SEMEN CHARACTERISTICS, GONADAL SPERM RESERVES, TESTICULAR HISTOLOGY AND HAEMATOLOGICAL PARAMETERS OF RABBIT BUCKS ADMINISTERED *Cissus quadrangularis* SUPPLEMENT DURING THE HOT-DRY SEASON

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DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION

FACULTY OF VETERINARY MEDICINE

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN THERIOGENOLOGY AND PRODUCTION

DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION

FACULTY OF VETERINARY MEDICINE

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

NOVEMBER, 2017

DECLARATION

I declare that the work in this thesis entitled “Semen characteristics, gonadal sperm reserves, testicular histology and haematological parameters of rabbit bucks administered*Cissus quadrangularis* supplement during the hot-dry season” has been carried out by me in the Department of Theriogenology and Production, Ahmadu Bello University Zaria. 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 this or any other institution.

Hyacinth Ndabatsado KOLO	----------------------------	--------------------------
Name of Student	Signature	Date

CERTIFICATION

This thesis entitled, “SEMEN CHARACTERISTICS, GONADAL SPERM RESERVES, TESTICULAR HISTOLOGY AND HAEMATOLOGICAL PARAMETERS OF RABBITBUCKS ADMINISTEREDwith *Cissus quadrangularis*SUPPLEMENT DURING THE HOT-DRY SEASON” by Hyacinth Ndabatsado KOLO, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. E. K. Bawa -------------------------------- ------------------------------
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DEDICATION

This thesis is dedicated to God Almighty for his grace and fulfillment of His purpose over my life.

To my parents Chief and Mrs. Pius. N. Kolo for being the best that has happened to me.

Finally, in loving memory of my brother and best friend, Joseph Lawal Kolo.

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ABSTRACT

This study evaluated the effect of *Cissus quadrangularis* supplement on the semen characteristics, gonadal sperm reserves, testicular histology and haematological parameters of New Zealand rabbit bucks during the hot-dry season in Zaria, Nigeria. Twenty four rabbit bucks weighing an average of 2.2 ± 0.5 kg were randomly distributed into four groups (A, B, C and D) of six bucks each: A served as the control, while B, C and D were the experimental groups and administered with 125 mg/mL, 250 mg/mL and 500 mg/mL of the supplementrespectively for 12 weeks. The data collected during the period of study were mean dailyambient temperature and relative humidity, weekly semen samples and live body weight, bi-weekly blood samples for haematology and extraction of serum for analysis of testosterone and antioxidant biomarkers. They were evaluated for eight weeks pre-administration and 12 weeks post-administration. Thereafter, two bucks were humanely euthanized from each group for histological examination of the testis and gonadal and epididymal sperm reserves. The mean sperm volume, motility, concentration, pH, live ratio, spermatozoa morphological defects, gonadal and epididymal lengths, weights and sperm reserves of the experimental groups did not differ ($P > 0.05$) significantly, when compared with the controls. Also haematological parameters (PCV, RBC, WBC and haemoglobin values) did not differ significantly ($P > 0.05$) between the groups. The mean testosterone level ($P = 0.0197$ and 0.0107), antioxidant biomarker – mean of superoxide dismutase (SOD) ($P = 0.0038$) and malondialdehyde concentration ($P = 0.0055$) were significantly differentfrom the controls. Vacuolations and hyperplastic Leydig cells were observed inall treated groups,indicative of testicular degeneration. It was concludedthat the supplement of *Cissus quadrangularis* has some ameliorative effects on semen characteristics, gonadal sperm reserves, testicular histology and haematological parameters of New Zealand white rabbit bucks.

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Abbreviations

ANOVA	One-way analysis of variance
ATP	Adenosine triphosphate
AV	Artificial vagina
CAT	Catalase
CCl_4	Carbon tetrachloridee
CQ	*Cissus quadrangularis* Linn
DNA	Deoxyribonucleic acid
DTNB	Dithiobisnitrobenzoic acid
ER-α	Estrogen receptors-alpha
ER-β	Estrogen receptors-beta
FSH	Follicle-stimulating hormone
GPx	Gluthatione peroxidase
GST	Glutathione-S-transferase
h	Hour(s)
H_2O_2	Hydrogen peroxide
IRRG	International rabbit reproduction group
kg	Kilogram
LH	Lutinising hormone
MCF	Michigan Cancer Foundation
MDA	Malondialdehyde
NAPRI	National Animal Production Research Institute
NO	Nitric oxide
OH^-	Hydroxyl radical
O_2^-	Superoxide
POP	Phytosterol oxidation products
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SEM	Standard error of mean
SOD	Superoxide dismutase
TBA	Thiobabituric acid
TCA	Trichloroacetic acid
THI	Temperature-humidity index
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Plants have been an important source of quality medicine for thousands of years, mainly on traditional remedies and as folk medicines (Sathyaprabha *et al*., 2010). In Africa, phytotherapy still plays an important role in the management of diseases; especially among very low income populations where traditional medicine is used and relies on the use of a wide variety of plant species (Geoffrey and Kirby, 1996).

Cissus quadrangularis Linn (CQ) is one of the plant species widely used in folk medicine, belonging to the kingdom: *Plantae*; Division: *Magnoliophyta*; Class: *Magnoliopsida*; Order: *Vitales*; Family: *Vitaceae*; Genus: *Cissus*; Species: *quadrangularis*. Few other important species are *C. populnea, C. adnata, C. discolor, C. pallida*. CQ is a perennial climbing shrub with stout fleshy jointed quadrangular stems; simple, long and slender tendrils (Plate I); normal roots with some aerial roots arising from the jointed nodes grow downwards; small, greenish flowers; ovoid or globose fruits (red berries) and ellipsoid seeds (Sala, 1994). The stout fleshy stem of CQ is an edible plant part found throughout the tropical parts of India, Sri Lanka, Malaya, West Africa and Ceylon with hot climates (Udupa *et al*., 1970) and also found throughout Thailand (Pongboonrod, 1995). It is commonly known as “bone setter”; the plant is referred to as “Asthisamdhani” in Sanskrit; “Hadjod” in Hindi because of its use in healing of fractures (Shirwaikar *et al*., 2003); “Dodoriya” in Hausa (Blench, 2007) and “Nanigi” in Nupe. The plant is used as a common food supplement in southern India (Jainu *et al*., 2006c) and Sri Lanka, where the green stems are fried or curried before consumption (Sivarajan and Balachandran, 1994). It is widely used in Ayurvedic medicine for gout, syphilis, venereal diseases, piles, tumours, haemorrhoids, peptic ulcers, leucorrhoea, and aphrodisiac and in the Siddha system of medicine for the treatment of piles, diarrhoea and dysentery (Warrier *et al*., 1994; Yoganarisimhan, 2000; Shirwaikar *et al*., 2003; Nagani *et al*., 2011). The stem is bitter and the stem juice is used for treatment of scurvy, irregular menstruation, otorrhoea and epistaxis (Deka *et al*., 1994). The roots and stems are most useful for treatment of fracture of the bones (Kumbhojkar *et al*., 1991).

The sap with tamarind has been reported in East Africa for the treatment of gonorrhea (Burkill, 2000). The herb is fed to cattle to induce milk let-down. The ash of plant is used as a substitute for baking powder (Anon, 1992).A paste of the stem is given as a remedy for asthma, burns and wounds, bites of poisonous insects and for saddle sores of horses and camels (Anon, 1992; Sharma *et al*., 2001).The dried and powdered leaves and young shoots are reportedly used as powerful alternative medicines in certain bowel infections connected with indigestion (Rastogi *et al*., 1993).The leaves, stem and roots are also reported to be used traditionally for helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, haemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellings. The stem is used for treatment of gastritis, constipation, eye diseases, pile, anaemia, fertility (Quattrocchi, 2012) and the Rongas of East Africa apply the pounded stem to wounds (Asolkar *et al.*, 1992; Kirtikar and Basu, 1996).

Phytochemical studies of CQ have shown several phytochemical constituents such as ascorbic acid, carotene, anabolic steroids, calcium, β-sitosterol, δ-amyrin, δ-amyrone, flavonoids, triterpenoids, alkaloids, tannins, carotenes, resveratrol, piceatannol, pallidol, parthenocissin, quadrangularins, phytosterol substances,antioxidant enzymes, nicotinic acid, tyrosin and linoleic acid (Bhutani *et al*., 1984, Gupta and Verma, 1990, Saburi *et al*., 1999; Mehta *et al*., 2001; Murthy *et al*., 2003; Joseph and Raj, 2011) and various secondary metabolites (Sen, 1964, Sen, 1966; Adesanya *et al*., 1999). Pharmacological studies of the fresh leaves and roots have shown that CQ has antioxidants, antibacterial, analgesic and neurosedative activities (Amos *et al*., 2001, Murthy *et al*., 2003; Viswanatha *et al*., 2006).

