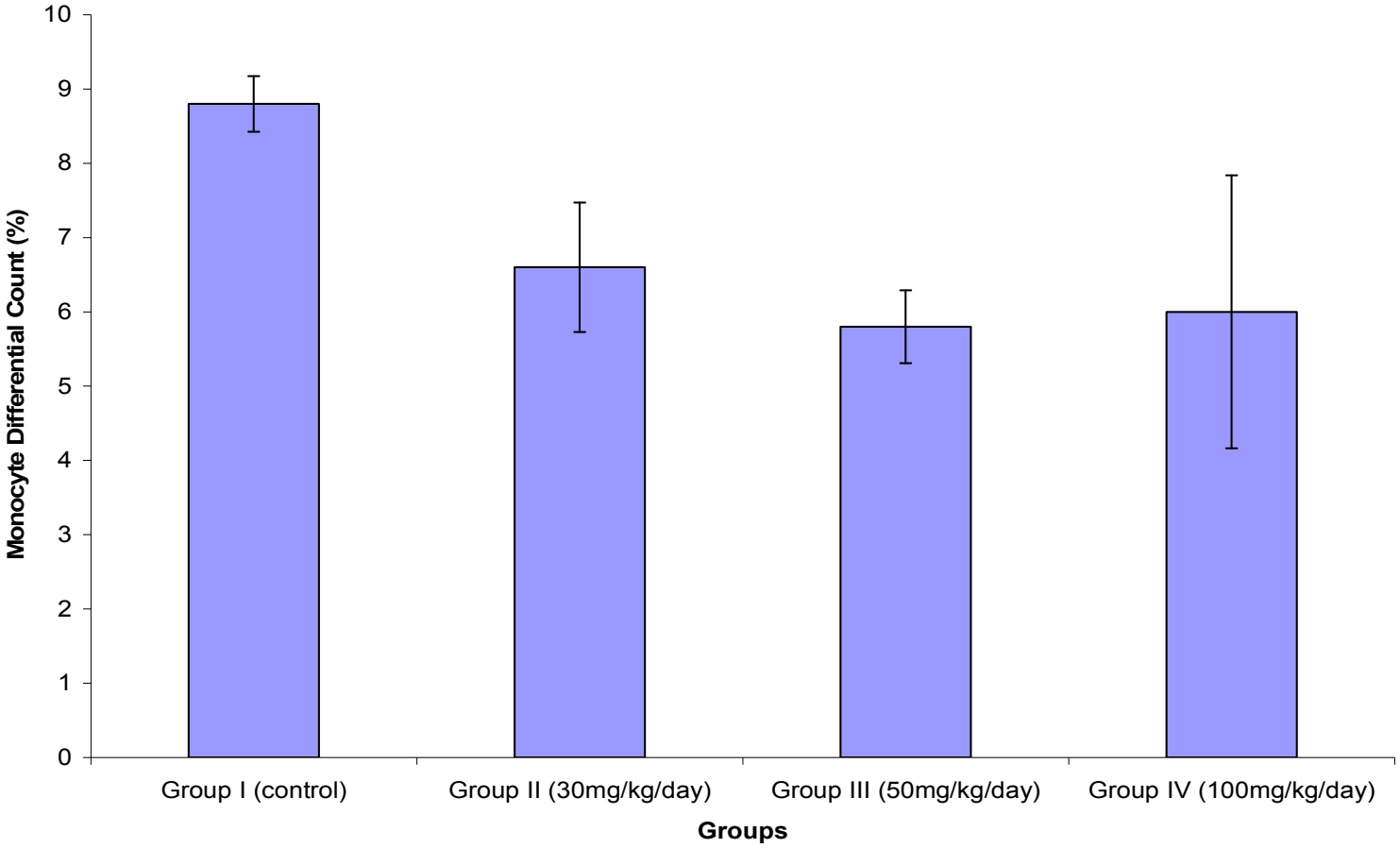


Fig. 4.8 **Showing Mean and Standard Error of Mean of Monocytes of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe***

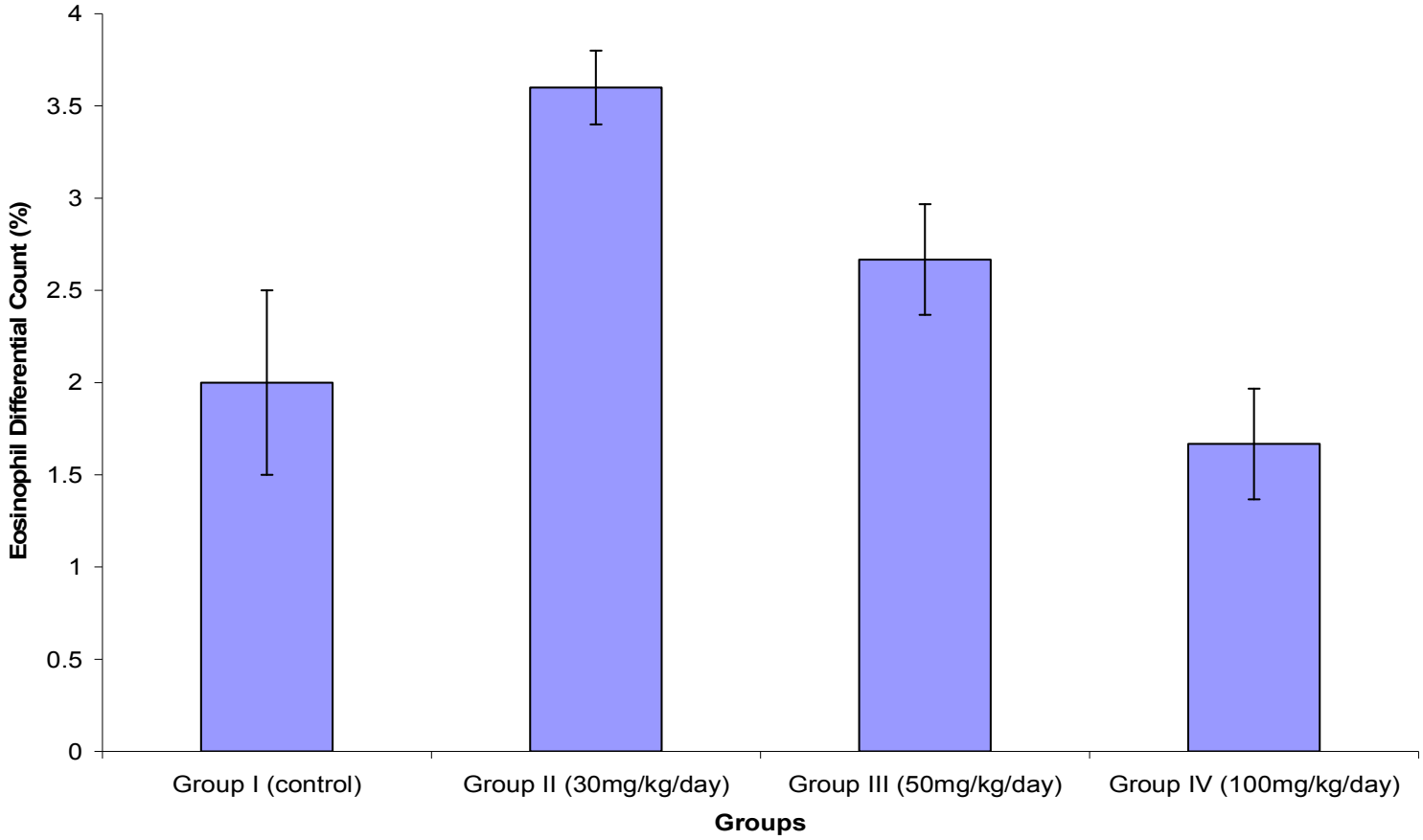


Fig. 4.9 Showing Mean and Standard Error of Mean of Eosinophils of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe*

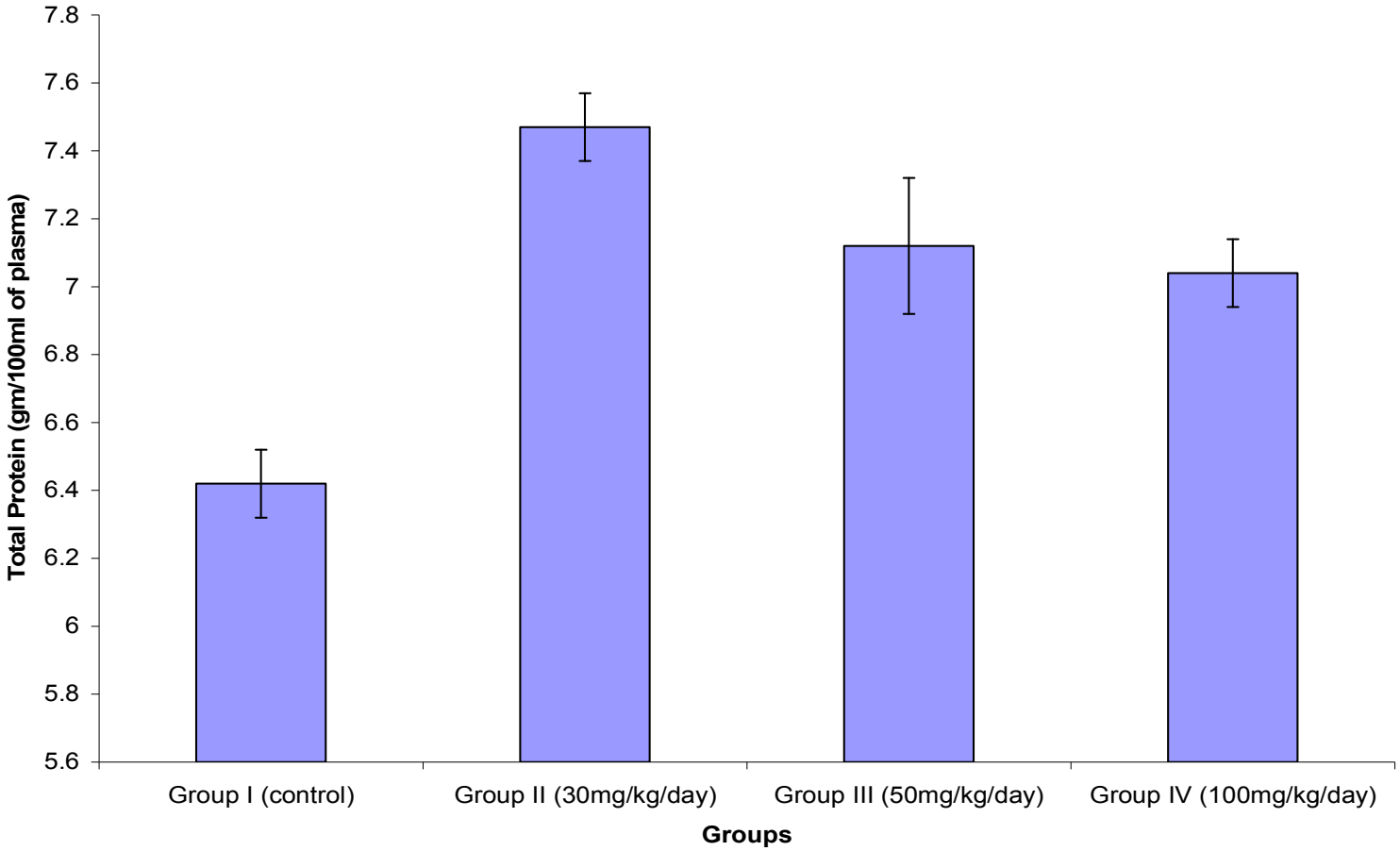


Fig. 4.10 Showing Mean and Standard Error of Mean of Total Protein (TP) of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe*