1.2 Statement of Research Problem

Cissus quadrangularis has been associated with a myriad of traditional and medicinal uses in different parts of the world. In Nigeria, the aqueous extract of the stem bark is reported to have aphrodisiac/fertility potentials by the Yoruba and Nupe tribes of Nigeria. Men are reported to consume the aqueous and ethanolic extracts copiously and consistently for prolong periods either in monoherbal or polyherbal formulations for aphrodisiac purposes (Ojekale *et al*., 2006). The use of this plantas an aphrodisiac and fertility enhancer by males has been attributed to the declining fertility in males and increasing levels of erectile dysfunction (Joint Report, 2004). Previous studies have focused on other pharmaceutical and medicinal uses (Iwe *et al*., 2004).

1.3 Justification of the Study

Cissus quadrangularis has been reported widely in India, Africa and other Asian countries because of health and medicinal benefits associated with it. Despite its growing acceptability to man, no study has been carried out to the best of our knowledge to ascertain its effect on reproductive parameters. Few studies have reported on its effect on male as an aphrodisiac (Aswar *et al*., 2010; Anon, 2016). The herbal dietary supplement (Super Cissus RX®) of CQ have been reported as a supplement to improve male sexual performance with an improved erectile function, a decrease in premature ejaculation, low libido and other symptoms of sexual dysfunction (Anon, 2016).However, its effects on spermatogenesis have not been reported to the best of our knowledge, as there is limited information on the effect of CQ on reproductive organs and reproduction parameters in animals and human particularly in the male. There is also a paucity of information on male reproductive hormones (Chopra *et al*., 1976). Therefore,this study may enable us to investigate the effect of the dietary supplement of CQ (Super Cissus RX®) on reproductive parameters of male rabbit and to establish baseline data for future uses of the plant extract for enhancing reproduction in livestock.

1.4 Aim of the Study

The aim of the study was to evaluate the effects of supplement of *Cissus quadrangularis* (Super Cissus®) on some reproductive parameters and haematological parameters of rabbit bucks during the hot-dry season.

1.5 Objectives of the Study

i. To evaluate the effect of CQ supplement (Super Cissus®) on semen characteristics,gonadal and extragonadal sperm reserves of rabbit bucksduring the hot-dry season.
ii. To determine the haematological parameters and testosterone profile of rabbit bucks following administration of CQ supplement (Super Cissus®) during the hot-dry season.
iii. To evaluate serum antioxidant biomarkers of rabbit bucks following administration of CQ supplement (Super Cissus®) during the hot-dry season.
iv. To determine histological changes in the testis of rabbit bucks following administration of CQ supplement (Super Cissus®) during the hot-dry season.

1.6 Research Questions

Does the dietary supplement (Super Cissus®)of *Cissus quadrangularis* have any effects on semen characteristics, gonadal, extragonadal sperm reserves, haematological parameters and testicular histology of rabbit bucks during the hot-dry season?

Plate I: The plant*Cissus quadrangularis*from Doko district of Lavun Local Government, Niger state, Nigeria, taken 23rd August, 2016.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Medicine

Knowledge can be derived from scientific or traditional sources (Santos García-Alvarado *et al*., 2001), traditional knowledge has been described as a cumulative body of knowledge, practice and belief, evolving through adaptive processes and handed over through generations by cultural transmission (Berkes *et al*., 2003). Traditional medicine is an ancient medical practice used throughout the world as it is heavily dependent on locally available plant species and plant based products and capitalizes on traditional wisdom-repository of knowledge (Awas and Demissew, 2009). Many cultures around the world rely heavily on traditional medicine as alternative medicines for the treatment of certain type of diseases (Barnes *et al.*, 2008), and the wide spread use could be attributed to cultural acceptability, economic affordability and easy accessibility. It is the easy source of treatment in the primary health-care system of resource poor communities around the world and efficient against certain type of diseases as compared to modern medicines. Thus, different local communities in countries across the world have indigenous experience in various medicinal plants, where they use their perceptions and experiences to categorize plants and plant parts to be used when dealing with different ailments (Omoruyi *et al*., 2012).

The World Health Organisation (WHO, 2008) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, 2008). The WHO estimates that about 80 percent of world population relies on traditional medicine for primary health-care, the use medicinal plants in the treatment of diseases (WHO, 2001). Available reports show that more than 300 distinct ethnic groups in Nigeria have their own unique indigenous healing heritage, which have evolved in response to the specific experiences and needs of the people. Currently, it is estimated that traditional medicine is the only health-care resource accessible to a third of all Nigerians (Ogunbodede, 1997).

During the past decades, the developed world has also witnessed an ascending trend in the utilisation of contemporary alternative medicine, particularly herbal remedies (Chintamunnee and Mahomoodally, 2012). It is interesting to note that 25 per cent of modern medicines are derived from plants that were used traditionally. Many of the current drugs either mimic naturally ocuring molecules or have structures that are fully or in part derived from natural motifs (Yates, 2002; Joseph and Raj, 2010a). In the traditional system of medicine, various plants parts such as stem back, root back, aerial root, vegetative bud, leaves, fruits and latex are used in the treatment of variety of ailments (Davies and Evans, 2008).The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients (WHO, 2000).

Plants typically contain mixtures of different phytochemicals, also known as secondary metabolites that may act individually, additively, or in synergy to improve health. Indeed, medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of the individual constituents. The combined actions of these substances tend to increase the activity of the main medicinal constituent by speeding up or slowing down its assimilation in the body. Secondary metabolites of plant origins might increase the stability of the active compound(s) or phytochemicals, minimise the rate of undesired adverse side effects and an additive, potentiating, or antagonistic effect. It has been postulated that the enormous diversity of chemical structures found in these plants is not waste products, but specialized secondary metabolites involved in the relationship of the organism with the environment(Chintamunnee and Mahomoodally, 2012). For example, attractants of pollinators signal products, defensive substances against predators and parasites, or in resistance against pests and diseases. A single plant may, for example, contain bitter substances that stimulate digestion and possess anti-inflammatory compounds that reduce swellings and pain, phenolic compounds that can act as an antioxidant and venotonics, antibacterial and antifungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste products and toxins, and alkaloids that enhance mood and give a sense of well-being (Gurib-Fakim, 2006; WHO, 2008; Chintamunnee and Mahomoodally, 2012;Nunkoo and Mahomoodally, 2012; Shohawon and Mahomoodally, 2013).

Some may view the isolation of phytochemicals and their use as single chemical entities as a better alternative to the use of plant extracts, however there is the view that there may be some advantages of the medical use of crude and/or standardized extracts as opposed to isolated single compound. Many traditional medicines and herbal preparations come in the crude form of plant as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1997; Samuelsson, 2004). Most plant-derived drugs were originally discovered through the study of herbal cures and folk knowledge of traditional people (Gilani andAtta-ur-Rahman., 2005).

2.2 Plants from the Genus Cissus

The genus *Cissus* contains approximately 300 species distributed in all major tropical regions (Wen, 2007a;Xiu-Qun *et al*., 2013) and represents the largest of the 14 genera of *Vitaceae* (Lombardi, 1997, 2007; Wen, 2007a; Wen *et al*., 2007). The genus has about 135 species in Africa, 85 species in Asia, 12 species in Australia, and 65 species in the Neotropics (Wen, 2007a). Many cultures in Asia, both East and West Asia, have used locally available species of *Cissus* to treat several medical problems. In China and the Far East, *C. assamica*, is used as anti-snake venom (Yang *et al*., 1998), while in South-East Asia, including the Indian subcontinent and Sri

Lanka, *Cissus quadrangularis* is used for fracture treatment (Udupa and Prasad, 1962) and as an anti-obesity agent (Oben *et al*., 2006). Several countries in Africa use different species of *Cissus* in their traditional medicinal practices: In Cameroon, *C. aralioides* is used as anti-microbial and toxicological agent against microorganisms of the gastrointestinal and urogenital tracts (Assob *et al*., 2011). In Gabon, alcoholic extracts of *C. debilis* showed antiproliferative activity on human colon cancer cell line (CaCo-2 cells) (Line-Edwige *et al.*, 2009). In Nigeria, few species such as *C. populnea, C. ibuensis and C. quadrangularis* are used as native medicine; *C. populnea* has anti-sickling, anti-bacterial properties (Moody *et al.*, 2003; Kone *et al*., 2004) and for treatment of trypanosomiasis (Atawodi *et al*., 2002).