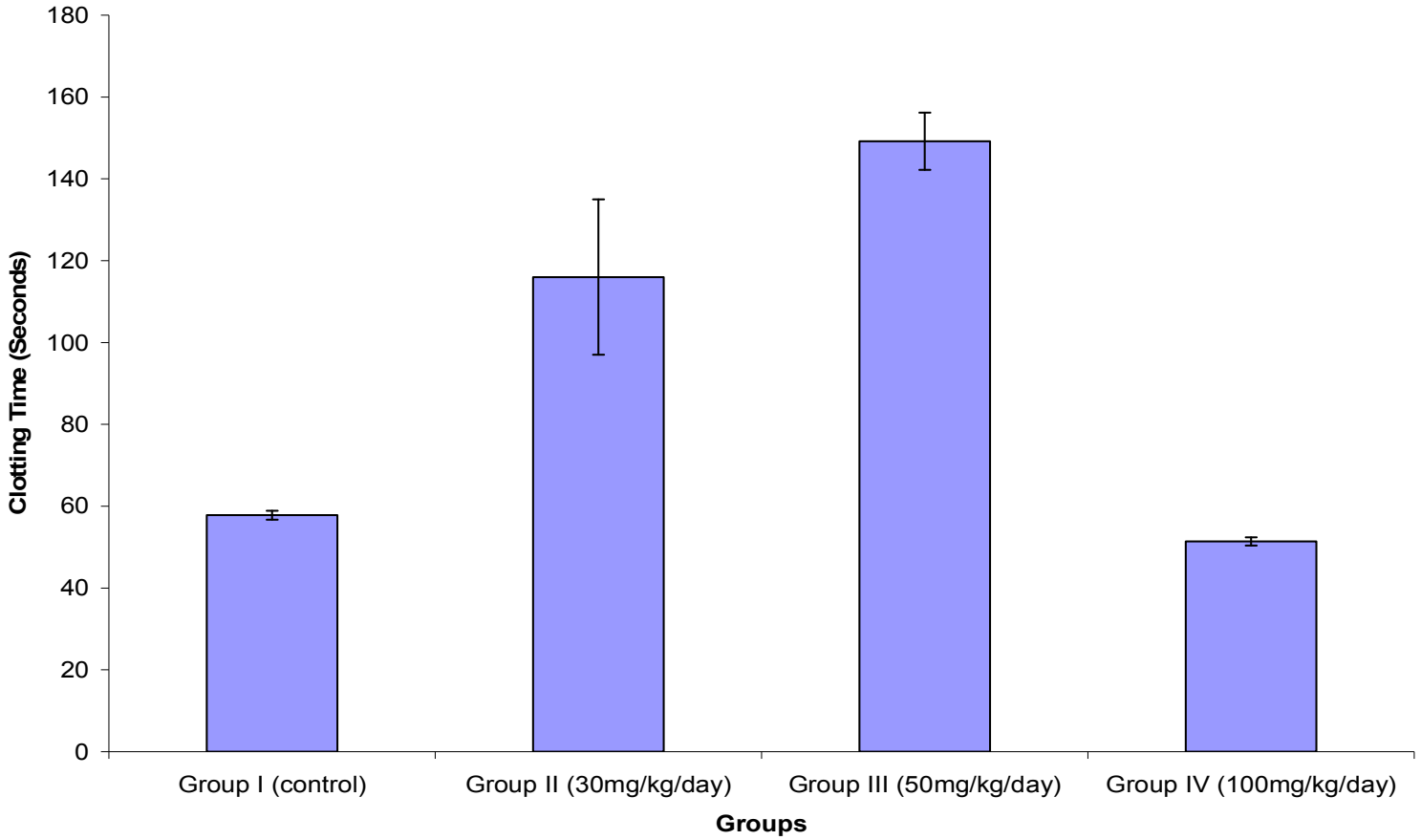


Fig. 4.11 Showing Mean and Standard Error of Mean of Clotting Time (CT) of Male Wistar Rats after Oral Administration of Crude Extract of *Pausinystalia yohimbe*

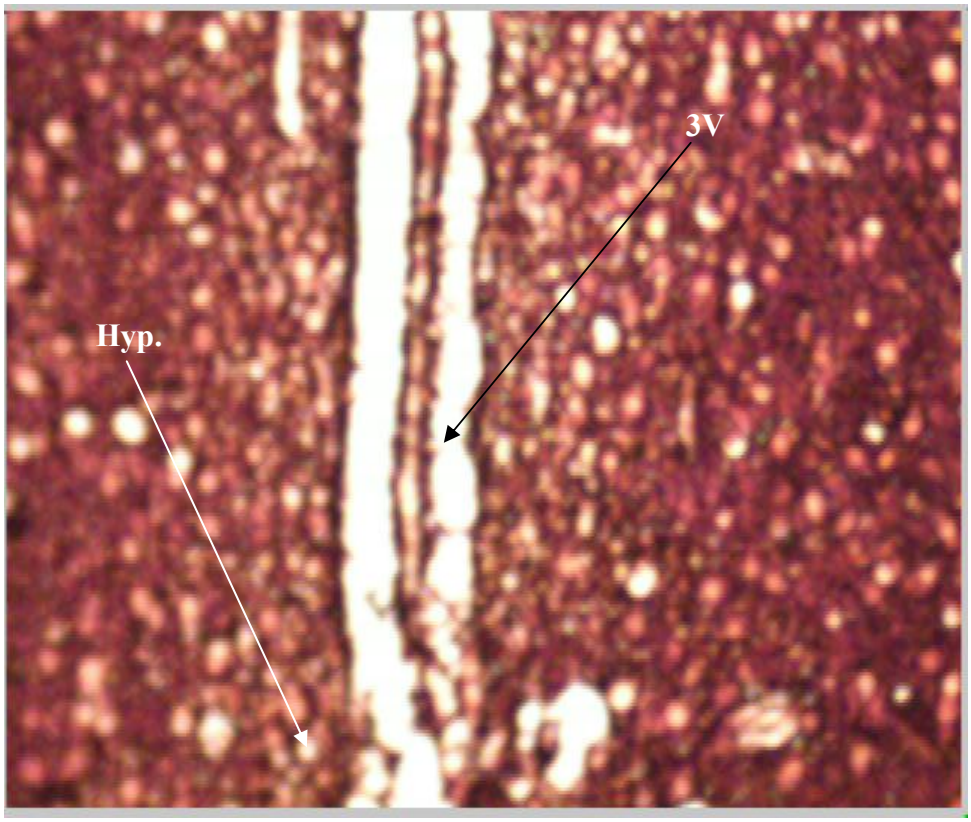


Plate 1a: Coronal section of Brain. Group I (Control) Note Hypothalamic Nuclei (Hyp.n), Third Ventricle (3V), Silver stain (Glee's method, 1954) x 100

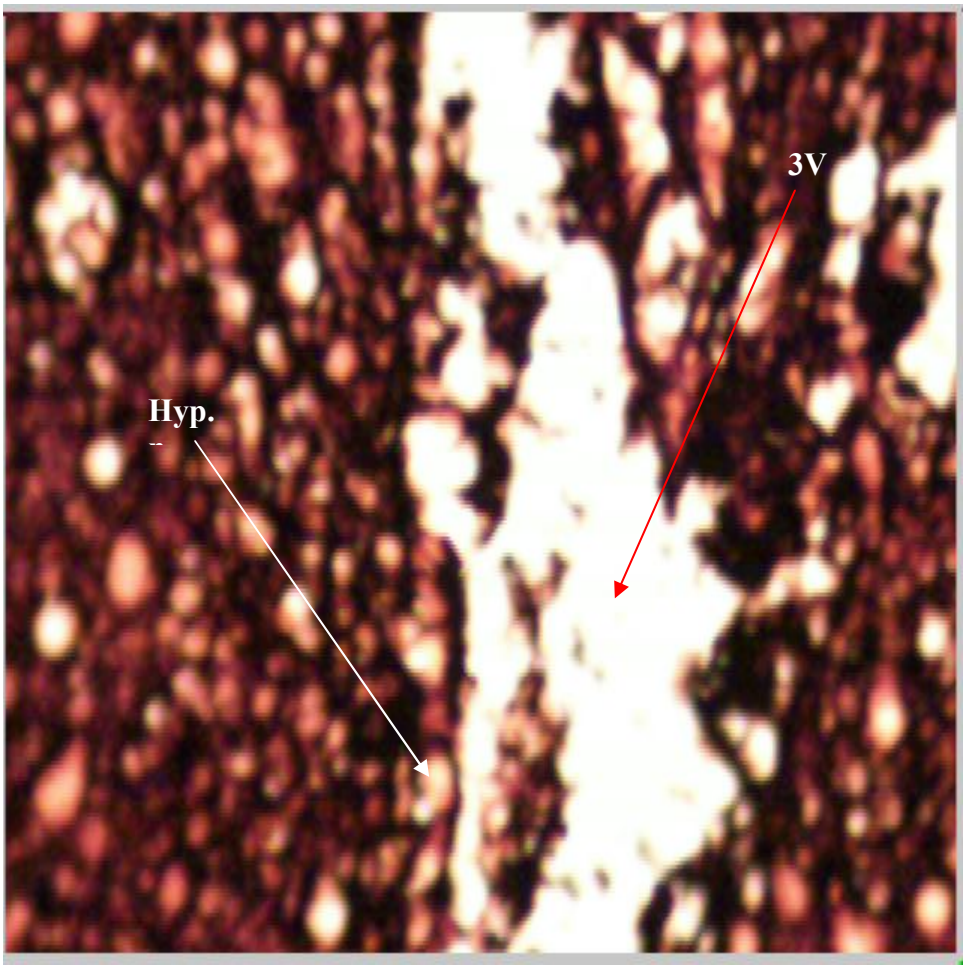


Plate 1b: Coronal section of Brain. Group I (Control) Note Hypothalamic Nuclei (Hyp. n), Third Ventricle (3V). Silver stain (Glee's method, 1954) x 250

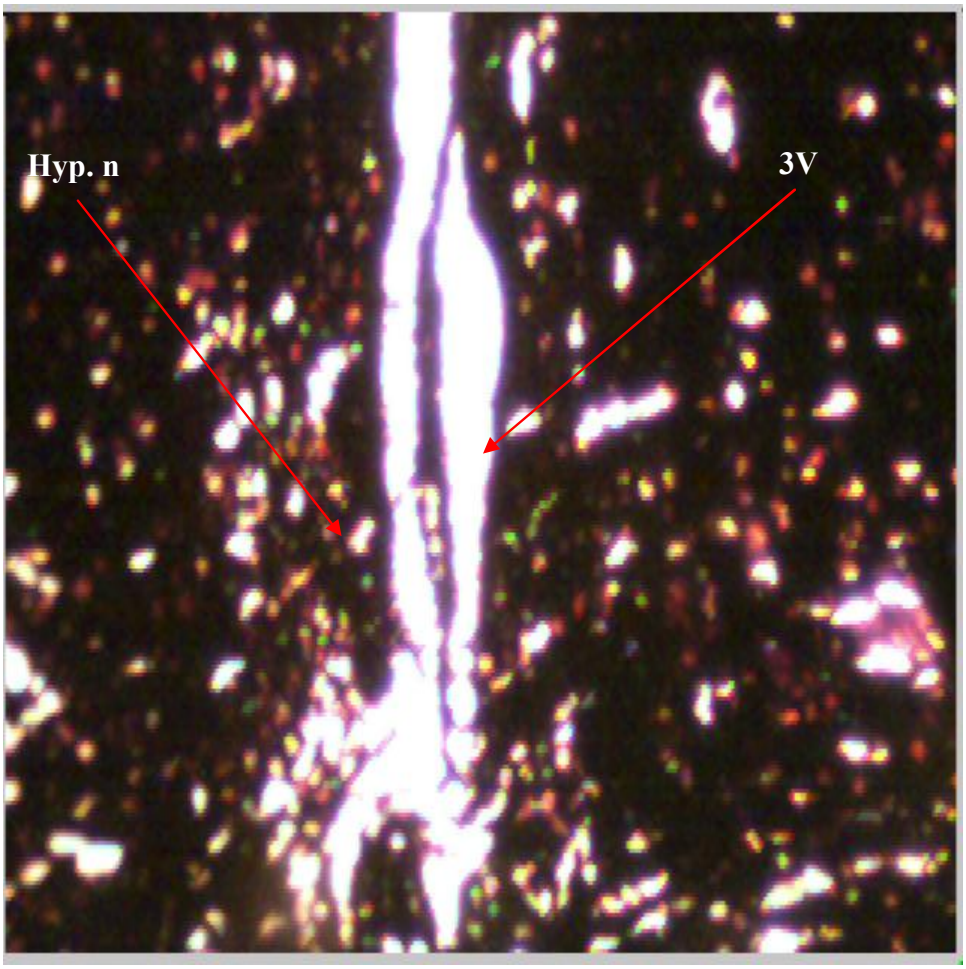


Plate 2a: Coronal section of Brain. Group II (30mg/kg/day). Note Prominent Hypothalamic Nuclei (Hyp. n), Third Ventricle (3V). Silver stain (Glee's method, 1954) x 100

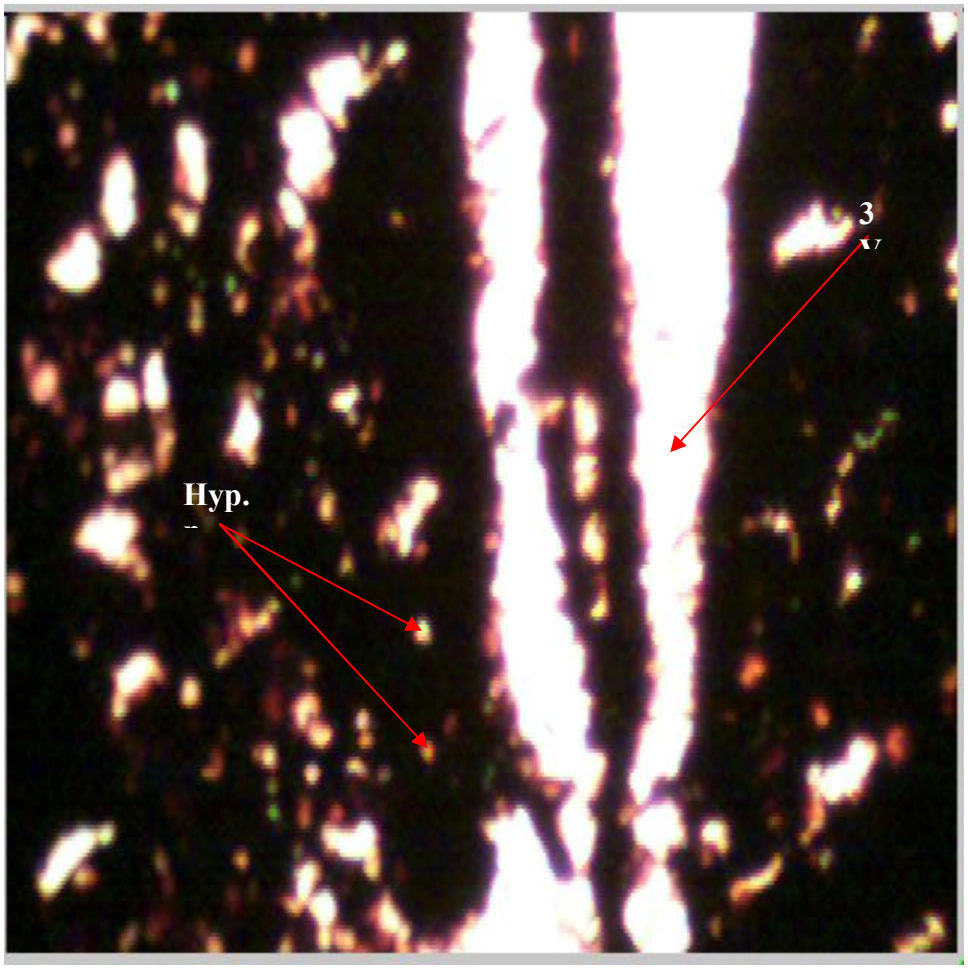


Plate 2b: **Coronal section of Brain. Group II (30mg/kg/day). Note Prominent Hypothalamic Nuclei (Hyp. n), Third Ventricle (3V). Silver stain (Glee's method, 1954) x 250**