Methanolic extracts of *C. populnea* have been reported to increased proliferation of sertoli cells TM4 in in vitro studies (Osibote *et al.*, 2011) but not in humans treated for 72 days (Ojekale *et al*., 2006). *C. populnea* had no adverse side effects after long term administration to Rabbits (Ojekale *et al*., 2007); *C. ibuensis* is used to treat gastrointestinal problems (Irvine, 1961), rheumatism and arthritis (Dalzeil, 1958). In Congo, *C. rubiginosa,* is used for the treatment of dysentery and diarrhoea (Otshudi *et al*., 2000). *Cissus rotundifolia* from Africa and Asia shows anti-diabetic (Onyechi *et al*., 1998) as well as anti-parasitic properties against intestinal parasites (Alzoreky and Nakahara, 2003). In Australia, Bush Medicine Practitioners use *C. hypoglauca* to treat sore throat (Lassak and McCarthy, 1997). In the Caribbean islands of Trinidad and Tobago, *C. verticillata* is used as an anti-diabetic agent and to treat urinary problems (Lans, 2006). In Brazil, *Cissus sycoides* is commonly used as vegetal insulin (Salgado *et al*., 2009). Of these reports, the most studied are *C. quadrangularis* for obesity, treatment of bone fracture and other bone related conditions (for example Arthritis) and *C. sycoides* as an anti-diabetic agent.

2.3 Physical Description of *Cissus quadrangularis*

Cissus quadrangularis is a traditional medicine plant reported to come from Ayurveda (an Indian folk medicine) but appears in other locations worldwidewhich have used it medicinally. It is a perennial succulent vine found in the tropics and subtropics of Asia and Africa belonging to the family *Vitaceae* (Kothari *et al*., 2011; Rasale, 2014; Suhashini and Helan Chandra, 2015). The plant grows best in warm tropical climate and it survives throughout the year in different seasons. It can be cultivated in plains coastal areas, jungles and wastelands up to 500 m elevation. It is planted as hedges and boundary walls of gardens, farms and cultivated fields and also in pots (Atram, 2015). The plant occurs as pieces of varying lengths; stem quadrangular, 4-winged, internodes 4-15 cm long and 1-2 cm thick. The surface is smooth, greenish tinge colour, angular portion reddish-brown; no taste and odour. The leaves are simple 2.5-5 cm long, broadly ovate, sometimes 3-7 lobed and 3-foliate, rounded edges, truncate at the base; petioles 6-12 mm long; stipules small. Flowers are small greenish white, bisexual, tetramerous, opposite to the leaves andare in shortly peduncle cymes with spreading umbellate branches. Calyx is cup shaped, truncate or very obscurely lobed. Petals are 4, ovate-oblong, short, stout. Fruit is ovoid fleshy berries,scarcely 6 mm, long apiculate, red when ripe and 1- (very rarely 2) seeded (Rajpal, 2005; Rasale, 2014; Nathar and Yatoo, 2015).

2.3.1 Classification of the plant (Taxonomy)

Kingdom:*Plantae* or green plants

Subkingdom:*Tracheobionta*: Vascular plants

Super division:*Spermatophyta*: Seed bearing plants

Division:*Magnoliophyta* (Flowering Plants)

Class:*Magnoliopsida*

Subclass:*Rosidae*

Order:*Vitales*

Family:*Vitaceae*

Genus:*Cissus* L.

Species:*quadrangularis* Linn. (Veldt-grape) Stems and branches acutely winged

Botanical name:*Cissus quadrangularis* Linn.

Classical Name: Asthisamhari (Raj and Joseph, 2011; Shah, 2011).

Other names: The plant is commonly known in English as, Veld grape, Veldt grape, Edible stemmed vine or bone setter. The Hindi name, *Hadjod* (Bone setter) is given because of its ability to join bones (Shirwaikar *et al*., 2003); Sanskrit names, *Asthisamhrta*, *Asthisamhaara,Asthi-samyojaka* also explain its bone setting properties (Sivarajan and Balachandran, 1994). It is also referred by other names like Vajravalli and Chaturdharaa (four liners). Other vernacular names include, *Mangaravalli* in Kannada, *Nelleru* in Telgu, *Perandai* in Tamil, *Hasjora* in Bengali and *Chaudari* or *Kandavela* in Marathi, *Dodoriya* in Hausa (Blench, 2007) and *Nanigi* in Nupe.

2.3.2 Morphological characters

Cissus shows remarkable morphological diversity (Jackes, 1988; Lombardi, 2007), and is generally characterized by well-developed thick and undivided floral disks, four-merous flowers, one-seeded fruits, and seeds with a long and linear chalaza (Descoings, 1960; Wen, 2007a; Chen and Manchester, 2011). It is a low-growing shrub with a characteristic, four-sided stem. It is a climbing plant, often found growing over lower-growing vegetation (Raj and Joseph, 2011).

2.3.3 Geographic distribution

Cissus quadrangularis is a vining plant native to Asia and Africa. It grows as a wild plant throughout the tropical warmer parts of India, Sri Lanka, Pakistan, Bangladesh, and Malaysia. It is also found in Tropical Africa in Nigeria, Cameroun to Namibia, South Africa, and Madagascar;

naturalized in Thailand, Java and the Philippines (Mabberley, 2008; Shah, 2011). It is also spread in drier parts of Arabia (Sivarajan and Indira, 1996).

2.4 Phytochemical Constituents

The phytochemical studies of CQ have shown the presence of various versatile constituents such as flavanoids (quercitin and quercitrin), triterpenoids, ascorbic acid (vitamin C), stilbene derivatives (quadrangularins A, B and C), anabolic steroids, phytosterol (β-sitosterol, ketosteroids, daucosterol, d-amyrin, d-amyrone, tarexerol and freidalin) and many others, e.g. resveratrol, piceatannol, pallidol parthenocissin, balsam ketone, and amyrin (Shirley and Sen 1966; Bhutani *et al*., 1984; Adesanya *et al*., 1999; Enechi and Odonwodo, 2003; Jainu and Devi, 2004; Singh *et al*., 2007). Others are two iridoids 6-O-[2, 3-dimethoxy]-Trans-cinnamoyi catalpol and 6-O-meta-methoxy-bezoyl catalpol (Singh *et al*., 2007) and acetyl taraxerol and lipids (Gupta and Verma, 1991). Out of which ascorbic acid, triterpene, β-sitosterol, ketosteroid, two asymmetrical tetracyclic triterpenoids and calcium were identified as major constituents of this plant (Ghouse, 2015).

The presence of phytochemicals confers different potential metabolic and physiological effects to plants (Jakikasem *et al*., 2000; Jainu and Devi, 2004). It is unclear specifically which chemical constituents are responsible for the physiological effects produced by CQ extracts. Most *Cissus* extracts are frequently standardised to 2.5 or 5% ketosteroids and the assays commonly used to determine ketosteroid levels are non-specific, and as a consequence, the actual level of ketosteroids present in *Cissus* extracts may be much lower than stated (Bhutani *et al*., 1984; Mehta *et al*., 2001; Singh *et al*., 2007; Chi *et al*., 2010;Mishra *et al*., 2010; Rao *et al*., 2011; Aswar *et al*., 2012).

Analysis of the air-dried CQ plant reported to contain moisture 13.1, protein 12.8, wax 1.0, fiber 15.6, carbohydrate 36.6, mucilage and pectin 1.2 and ash 18.2%. The root powder contain a rich source of mineral elements (mg/100g dry matter): potassium 67.5, calcium 39.5, zinc 3.0, sodium 22.5, iron 7.5, lead 3.5, cadmium 0.25, copper 0.5 and magnesium 1.15 (Mishra *et al*., 2010). Analysis of the toxicants revealed the presence of oxalate, tannin, phytate, saponin contents (135, 0.3, 20, 0.16mg/100 g of dry matter, respectively) (Mishra *et al*., 2010). The ash formed from the plant contains mostly carbonates and to a smaller extent phosphates of sodium, potassium, magnesium and calcium. Presence of potassium tartarate is also reported.Stem of the plant is reported to contain a water-solukble glycoside, which produces a fall in blood pressure in anaesthetised cats. Fresh stem of CQ produces irritating action on the skin, which may be attributed to the presence of calcium oxalate and 31 methyl tritiacontanoic acid along with taraxeryl acetate, taraxerol and iso-pentacosanoic acid. The stem extract ofCQ plant contains a high percentage of calcium ions (4% by weight) and phosphorous (Mishra *et al*., 2010). The nutrient composition of dried CQ stem shows (1.451) phytosterols, (0.212) ascorbic acid, (0.488) phenols, (9.03) calcite, (0.205) iron, (0.175) selenium (g/100g) on dry weight basis (Karadbhajne *et al*., 2014).