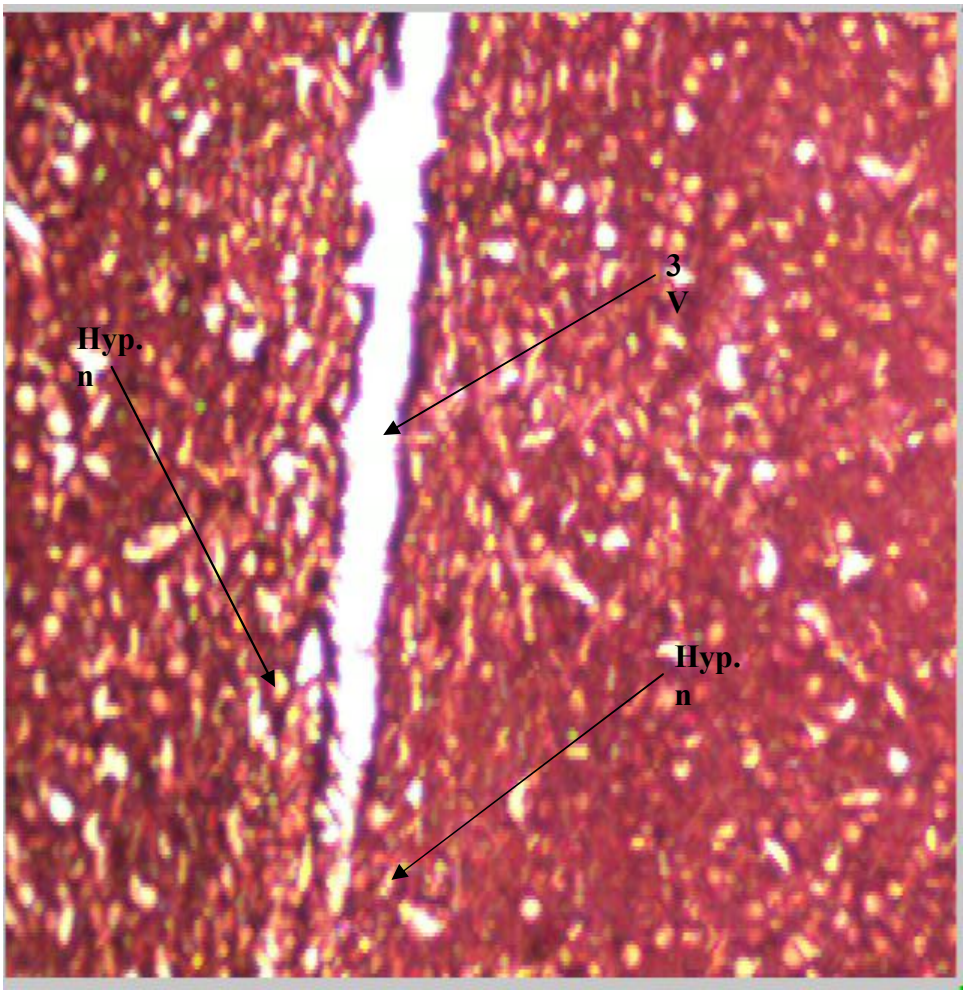


Plate 3a: Coronal section of Brain. Group III (50mg/kg/day). Note Prominent Hypothalamic Nuclei (Hyp. n), Third Ventricle (3V). Silver stain (Glee's method, 1954) x 100

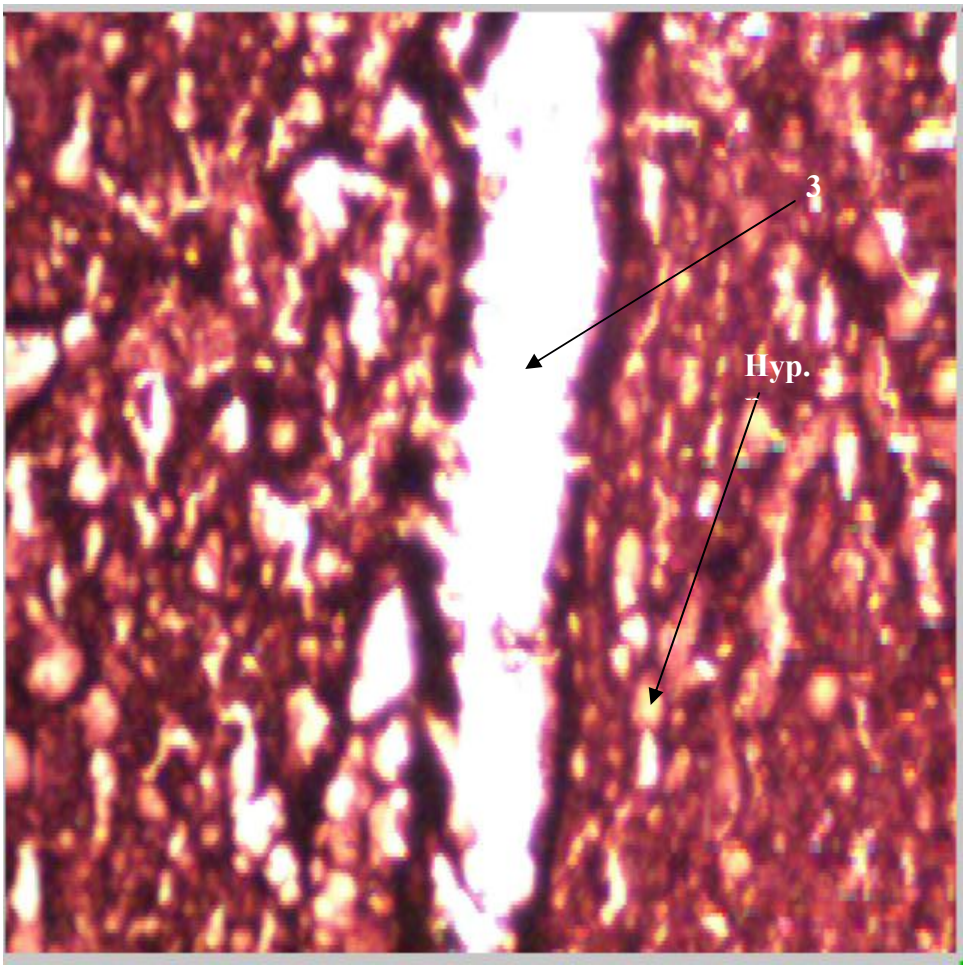


Plate 3b: Coronal section of Brain. Group III (50mg/kg/day). Note Prominent Hypothalamic Nuclei (Hyp. n), Third Ventricle (3V). Silver stain (Glee's method, 1954) x 250