2.4.1 Phytosterols

Phytosterols are a group of naturally-occurring compounds present in the lipidic portion of plants for a variety of biological purposes (Couto *et al*., 2014; Dykstra *et al*., 2015). As one type of plant-derived natural compounds, they are triterpenoids that serve the same function in plants as the animal steroid and cholesterol (Bowsher *et al.,* 2008) through the stabilisation of phospholipid bilayers in cell membranes and also due to their inherent molecular structure, and close structural similarity to cholesterol only by minor modifications with an extra methyl or ethyl group (Kmiecik *et al*., 2011; O'Callaghan *et al*., 2014). Given their similarities in molecular structure, it is not surprising that many of the biological functions of phytosterols in plants mirror the functions of cholesterol in humans (Piironen *et al*., 2000; Wong, 2014) and animals (Akhisa and Kokke, 1991). Phytosterols are usually not synthesised in the human body so that they are completely derived from dietary sources. Appreciable amounts of phytosterols are found in lipid-rich plant foods, that is, vegetable oils, nuts, legumes and other edible seeds, whereas cereal grains, fruits and vegetables contribute to a certain extent to the daily intake of these phytoconstituent (Piironen and Lampi, 2004; Jiménez-Escrig *et al*., 2006) and also contained in herbal food plants e.g. *Parax ginseng* (Lee *et al*., 2004), *Carthamus tinctiris* L (Hamrouni-Sellami *et al*., 2007) as well as plants of the family *Vitaceae* such as *Vitis vinifera* (Ruggiero *et al*., 2013) and *Cissus quadrangularis* (Sharma *et al*., 2011). More than 40 phytosterols have been identifiedin many plant species, from themβ-sitosterol, campesterol, and stigmasterol account for more than 95% of total phytosterol with β-sitosterol as the most abundant (Calpe-Berdiel *et al*., 2009; Dykstra *et al*., 2014).

Phytosterols are reported to have cholesterol-lowering properties, anti-atherogenic and cardioprotective effects (Trautwein and Demonty, 2007), anticancer, anti-inflammatory and immunoregulatory activities (Ling and Jones, 1995; Woyengo *et al*., 2009; Jones and Abumweis, 2009; Carr *et al*., 2010; MacKay and Jones, 2011) and act as a chemo-preventive agent (Paniagua-Pérez *et al*., 2008). Other effects include endothelial function, oxidative stress and antioxidant status, coagulation and platelet aggregation, inflammation, neurocognitive function, eye disease and anti-osteoarthritic (Brüll *et al*., 2009; Jones and Abumweis, 2009; Derdemezis *et al*., 2010; Gabay *et al*., 2010; Rudkowska, 2010). However, research into their potential role in reproduction, and specifically male fertility and spermatogenesis, has received comparably little attention. Some studies have suggested that the effect of phytosterol on reproduction could be one of reproductive enhancement (Nieminen *et al*., 2004; Ryokkynen *et al*., 2005b) possibly due to the modification of β-sitosterol into sex steroids (Moghadasian, 2000). On the contrary, in some studiesthe effects β-sitosterolseems to be minor and probably harmless.For example, no responses have been noted in mice after a five-generation study (Ryokkynen *et al*., 2005a), β-sitosterol caused transitory decreases in plasma estradiol levels in female raccoon dogs (*Nyctereutes procyonoides*; Nieminen *et al*., 2003), increased estradiol concentrations in European polecats (*Mustela putorius*; Nieminen *et al*., 2002) and slightly increased litter size without clear effects on plasma sex steroid levels in female American mink (Ryokkynen *et al*., 2005b).

In European polecats (*Mustela putorius*), phytosterol exposure leads to increases in the circulating estradiol and thyroid hormone levels (Nieminen *et al*., 2002), which may be caused by phytosterol being used as precursors of sex steroids in gonads (Moghadasian, 2000). In fish, β-sitosterol caused decreased plasma sex steroid concentrations and increased vitellogenin expression (MacLatchy and Van Der Kraak, 1995; Mellanen *et al*., 1996). In rats (*Rattus norvegicus*) β-sitosterol reduced sperm count and testicular mass (Malini and Vanithakumari, 1991), while some tests have found no estrogenic effects of phytosterol in immature female rats (Baker *et al*., 1999). A reproduction study found no adverse effects of orally administered phytosterol esters in 2 successive generations of Wistar rats (Sanders *et al*., 2000). In a five-generation exposure of mice (*Mus musculus*) to β-sitosterol, it did not have any clear effects on fertility (Ryokkynen *et al*., 2005a). In contrast, the tundra vole (*Microtus oeconomus*)a small rodent with a circumpolar distribution (Tast, 1966)has potential as a bioindicator of possible endocrine disruption in arctic and boreal nature showed an increase in the circulating testosterone concentrations of male voles and the liver glycogen phosphorylase activities of both sexes following a two-week exposure to phytosterol (Nieminen *et al*., 2003) had an increased percentage of reproducing pairs after chronic per os β-sitosterol (Nieminen *et al*., 2004).

Phytosterols themselves usually have very limited antioxidant capacity. Currently due to growing concerns about the safety of the synthetic antioxidants, food scientists are interested to look for safer naturally derived alternatives. Therefore, it is important to incorporate phenolic acids with phytosterols through esterification and accordingly confer their excellent antioxidant activities to phytosterols (Fu *et al*., 2014). However, phytosterols undergo oxidation. The presence of double bonds in phytosterol molecules makes them sensitive to the effect of light, metal ions, pigments, enzymes and elevated temperature. Under the influence of these factors, phytosterol oxidation occurs, most frequently as a result of autoxidation reactions, leading to the formation of phytosterol oxidation products (POPs), such as 7-hydroxy, 7-keto, epoxy, 25-hydroxy and triols of sterols (Soupas, 2006). Studies have shown the possible biological effects of POP as cytotoxic effects(Meyer *et al*., 1998;Adcox *et al*., 2001;Maguire *et al*., 2003),mutagenic potential (Peterson*et al*., 1988;Cheng *et al*., 2005; Kothari *et al*., 2011), putative ill-effects (Suzuki *et al*., 2002; Ikeda *et al*., 2006)and possible endocrine-reproductive effects (Van den Heuvel*et al*., 2006;Christianson-Heiska *et al*., 2007) such as increased plasma sex steroid, acceleration of spermatogenesis in males, and increased ovarian atresia in females.However, the mechanism of toxicity and action are unknown.

2.4.2 Resveratrol

Resveratrol is a natural polyphenol that belongs to the chemical group of stilbenoids, low-molecular-weight phenol compounds, characterised by two aromatic rings linked by an ethane or ethylene residue (Risuleo, 2016). Resveratrol was originally found in the berries of the wine grape (*Vitis vinifera*), but it is also present in the roots, seeds, and stalks of the plants (Eleawa *et al*., 2014). This makes it available in plantsof the family *Vitaceae*, which *Vitis* and *Cissus* species belongs. The highest concentration is found in the peel of the berries, although the content may vary significantly depending on the grapes type (Risuleo, 2016). It is found in numerous plant species and fruits which are part of the human diet; including mulberries, bilberries, blueberries, cranberries, peanuts and other nuts, seeds, legumes and can be extracted from several other natural sources (Kundu *et al.*, 2006; Goswami and Das, 2009) and particularly in dried roots of plant *Polygonum cuspidatum* (Jang *et al*., 1997; Mukherjee *et al*., 2010; Hu *et al*., 2013), but is relatively low in fruits and vegetables (Soleas *et al*., 1997).

Many reports have shown that resveratrol possesses cardiovascular protective (Hung *et al*., 2000), antiplatelet (Kirk *et al*., 2000), antioxidant (Valdecantos *et al*., 2010), anti-inflammatory (De la

Lastra and Villegas, 2007) blood glucose-lowering (Sadi *et al*., 2014) and anticancer (Vanamala *et al*., 2010) activities.By increasing the production of nitric oxide, resveratrol inhibits platelet aggregation and stimulates vasodilation (Cucciolla *et al*., 2007). Reports have shown that resveratrol protects against some neurodegenerative diseases, such as Alzheimer's disease (Sun *et al*., 2010), and obesity (Alberdi *et al*., 2011; Lasa *et al*., 2012) as well as is effective in the management of osteoporosis in post-menopausal women without an increased risk of breast cancer (Su *et al*., 2007).