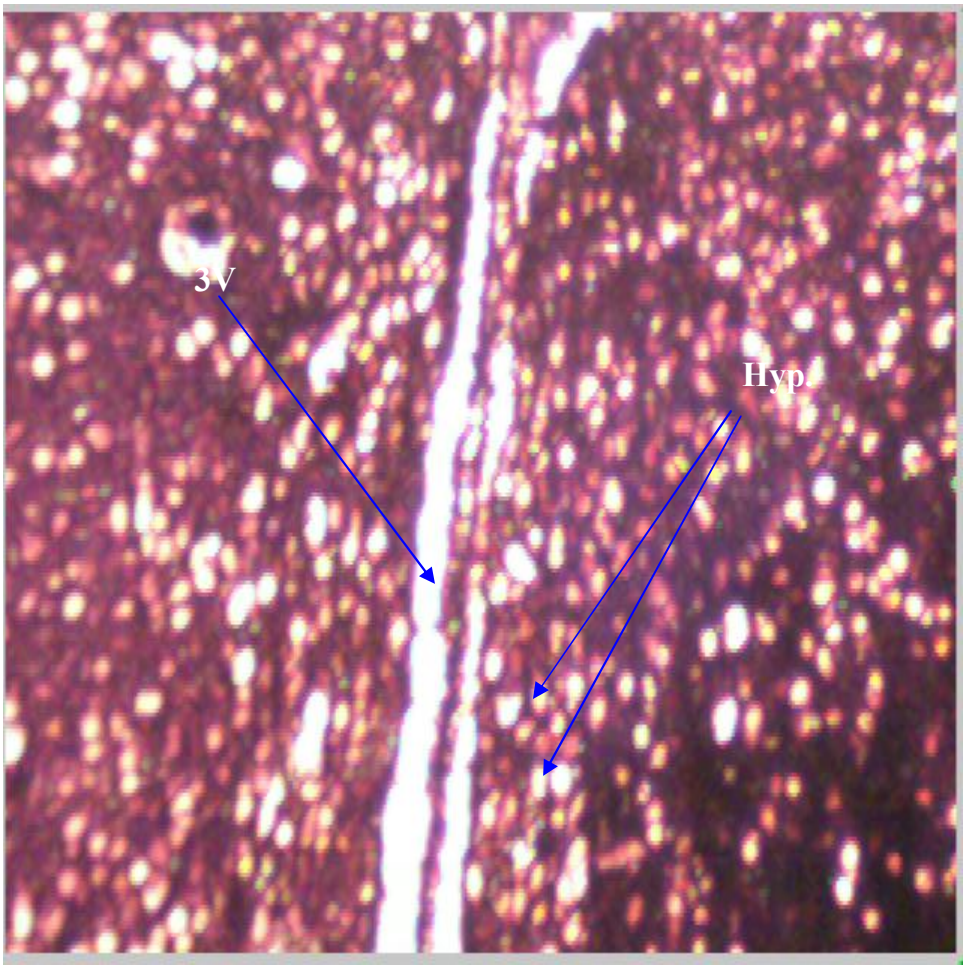


Plate 4a: Coronal section of Brain. Group IV (100mg/kg/day). Note the More Prominent Hypothalamic Nuclei (Hyp. n), Third Ventricle (3V). Silver Stain (Glee's method, 1954) x 100

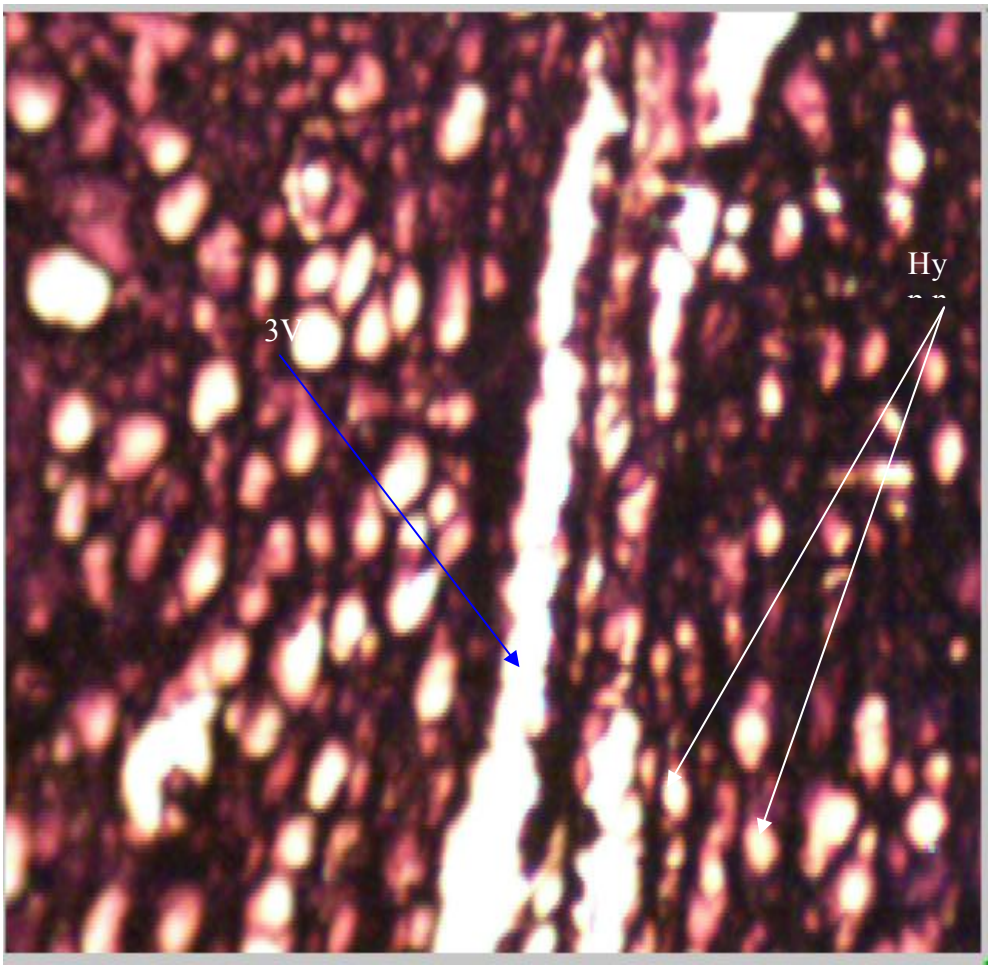


Plate 4b: **Coronal section of Brain. Group IV (100mg/kg/day). Note the More Prominent Hypothalamic Nuclei (Hyp. n), Third Ventricle (3V). Siliver Stain (Glee's method, 1954) x 250**

4.12 MICROSCOPIC EXAMINATION OF TISSUE

The hypothalamus (brain) was studied microscopically using the Glee's method for paraffin section (Marsland, Glees and Erikson, 1954).

4.12.1 Glees' Methods

The result of the microscopic examination of the hypothalamus stained using the Glees' methods of silver stains are shown in plates 1 to 4.

Plate 1a and b, with the control Group I administered distilled water orally. Here it reveals the general outline of the normal histology of the hypothalamus (brain). The third ventricles (3V) and the hypothalamic nuclei (Hypo Nu) were clearly presented.