In reproduction, it has phytoestrogenic properties by binding to estrogen receptors alpha and beta (ER-α and ER-β) with similar affinities, but this interaction is 7000 times less powerful than that of estradiol (Bowers *et al*., 2000). Molecular studies have shown that the union of resveratrol to ER-α is stereoselective, that is, that the trans-isomer shows more affinity for this receptor than the cis-isomer (Abou-Zeid and El-Mowafy, 2004). The chemical structure of resveratrol is similar to that of 17-β-estradiol or synthetic estrogens like diethylstilbestrol. Thus, several studies have been carried out in order to test its ability to act as a phytoestrogen (Gehm *et al*., 1997; Kopp, 1998; Ashby *et al*., 1999; Turner *et al*., 1999). Juan *et al*. (2005) were the first to find that long-term (90 days) oral trans-resveratrol supplementation (20 mg per kg of body weight) increases sperm production in healthy rats. They also noted a significant increase in serum concentrations of gonadotropins and testosterone (Juan *et al*., 2005). Another study conducted on mice given an oral dose of resveratrol (50 mg/kg) for 28 days also observed a 51.6% increase in blood testosterone concentrations (Shin *et al*., 2008).

2.4.3 Vitamin C

Water-soluble vitamins, such as vitamin C (ascorbate/L-ascorbic acid) and the B-complex vitamins are produced by plants and microorganisms (Engelking, 2015) for their normal metabolic functioning. It is an important antioxidant substance in biological systems (Duarte and Lunec, 2005). It is a water-soluble micronutrient, well absorbed by the gastrointestinal tract and required for multiple biological functions and biochemical reactions in humans and animals and it is an important element for the body (Li and Schellhorn, 2007). It has been shown to be important for reproduction in several other mammalian species (Luck *et al*., 1995) and earlier works showed a beneficial effect of subcutaneous injections of ascorbic acid to sub-fertile bulls (Phillips *et al*., 1940), cows (Phillips *et al*., 1941) and stallions (Ralston *et al*., 1988) by increased plasma and seminal concentrations as well as improved their fertility.

In the male reproductive system, vitamin C is known to protect spermatogenesis and it plays a major role in semen integrity and fertility both in men (Agarwal *et al*., 2005; Eskenazi *et al*., 2005) and animals, increases testosterone levels (Sönmez *et al*., 2005) and prevents sperm agglutination. It has also been reported to be associated with fertility as it is very concentrated in the epididymal fluid and seminal plasma. Vitamin C intake closely associates with sperm numbers, concentration and motility. Its protective role within the epididymis is manifested through its reductive property which prevents sperm from oxidative impairment of DNA, thereby maintaining the genetic integrity of sperm (Begum *et al*., 2009). Exogenous supplementation with ascorbic acid brings a marked increase in the concentration of ascorbic acid in the testes and blood plasma, while a significant reduction level of lipid peroxidation is observed in these locations (Cheah and Yang, 2011).

Vitamin C supplementation decreased free radical formation in human seminal plasma (Dawson *et al*., 1992) and decreased the incidence of secondary sperm abnormalities by reducing free radical formation (Sikka, 1996). Vitamin C is also involved in the synthesis of sex steroids such as testosterone, and peptide hormones; hydroxylation of steroids is especially vitamin C-dependent (Luck *et al*., 1995; Weber *et al*., 1996). The beneficial effects of vitamins for a suitable development of the male reproductive system are widely studied and are mostly emphasised with respect to their oxidative resistance. It remains unknown whether excessive vitamin intake may bring deleterious effect to sperm and testicular function and the molecular mechanisms of vitamins in improving spermatogenesis and testicular development are yet little understood (Cheah and Yang, 2011).

2.5 Secondary Metabolites

Secondary metabolites, which may also be referred to as phytochemicals, are chemicals produced by means of secondary reactions resulting from primary carbohydrates, amino acids and lipids (Kayani *et al*., 2007). Such metabolites are known to participate in plant defence mechanisms (against herbivores, pathogens, and allelopathy) by their repellent or attractive properties, protection against biotic and abiotic stresses - which includes adaptation to changing environments and the maintenance of structural integrity (Edvera *et al*., 2008; Achakzai *et al.,* 2009; Sa` *et al*., 2009). The most common classes of these chemicals are saponins, tannins, anthraquinones, flavonoids, and alkaloids which are widely distributed amongst various plant families in abundant quantities. It is these secondary metabolites which attract so much attention from biological scientists due to their ability to inhibit the growth of microbes pathogenic to man (Pereira *et al*., 2009).

2.5.1 Alkaloids

Alkaloids are one of the most abundant and diverse group of secondary metabolites found at a minimum concentration in almost all plants (Kumar and Sachin, 2013). Alkaloids are naturally occurring, nitrogen-containing organic compounds with the exception of amino acids, peptides, purines and derivatives, amino sugars, and antibiotics (Wansi *et al*., 2013). The majority of alkaloids are true alkaloids which are derived from alpha-amino acid precursors. Other alkaloids derived from terpenes and steroids are named pseudo-alkaloids because the relatively late amination process occurs in a transamination reaction by donating a nitrogen atom of an amino acid source (Wansi *et al*., 2013).

Alkaloids have complex molecule structure and they have significant pharmacology activities. They are widely distributed in higher plants and are used as phytomedicine or as weapons toxins. Some alkaloids focus their range in a particular part of the body, though many of them can act in several fronts at once. They have also been reported in lower plants, insects, marine organisms, and microorganisms. Alkaloids are known to display arrays of pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals but many of them are toxic to other organisms. They are local anesthetics and stimulants (cocaine), psychedelics (psilocin), stimulants (caffeine, nicotine), analgesics (morphine), antibacterials (berberine, kokusaginine, nkolbisine), anticancer drugs (vinblastine, vincristine), antihypertensive agents (reserpine), cholinomimerics (galantamine), spasmolysis agents (atropine), vasodilators (vincamine), antiarrhythmia (quinidine), antiasthma therapeutics (ephedrine), antimalarials (quinine) (Kuete *et al.*, 2008; Kuete and Efferth, 2010; Wansi *et al.*, 2013; Zofou *et al.*, 2013).

Some alkaloids like caffeine exert stimulating role on nervous system and ricinine leads to toxicity in digestive system causing violent irritation with typical manifestations such as diarrhoea, vomiting etc. Most of the alkaloids act as antibiotics (García *et al.*, 2011) and inhibits the translation process in various organisms. Alkaloids also possess antimitotic (Tits *et al.*, 1984) and allergic (Chukaew *et al.*, 2008) effects at cellular level. These functions of alkaloids are well known for their toxic (Verissim *et al.*, 2011) and sometimes psychomimmetic (for example, Ergot), euphoric (for example, Harmala), and hallucinogenic (for example,tryptamine alkaloids) properties. They also possess hypoglycemic effects (Nguyen *et al.*, 2012; Wang *et al.*, 2012; Zhang *et al.*, 2012), antibacterial and antiviral insecticidal effects (Meng, *et al.*, 2009; Atal *et al.*, 2012; Kaur *et al.*, 2013), anti-inflammatory (Lee *et al.*, 2012; Fu *et al.*, 2013) and central nervous system effects (Shi *et al.*, 2012; Farias *et al.*, 2012). Many alkaloids, though poisonous, have physiological effect that renders them valuable medicine against various diseases including malaria, diabetics, cancer and cardiac dysfunction. These are also used in local anaesthesia and relief of pain while some influence the reproductive system of animals such as antifertility, antiovulatory, contraceptive andabortifacient, antiprogesterogenic activity, anti-implantation effects, antispermatogenic and antisteroid-ogenic activities(Takrouri *et al.*, 1999).

2.5.2 Flavonoids

Flavonoids are extremely common and widespread in the plant kingdom. They function as plant pigments and are responsible for the colours of many flowers and fruits (Bone and Miles, 2013). Compared to other active plant compounds, they are low in toxicity. A number of types of flavonoids have been shown to have different pharmacological effects such as antioxidants (Fremont *et al.*, 1998; Cazarolli *et al.*, 2008),hepatoprotective and antiulcer (Mota *et al.*, 2009; Zhu *et al.*, 2012), antibacterial (Mishra *et al.*, 2013), analgesic activity *in vivo* (Havsteen, 2002),anti-inflammatory effects (Tunon *et al.*, 2009; Serafini*et al.*, 2010), anticancer (Elangovan *et al.*, 1994; Koen *et al.*, 2005), antiviral (Zandi *et al.*, 2011).Various types of flavonoids have been known to have positive and negative effects on male reproduction. Flavonoids have been shown to produce anti-androgenic activity and affect male fertility in dogs (Bhargava, 1989) whereas coumarin, a well-known liver toxicant (Born *et al.*, 2000), has been reported to have antifertility activity in mature female rats (Ulubelen *et al.*, 1994). Sitosterol, a weak estrogenic phytosterol used for lowering cholesterol and treating benign prostatic hyperplasia (Kritchevsky and Chen, 2005), was reported to have caused decreased sex steroid concentrations in fish (MacLatchy and Van Der Kraak, 1995) and lowered sperm counts in rats (Malini and Vanithakumari, 1991). Chrysin used as treatment of male infertility (Ciftci *et al.*, 2012). Excessive dose of icariin may cause tissue and organ oxidative damage, consequently damaging reproductive functions (Chen *et al.*, 2014), while some others do not have no effect on male reproduction (Lucinda*et al.*, 2010; Becho *et al.*, 2016).