Plate II a and b, showed the treated Group II which was administered orally 30mg/kg/day of the crude ethanolic extract of *Pausinystalia yohimbe* bark. Here there was more appearance of the cells (nuclei), a condition in cellular proliferation known as cell hyperplasia. The nuclei are more prominent when compared with the control Group I who was only administered distilled water. The third ventricle and hypothalamic nuclei were represented as 3V and Hypo Nu respectively.

Plate III a and b, showed the treated group III which was administered orally 50mg/kg/day of the crude ethanolic extract of *Pausinystalia yohimbe* bark. Here the nuclei appear more pronounced and also showed more increase in cell histological when compared with the control group which was only administered distilled water orally. Group IV (100mg/kg/day), shown in plates IV a and b and plates.

The plates showed the third ventricles (3V) and the hypothalamic, nuclei, (Hypo Nu), clearly presented. And it shows more increase in the nuclei histologically when compared with groups III and II and most especially when with the control group.

CHAPTER FIVE

5.0 DISCUSSION

The result of the study show that, *Pausinystalia yohimbe* administered at 30mg/kg body weight/day 50mg/kg body weight/day and 100mg/kg body weight/day and for the duration of the experiment appears to be responsible for the weight loss seen in this investigation. And this agrees with the report from Health Notes, 2000, which says that *Pausinystalia yohimbe* bark is used in weight reduction. And this might have occurred probably by the presence of saponins found in the stem bark extract. Saponins are known to be toxic to body systems (Watt and Breyer-Brandwijk, 1962). The effect of saponins may have contributed to the clinical signs of loss of appetite and loss of weight observed in all the experimental animals administered with the *Pausinystalia yohimbe* bark extract. It could be that saponins possibly have an inhibitory effect on food assimilation and metabolism. Furthermore, the plant extract could also have some other constituents that have effect on the feeding centre in the hypothalamus. Hypothalamus plays an important role in the control of many functions that are vital for the survival of an animal (Sambuligam *et al.,* 2001). Hypothalamus is also responsible for the feeling of hunger and of satiety, and this determines whether the animal will accept or refuse food. It has been observed that the stimulation of the medial zone hypothalamic nuclei produces satiety has shown that a feeding centre has been described in the lateral hypothalamic nucleus, and satiety in the ventromedial (Singh 1999; Guyton, 2000).

The *Pausinystalia yohimbe* bark extract found to bring significant increase in the erythrocytes (red blood cells) counts values. There was general increase in red blood cell count in the treated animals when their erythrocyte counts values were compared with the

control animals. This increase could possibly be due to the presence of some of the phytochemical constituents like cardiac glycoside, alkaloids, which That may have effects on the red blood cells production system in the body of the animals. Generally, increased in erythrocytes count value was referred to as Polycythemia (Ganong, 2005). And possible causes of Polycythemia have been attributed to physiological processes like lack of oxygen and erythropoietin hormone secretion. This could also result from muscular exercise and emotional state produced by sympathetic stimulation which squeezes the spleen in hot weather, (Sembulingam, *et al,* 2000). The alkaloid known as yohimbine which is the primary active constituent in yohimbine is known or reported to induced central nervous system adrenergic activation which squeezes the spleen and thereby could possibly bring about increase in erythrocytes volume (Oliver, *et al,* 2000).

The plant extract seem not to have any significant effect on the packed cell volume as seen in this experiment. The packed cell volume is the percentage ratio of erythrocyte volume to the total blood volume (Guyton *et al,* 2000, Ganong, 2005). Also, the haemoglobin concentration (Hb) was not significantly affected during the period of the administration of *Pausinystalia yohimbe* bark extract.

The result of this study showed that, the bark extract of *Pausinystalia yohimbe* administered at the dosages used and for the duration of the experiment appear to have significant effect on the leucocytes count values and the differential leucocytes counts values. There was a reduction in the lymphocytes, monocytes percentages, while there was increase in the neutrophil percentage in the treated animals, as compared with the control. The reduction may have occurred due to lysis of blood cells and probably suppression of blood cell synthesis by saponins found in the bark extract. Saponins are

known to be toxic to body systems (Watt and Breyer-Brandwijk, 1962). The effect of saponins as earlier stated contributed to the loss of weight observed in all the animals administered with the plant extract. The decrease in some of these differential leucocytes parameters, like the lymphocytes and monocytes, agrees with the work of (Siddiqui, *et al,* 1996), which stated that agranulocytosis was associated with yohimbe use. Agranulocytosis is a haematological condition in which there is severe acute deficiency of certain blood cells as a result of bone marrow damage by toxic substances or chemicals. Interstingly *pausinystalia yohimbe* was found to contain many substances, which when their individual activities is examined, may likely be responsible for this reduction and increase in the haematological parameter as the case may be.

Also the result showed that oral administration of *Pausinystalia yohimbe* bark extract seem to cause increased in the amount of the plasma proteins. The main types of plasma proteins are albumin, globulins, fibrinogen, prothrombin, (Guyton *et al,* 2000, Ganong, 2005). Albumin, fibrinogen and prothrombin are formed in the liver as evidenced by the marked decrease of these proteins in liver diseases, for example, infective hepatitis (Guyton and Hall, 2000), or after hepatectomy in animals. While globulins are formed by the plasma cells and the cells of the reticuleondothelial system present in the lymph nodes, spleen, bone marrow and liver, (Ganong, 2005). Plasma proteins are known to play important role in blood clotting, osmotic function, blood viscosity, defensive function, buffering action, capillary permeability, conservative and transport function and as a reserve proteins. They are also responsible for the transport of water-soluble hormones e.g. peptides and cathecolamines and also the steroid hormones. (Guyton and Hall, 2000). The fact that the plasma protein was not reduce also indicated

that the extract did not have necrotic effect on the liver; this could mean that extract had inhibitory/facilitatory effect of activating blood clotting factors which plays an activating role in blood coagulation especially in the intrinsic system (Ganong, 2005).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.0 SUMMARY

The effects of oral administration of the crude extract of *Pausinystalia yohimbe* on the weight, heamatological parameters and histology of the brain (hypothalamus) of male wistar rats was investigated, and was found to cause decrease in weight, it was also found to have statistical significant difference on the erythrocytes counts, total proteins, clotting time, and also in the lymphocytes, monocytes, eosinophils and neutrophils of the white blood cells, at $P < 0.05$. While the histological studies shows that the crude extract of *Pausinystalia yohimbe elicit* excitatory/facilitatory effect thus bringing about increase in cell mass hyperplasia.

The investigation showed that the oral administration of the crude extract of *Pausinystalia yohimbe* have negative effect on the haematological parameters and the hypothalamic nuclei of adult male wistar rats. The changes seen on the haematological parameters are statistically significant at $P < 0.05$; moreover, the histological studies show that the crude extract of *Pausinystalia yohimbe* has excitatory effect on the brain (hypothalamus).

6.1 RECOMMENDATIONS

It is recommended that the different chemical components of crude extract of *Pausinystalia yohimbe* should be analyzed and the active ingredient responsible for the excitatory effect be isolated and used for further studies.