2.5.3 Saponins

Saponins are naturally-occurring surface-active glycosides with distinctive foaming characteristics found naturally in many plant species, including wild plants and cultivated crops (Francis *et al*., 2002 and Stegelmeier *et al*., 2013).They have long been used as herbal medicines (Ohtsuki, 1984) with numerous pharmacological actions (Van Heerden *et al*., 2007).There are several reviews on the biological actions of saponins (Yoshiki *et al*., 1998; Francis *et al*., 2002; Thakur *et al*., 2011; Begum *et al*., 2014; Soetan *et al*., 2014). They are generally considered non-toxic, however, under some poorly defined conditions and stress, saponin-containing plants have been associated with livestock poisoning.Saponins are commonlyas either triterpenoid or steroidal in character (Stegelmeier *et al*., 2013). The triterpenoid saponins are generally predominant in cultivated crops, while steroid saponins are common in plants used as herbs or for their health-promoting properties (Fenwick *et al*., 1991).A large number of the biological effects of saponins have been ascribed to their action on membranes and they possess surface active characteristics because of the amphiphillic nature of their chemical structure, which destroys the structural integrity of the cell membrane, increases its permeability (Ohtsuki, 1984) and alter cellular function (Stegelmeier *et al*., 2013).

Studies have shown the lytic action of saponins on erythrocyte membranes (Glauert *et al*., 1962; Segal *et al*., 1974; Segal and Milo-Goldzweig, 1975; Gee and Johnson, 1988; Yamasaki, 1996;Bone and Miles, 2013)and this property has been used for their detection. The haemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for membrane sterols, particularly cholesterol (Glauert *et al*., 1962) which is also present in sperm cells and they form insoluble complexes (Bangham and Horne, 1962).The interaction between saponins and membrane lipids thus seems to be complicated, with the composition of the target membrane, the type of side chain, and the nature of the aglycone to which these are attached all appearing to be necessary to produce a permeabilising effect (Gee *et al*., 1998; Attele *et al*., 1999). The precise details of the interactions between saponins and membranes need more elucidation so that the molecular mechanisms involved could be better understood (Francis *et al*., 2002).However, little is known regarding the function of steroidal saponins.

The negative effects of saponins on animal reproduction have long been known and have been ascribed to their abortifacient, antizygotic and anti-implantation properties (Tewary *et al*., 1973; Stolzenberg and Parkhurst, 1976). Saponins were found to be extremely strong stimulators of luteinising hormone release from cultured hypophysial cells (El Izzi *et al*., 1989; Benie *et al*., 1990) but their action was neutralised in the presence of serum indicating a passive membrane-permeabilising effect (El Izzi *et al.,* 1992). The steroid saponin was found to directly inhibit the genes responsible for steroidogenesis, and also suppress the proliferation of follicle-stimulating hormone-modulated granulosa cells in the ovarian follicle (Francis *et al*., 2002).

Saponins have also been shown to have both positive and negative effects on the viability of human sperm cells *in vitro* with some ginseng saponins increasing motility as well as progression of sperm (Chen *et al*., 1998) while *Sesbania sesban* saponins were spermicidal (Dorsaz *et al*., 1988). The *in vivo* effects of saponins on the reproductive functioning seem to indicate more than a simple permeabilising effect on secretary cell membranes and could possibly be linked to interactions between saponins and steroid receptors given the similarities between the basic chemical structures of saponins and steroid hormones (Francis *et al*., 2002). Little is known about CQ containing saponins and the mechanism of action in these in vitro effects of dietary saponins remains to be clarified as there are relatively few reports in this regard.

2.5.4 Anthraquinones

Anthraquinones are aromatic organic compounds and is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is fairly stable under normal conditions. Anthraquinones naturally occur in some plants, fungi, lichen and insects, wherein they serve as a basic skeleton for their pigments.Anthraquinone derivatives, among which emodine, physcione, aloe-emodine, Rheine and chrysophanol, are recognized as important biologically active components and their presence is oftenused as criteria in the quality control of plants used for medicinal purposes (Locatelli, 2011).Anthraquinones exert a wide range of biological activities including anti-fungal (Agarwal *et al*., 2000; Manojlovic *et al*., 2005; Mendoza *et al.*, 2005), anti-microbial (Ifesan *et al*., 2009;Miethbauer *et al*., 2009 and Yadav *et al*., 2010), anti-cancer (Chen *et al.*, 2007 and Park *et al*., 2009), antioxidant (Iizuka *et al*., 2004 and Cai *et al*., 2004), and anti-viral activities (Barnard *et al*., 1995), other than the well-known actions on the gastrointestinal apparatus (Zhang *et al*., 2005). There are little or no information on the effects of anthraquinones on animal reproduction.

2.5.5 Cardiac glycosides

Cardiac glycosides are component of plants that are used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants and in some animals. Some of these compounds are used as arrowhead poisons in hunting (Filippos *et al*., 2007).

2.5.6 Tannins

Tannins are plant secondary metabolites widely synthesized and distributed in many plant species. They play a role in plant growth regulation and also in protection from predation due to their astringent character rendering plant tissues inedible (Bone and Miles, 2013). Its polyphenolic compound binds toand precipitates proteins and various other organic compounds including amino acids and alkaloids (Bone and Miles, 2013; Ky *et al*., 2016). Indeed, tannins are divided into four groups with two majors: the condensed tannins or proanthocyanidins and the hydrolyzable tannins (Ky *et al*., 2016). Tannins have traditionally been considered antinutritional, but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage (Muller-Harvey and McAllan, 1992).

2.6 Medicinal Importance of *Cissus quadrangularis*

2.6.1 Traditional uses

It is a medicinal plant used in Ayurveda since the time of Bhavprakash Nighantu written by Acharya Bhavprakash in 16th century, the plant beneficial for healing the fracture of bone. The plant also documented in Ayurveda for treatment of osteoarthritis, rheumatoid arthritis and osteoporosis (Paulsen *et al*., 2007), scurvy, menstrual disorders, epistaxis (Anonymous, 2008). In East Africa it is used with tamarind for the treatment of gonorrhoea (Burkill *et al*., 2000). A stem paste is useful in burns, wounds, bites of poisonous insects and for saddle sores of camels and horses (Sharma *et al*., 2011). The stem of CQ is used for the treatment of gastritis, constipation, eye diseases, piles and anemia. Useful in stomachic when preserve is made with stem and lime water. Other Ayurveda names for certain conditions are as follows: *Dipana* (appetiser), *Pachaka* (digestant), *Raktashodhaka* (blood purifier), *raktastambhaka* (arrest bleeding), *Bhagna-Ashtibhagna* (it uses in bone fracture healing). *Krimighna* - It eliminates the Krimi (work in worm infestation), *Arshoghna* - It helps to cure piles. *Akshirogajit* - It is used in ophthalmic disease. *Vrishya* - It is aphrodisiac (Pandey, 2005).

In addition to the above mentioned medicinal conditions, CQ is used in various countries for numerous ailments as follows:

a) In India, an infusion of the plant is considered as a purgative.

b) In Thailand, thin slices of the stem covered with banana pulp and swallowed without chewing (to prevent irritation of the mouth) as a remedy for haemorrhoids.

c) In Senegal, a decoction of the stems and leaves is rubbed into the skin and also added to the water used for washing in patients suffering from fever and malaria.

d) A root infusion is used for chest pain in Northern Kenya.

e) In ear-ache the juice of the stem is dripped into the ear in East Africa.

f) An infusion of the leaves is ingested for sexually transmitted diseases in Central and West Africa.

g) A decoction of the stem is applied for muscle pain and swellings in South Africa.

h) In India, Thailand, Java and Southern Africa, the juice of the stem is applied for rheumatism and to relief pain in fractures. It also hastens the recovery of fractures.

i) In India and Indonesia the root powder is used internally for fractures and indigestion.

j) Massai people of Kenya, as herbal remedy for malaria.

k) In Zimbabwe, pulp of the whole plant is applied for wounds with maggots (Samaranayake *et al*., 2015).

l) In Nigeria, among the Nupe tribe it is ingested for sexual and fertility enhancement.

2.6.2 Therapeutic uses of CQ

2.6.2.1 Bone health activity

A lot of studies have demonstrated the ability of *Cissus* to accelerate healing of bone fractures (Udupa and Prasad, 1962, 1964a, 1964b; Prasad and Udupa, 1963; Singh and Udupa, 1962). Deka *et al*. (1994) similarly demonstrated that a dried methanolic extract of *Cissus* given subcutaneously to dogs accelerated the healing process of experimentally fractured radius and ulna. Using an ovariectomised rat model of osteoporosis, Shirwaikar *et al*. (2003) observed anti-osteoporotic activity based on biochemical and histopathological parameters using ethanolic extract of *Cissus*. A series of studies by Potu *et al*. (2008, 2009a, 2009b, 2010, 2011) have provided additional evidence of the ability usinga petroleum ether extracts of *Cissus*to stimulate bone growth and healing, and prevention and reversal of osteoporosis in all these studies.

Banu *et al*. (2012) and Aswar *et al*. (2012) demonstrated the ability of an ethanolic extract to inhibit bone loss using an ovariectomised mouse and rat model. The constituents responsible for these effects were not determined and are not known. Using primary cultures of osteoblasts, Kumar *et al*. (2010a) have shown that various constituents of several plant species including

Cissus increased osteoblast differentiation and mineralisation in rat osteoblasts. The *Cissus* constituent 6-O-trans-cinnamoyl catapol was shown to exhibit anti-osteoporotic activity in the rat osteoblasts (Kumar *et al*., 2010a).

More recently, Mishra *et al*. (2010) and Singh *et al*. (2011) conducted a human clinical evaluation of the ability of a *Cissus* extract. Pain, swelling, tenderness and healing times were reduced in most of the subjects with a shortened the duration of bone healing by about two to three weeks, demonstrating clinical efficacy in decreasing fixation time.

2.6.2.2 Antioxidant activity

A number of studies have demonstrated that various organic extracts of *Cissus* exhibit antioxidant activities that are integral in association with fracture and tissue healing.

Chidambara *et al*. (2003) and Patarapanich, (2004) demonstrated that ethyl acetate fraction of *Cissus* stems exhibited significant antioxidant activity in several *in vitro* systems while methanol and water extracts exhibited lesser activities respectively. Another study was performed to evaluate the effect of the methanolic extract of CQ against free-radical damage and confirmed to exhibit a strong antioxidant and free radical scavenging activity *in vitro* and *in vivo* systems mainly due to the presence of β-carotene (Jainu and Devi, 2005).

Jainu and Devi (2004, 2005a, 2005b, 2006) and Jainu *et al*. (2006) have conducted a series of studies on the tissue protective effects of extracts of *Cissus* stems. A methanolic extract of *Cissus* was shown to inhibit lipid peroxidation and free-radical production and increase antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) and reduced glutathione in rat erythrocyte and liver systems (Jainu and Devi, 2005a). Jainu and Devi (2004) demonstrated its ulcer protective effect in rats. The extract increased mucin secretion, mucosal cell proliferation, glycoprotein secretion and life-span of the mucosal cells (Jainu and Devi, 2005b).

The stem part of CQ contain vitamin C, carotenoid, calcium, steroidal and these are known to be excellent antioxidants and numerous studies that dietary intake of plant polyphenol antioxidant may have positive effect in oxidative stress related pathogenesis extract of CQ were tested for antioxidant activity by β-carotene linoleic acid model (Atram, 2015). Also Quercetin and resveratrol isolated from CQ(Adesanya *et al.*, 1999; Singh *et al.*, 2007; Thakur *et al.*, 2009) are natural antioxidants (Leonard *et al.*, 2003; Boots *et al.*, 2008). Quercetin possessed cytoprotective ability by increasing superoxide dismutase (SOD) activity and nitric oxide release in homocysteine-injured human umbilical vein endothelial ECV304 cells (Lin *et al.*, 2007). Quercetin prevented oxidative stress in carbon tetrachloride (CCl_4) - induced cirrhosis in rats by increasing the activity and protein expression of antioxidant enzymes, SOD, catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) (Ama´lia *et al.*, 2007). Quercetin attenuated phenylephrine-induced contraction in rat aortic vessels by nitric oxide dependent mechanism through the stimulation of endothelial nitric oxide synthase (eNOS) phosphorylation with a concomitant increase in nitric oxide production in bovine aortic endothelial cells (Khoo *et al.*, 2010).

2.6.2.3 Anti-inflammatory activity

Begum and Sadique (1999) and Vijay and Vijayvergia (2010) demonstrated the anti-inflammatory activity in a carrageenan-induced rat paw swelling model using *Cissus* stem powder and ethanolic extract of *Cissus*, respectively. The extract significantly reduced oedema within 1 to 5 h at doses of 50–150mg/kg. The anti-inflammatory effect of an ethyl acetate extract of *Cissus* was explored in a macrophage cell system, with a potently inhibited nitric oxide production and inducing heme oxygenase-1 expression, which provided a mechanistic rationale for the anti-inflammatory activity of the extract (Srisook *et al.*, 2011).

Flavanoids inhibit the inflammatory process. They are inhibitor of lipooxygenase especially luteolin, which is compound of CQ is known to be inhibitor. The anti-inflammatory activity of β-sitosterol was demonstrated to have an inhibitory effect on oedema induced by both carrageen and arachidomic acid. It is suggested that CQ is dual inhibitor of arachidomic acid metabolism (De la Puerta, *et al*., 2000). Calcium oxalate, carotene, tetraterpenoids, β-sitosterol, amyrin and anabolic ketosteroids, which are responsible for acceleration of healing and possess anti-inflammatory and analgesic activity (Udupa and Prasad, 1963; Jaiswal *et al.*, 2004).

2.6.2.4 Antimicrobial activity

The ethyl acetate and methanolic extracts of the root and stem of CQ exhibited antimicrobial activity against Gram-positive organisms (Chidambara *et al*., 2003). Austin *et al*. (2003) showed that aqueous and organic solvent extracts (acetone, methanol and chloroform) of *Cissus* were effective against *Helicobacter pylori* human isolates. In a study of the antibacterial activity of various extracts of *Cissus* stems, a methanol extract was shown to exhibit greatest antibacterial activity as compared to extracts of ethyl acetate, petroleum ether, acetone, ethanol and water (Kashikar and Indu, 2006).

Methanol extract (90%) and dichloromethane extract of stems possess antibacterial activity against *S. aureus, E. coli,* and *P. aeruginosa* and mutagenicity against *Salmonella microsome* (Luseba *et al*., 2007). The alcoholic extract of aerial part was found to possess antiprotozoal activity against *Entamoeba histolytica* (Rajpal, 2005). Alcoholic extract of the stem showed activity against *E. coli* (Rao and Deshpande, 2005). Methanol and dichloromethane extract of whole plant were screened for in vitro antiplasmodial activity (Paulsen *et al*., 2007).

A stem extract of *Cissus* and synthesised silver nanoparticles prepared therefrom have been evaluated for their anti-parasitic activity (Santhoshkumar *et al*., 2012). The preparations were tested and effective against an adult haematophagous adult fly and cattle tick larvae at 7–50 mg/L concentrations range.

Suhashini and Helan Chandra (2015) also evaluated the antibacterial potential of the petroleum ether and ethyl acetate plant extract ofCQ on both gram positive organisms (*Bacillus cereus* and *Staphylococcus aureus*) and gram negative organisms (*Escherichia coli* and *Salmonella typhi*).The ethyl acetate plant extract was highly effective against all the organisms compared to petroleum ether extract. Among the bacterial strains, both the extracts showing maximum zone of inhibition against *Staphylococcus aureus*. However, the chemical components or constituents of various extracts responsible for these activities have not been specifically identified.

2.6.2.5 Analgesic activity

Although *Cissus* has been used for many years as an analgesic (Gupta and Sharma, 2008), one of the first scientific studies to demonstrate this activity was reported by Singh *et al*. (1984), who showed that an extract of the dried whole *Cissus* plant prepared with 70% ethanol exhibited significant analgesic activity in mice when given orally and intraperitoneally.

The analgesic, anti-inflammatory and venotonic effects of a methanolic extract of *Cissus* were determined by Panthong *et al*. (2007). Significant peripheral and central analgesia was demonstrated in mice,while the anti-inflammatory activity of *Cissus* extract was demonstrated in the rat ear oedema model. In another study (Mate *et al*., 2008), an extract was prepared using a combination of chloroform: water (20:80). The analgesic (anti-nociceptive) activity of the extract was demonstrated in mice, with the aqueous extract of *Cissus* root shown to exhibit analgesic and smooth muscle relaxant properties (Kumar *et al*., 2010).However, calcium oxalate, carotene, tetraterpenoids, β-sitosterol, amyrin and anabolic ketosteroids could be responsible for the analgesic activity as earlier stated (Udupa and Prasad, 1963; Jaiswal *et al*, 2004).