Moreso, the indiscriminate use of the crude extract of *Pausinystalia yohimbe* should be discouraged, by men who utilize it for penis erection because of its effects on the haematological parameters.

Further studies should be carried out to study the effect of the *Pausinystalia yohimbe* on the brain using electron microscope for ultrastructural study of the nuclei in the brain.

It is here suggested that *Pausinystalia yohimbe* can be used as a weight regulating drugs, due to the loss in weight observed in the animals following *Pausinystalia yohimbe* ingestion from this study.

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APPENDIX I: GROUP 1 (CONTROL DISTIL WATER)

S/no	Weight (g)	PCV (%)	RBC (10^{12}/l)	WBC (10^{9}/l)	HB con. (g/dl)	Total protein (g/100ml)	Clotting time (Sec.)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	145	47	4.85	1.13	15.7	6.50	60	60	31	9	-	-
2	145	43	3.16	8.40	14.3	6.50	55	55	35	10	-	-
3	133	45	4.12	7.80	15.0	6.75	59	59	30	8	3	-
4	184	51	2.19	11.80	17.0	6.35	60	60	30	8	2	-
5	129	40	5.20	8.70	13.3	6.00	55	55	35	9	1	-

* PCV = Pack Cell Volume (Hematocrit)
* WBC = White Blood Cell (Leucocytes)
* Hb = Haemoglobin
* RBC = Red Blood Cell (Erythrocytes)

APPENDIX II: GROUP 2 (30mg/kg/day. *Pausinystalia yohimbe*)

S/no	Weight (g)	PCV (%)	RBC (10^{12}/l)	WBC (10^{9}/l)	HB con. (g/dl)	Total protein (g/100ml)	Clotting time (Sec.)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	100	50	5.02	15.20	16.7	7.50	100	60	38	8	4	-
2	100	50	5.76	8.70	16.7	7.00	180	52	37	8	3	-
3	100	40	4.38	14.05	13.3	7.60	120	55	40	5	3	-
4	100	50	5.12	10.65	16.7	7.25	60	50	40	4	4	-
5	100	51	5.29	9.90	17.0	8.00	120	50	40	8	4	-

* PCV = Pack Cell Volume (Hematocrit)
* WBC = White Blood Cell (Leucocytes)
* Hb = Haemoglobin
* RBC = Red Blood Cell (Erythrocytes)

APPENDIX III: GROUP 3 (50mg/kg/day. *Pausinystalia yohimbe*)

S/no	Weight (g)	PCV (%)	RBC (10^{12}/l)	WBC (10^{9}/l)	HB con. (g/dl)	Total protein (g/100ml)	Clotting time (Sec.)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	125	47	5.01	8.00	15.7	7.00	170	47	43	5	2	1
2	127	50	6.15	13.00	16.7	6.80	160	45	50	5	-	-
3	127	46	6.00	9.85	15.3	6.50	125	50	45	5	-	-
4	131	50	5.44	10.45	16.7	7.30	141	45	45	7	3	-
5	131	50	5.06	5.60	16.7	8.00	150	50	40	7	3	

* PCV = Pack Cell Volume (Hematocrit)
* WBC = White Blood Cell (Leucocytes)
* Hb = Haemoglobin
* RBC = Red Blood Cell (Erythrocytes)

APPENDIX IV: GROUP 4 (100mg/kg/day. *Pausinystalia yohimbe*)

S/no	Weight (g)	PCV (%)	RBC (10^{12}/l)	WBC (10^{9}/l)	HB con. (g/dl)	Total protein (g/100ml)	Clotting time (Sec.)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	148	45	5.52	6.55	15.0	7.00	50	51	40	9	-	-
2	164	51	5.52	10.4	17.0	6.50	35	55	40	6	2	-
3	165	50	5.50	5.60	16.7	7.50	80	50	44	6	-	-
4	175	54	6.62	8.95	18.0	7.40	60	49	45	4	1	-
5	177	46	5.73	4.70	15.3	6.80	91	52	41	5	2	-

* PCV = Pack Cell Volume (Hematocrit)
* WBC = White Blood Cell (Leucocytes)
* Hb = Haemoglobin
* RBC = Red Blood Cell (Erythrocytes)

APPENDIX V

Composition of Wistar Rat Feed

(Vital Pelletised Growers Feed) KM 17, Zawan Roundabout, P.O. Box 13462, Jos, Plateau State, Nigeria.

Crude Protein	14.50%
Fat	7.00%
Crude Fibre	2.20%
Calcium	0.80%
Available Phosphorus	0.40%
Metabolisable Energy	2,500 kcal/kg

APPENDIX VI

Calculation:

A. Group 1 = control – distil water

$$\text{Dose} = \frac{\text{weight (kg) X dosage mg/kg}}{\text{Concentration mg/kg}}$$

$$= \frac{0.20\text{kg X } 100 \text{ mg/kg}}{100 \text{ mg/ml}}$$

$$= 0.20 \text{ ml}$$

i.e. an animal of 200g (0.20 kg) is administered 0.20ml of distill water.

B. Group 2 = 30mg/kg Body weight

$$\text{Dose} = \frac{\text{weight (kg) X Dosage (mg/kg)}}{\text{Concentration (mg/kg)}}$$

$$= \frac{0.180 \text{ kg X } 30 \text{ mg/kg}}{30 \text{ mg/ml}}$$

$$= 0.180 \text{ ml}$$

i.e. an animal of 180g (0.180kg) is administered 0.180ml of *Pausinystalia yohimbe* extract.

C. Group 3 = 50mg/kg Body weight

$$\text{Dose} = \frac{\text{weight (kg) X Dosage (mg/kg)}}{\text{Concentration (mg/ml)}}$$

$$= \frac{0.20\text{kg X } 50\text{mg/kg}}{50\text{mg/ml}}$$

$$= 0.2\text{ml}$$

i.e. an animal with 200g (0.20kg) is administered 0.2ml of 50mg of *Pausinystalia yohimbe* extract.

D. Group 4 = 100mg/kg Body weight

$$\text{Dose} = \frac{\text{weight (kg) X Dosage (mg/kg)}}{\text{Concentration (mg/ml)}}$$

$$= \frac{0.20\text{kg X } 100\text{mg/kg}}{100\text{mg/ml}}$$

$$= 0.20\text{ml}$$

i.e. an animal with 200g (0.20kg) is administered 0.20ml of 100mg of *Pausinystalia yohimbe* extract.

APPENDIX VII

1. White Blood Cell diluting fluid

Diluting fluid: WBC=D

Glacial acetic acid – 1.5 ml

1% aqueous solution of Gention violet -1.0ml

Distilled water - 98.0ml.

Other materials:

White blood cell pipette, simple light microscope, haemocytometer, cover slide

APPENDIX VIII

2. Red Blood Cell diluting fluid

Hayem's fluid.

Mercuric chloride 0.5 gm

Sodium chloride 1.0 gm

Sodium sulphate 5.0 gm

Distilled water to 200 ml.

Others materials:

Red blood cell pipette, haemocytometer (Neubar counting chamber), simple light microscope.