2.6.2.6 Anti haemorrhoidal activity

Phytochemical study of CQ revelled that its major compounds are flavanoids.A mixture of flavonoids, particularly diosmin and hesperidin, is used as a reference drug for the treatment of an acute symptom of haemorrhoid (Sarabia *et al*., 2001) by increasing venous tone (Panthong *et al*., 2007) and modulating oxidative stress in various cell systems (Cypriani *et al*., 1993; Chiou *et al*., 2008). The bioflavonoids particularly diosmin, hesperidin complex have demonstrated potential in the treatment of haemorrhoids. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well-known to be involved in pathology of vascular diseases and blood vessel weakness including haemorrhoid (Cai and Harrison, 2000). The bioflavonoids exhibit phebotonic activity venatonic activity, vasculo-protective effects and antagonistic effect on biochemical mediator of inflammation (Atram, 2015). The extract of CQ produce same activity can also be used as anti haemorrhidal drug. The herb also possesses analgesic effect which is very useful in painful haemorrhoids. Panpimanmas *et al*. (2010) examined the efficacy of a *Cissus* preparation in the treatment of haemorrhoids in human subjects based on previous animal studies demonstrating analgesic, anti-inflammatory and venotonic activities. At the end of the study, no differences in improvement in symptoms were observed between the treated and placebo groups. Hence, it was concluded that a longer term study was required.Quercetin and resveratrol are also likely to represent ones of bioactive constituents in CQ, contributing vascular protection for the healing effect of haemorrhoid, in a similar way to the reference drug containing diosmin and hesperidin (Sapsrithong *et al*., 2012).

2.6.2.7 Anti-ulcer activity

The CQ is rich source of arytenoids, triterpenoides and ascorbic acid, which plays an important role in human nutrition. Many studies have analysed and revealed the effects against gastric toxicity and gastro protective effect of CQ against the gastric mucosal damages induce by aspirin. The studies show that administration of aspirin increased lipid peroxidation status, xanthenes oxides, myeloperoxidase in gastric mucosa resulting in mucosal damages at both cellular level and sub-cellular level which more reversed by CQ extract. This finding suggests that the gastro-protective creativity of the plant extract was confirmed by histoarchitecture, which was comprised of reduced size of ulcer crater and restoration of mucosal epithelium possibly through its reduced neutrophil infiltration, antioxidant and anti-apoptotic effect (Jainu and Devi, 2006). Methanol extract was shown to have significant antiulcer activity in experimentally induced ulcer in rat model by decreasing gastric secretions and by enhancing glycoprotein levels (Rao *et al*., 2007). Methanol extract also produced healing effect on aspirin induced gastric mucosal damage in rats through its antioxidative mechanism (Jainu and Devi, 2003). Triterpenoids and β-sitosterol present in methanol extract were likely responsible for this activity as they possess anti-lipid peroxidising effect and thus prevent gastric damage (Somova *et al*., 2003).

Another study was performed to evaluate the effect of CQ extract after the application of acetic acid to the stomach enhanced the reduction of ulcer area in a dose-dependent manner which was confirmed by histoarchitecture and significantly increase in the 3H-thymidine incorporation and the levels of polyamines such as putrescine, spermine and spermidine in ulcerated rats. The extract offers gastroprotection in the ulcerated area and also reversed the changes in the gastric mucosa of ulcerated rats with significant elevation in mitochondrial tricarboxylic acid cycle enzymes. Based on these results, the healing effect of the extract on acetic acid induced gastric mucosal injury in rats may be confirmed (Jainu *et al*., 2010).

2.6.2.8 Wound healing activity

The stem extract of the plant was identified to have wound healing activity (Inngjerdingen *et al*., 2004). This activity was validated by Mohanty *et al*. (2010) on excision and incision wound models in albino rats using different extracts of CQ (The petroleum ether, chloroform and methanol extracts), with each incorporated in simple ointment base (5% w/w) and used for excision wound model externally. The results showed the methanol extract of the plant has more significant wound healing activity in excision and incision wound models as compared to petroleum ether and chloroform extract respectively and thus supporting the popular use of plant to open wound in folk medicine. Quercetin and vitamin C are responsible for this activity.

2.6.2.9 Weight management and metabolic syndrome activities

Most studies involving the use of *Cissus* in formulations for weight management and metabolic syndrome have been published by Oben *et al.* (2006, 2007,2008). The above studies suggest that *Cissus* extracts either alone or in conjunction with other ingredients are beneficial in supporting weight loss as well as aiding in the return of blood glucose, cholesterol and triglyceride levels to the normal range. Elevations of these properties are all characteristic of metabolic syndrome. However, the human studies have not been confirmed by other investigators. *Cissus* extracts also exhibited tissue protective effects associated with faulty lipid and carbohydrate metabolism. Again, the specific components of *Cissus* responsible for these beneficial effects have not been identified.

In a study involving rats fed a high fat-high fructose diet which produces insulin resistance (Chidambaram and Carani, 2010), the addition of an ethanolic extract of *Cissus* stem to the diet (10 g/100 g diet) for 60 days resulted in significantly improved insulin sensitivity, reduced liver damage and return of hepatic antioxidant levels and lipids towards normal.

2.6.3.0 Anti-tumour activity

*Cissus quadrangularis*through the whole water extract more than the methanol extract of antagonistic role of human liver (HepG2) cell proliferation, which as a traditional treatment for their cancer patients provide a scientific basis (Opoku *et al*., 2000). In addition, Resveratrol is an effective anti-cancer agent of natural chemicals from CQ that can trigger the human tumor cells, CD95 signalling-dependent cell death. Also has an entry that its anti-cancer activity attributed to its anti-cyclo-oxygenase activity (Clement *et al*., 1998). In another study, Ruskin *et al.* (2014) conducted an *in vitro* anti-cancer activity in CQ extract screening by MTT Cell Viability assay against Michigan Cancer Foundation (MCF) cell line(Breast cancer cell line) for the different solvent extracts of acetone,chloroform, ethanol, ethyl acetate and methanol. Ethyl acetate had the lowest percentage of viability and showed significant anticancer activity. All the other solvent extracts such as acetone, ethanol, methanol and chloroform had cytotoxic effect but not much that of significant anticancer activity. It was concluded that the ethyl acetate of CQ possess significant anticancer activity against *in vitro* studies (Renugadevi *et al*., 2012). The activity may be due to the presence of one or more phytochemical constituents present in the extract (Nalini *et al*., 2011). In another study, it was evident that the ethanol extracts and isolated flavonoid fraction of CQ possess effective antioxidant and anticancer activities due to the presence of phytochemicals like flavonoids such as quercetin and rutin (Vijayalakshmi *et al*., 2013). It has also been reported that phytosterols have anticancer properties, and act as immune system modulators (QuÍlez *et al*., 2003).

2.6.3.1 Antipyretic activity

The extract of the CQ administered orally in albino rats showed a reduction in hyperpyrexia induced by dried yeast injection with activity being pronounced in 18 h. This shows the antipyretic activity of CQ (Priyanka and Rekha, 2010).

2.6.3.2 Central nervous system activity

The root extract of the CQ possess central nervous system depressant activity as indicated by a decrease in exploratory behaviour (Adzu *et al*., 2002). Methanol extract of roots contains saponins which show potent sedative activity and also inhibit spontaneous motor activity in mice (Dubois *et al*., 1986).

2.6.3.3 Miscellaneous activity

Acetone and dichloromethane extract of the plant possess proteolytic activity against cysteine protease (Paulsen *et al*., 2007). The extract of plant exhibits cardiotonic and androgenic property (Anon, 2000).The leaf extract showed anti-fungal activity (Misra *et al*., 1949). Ethanol extract (50%) of aerial parts possess hypotensive activity and stem extract possess diuretic activity (Guhabakshi *et al*., 2001). Chloroform extract of the plant has some molluscidal activity with aconcentration of 0.4 mg/ml causing 16.66 % deaths for both larvae of *Anopheles gambiae* and *Culex quinquefasciatus* species (Hope, 2005).

2.6.3.4 Safety

Cissus powders and extracts have been used for many years, with little reference to adverse effects (Gupta and Sharma, 2008). Conversely, only a small number of studies have assessed the safety of *Cissus* either in animals, humans or cell culture systems under controlled conditions. A sub-chronic toxicity study involving a powder from dried stems of*Cissus* was conducted in Wistar rats (Attawish *et al*., 2002). The animals were dosed orally at 0, 0.03, 0.3, 3.0 and 30.0/kg body weight per day for 3 months. No toxicity was observed over this period of time. There were no differences in body weight, haematological parameters, serum chemistry or histopathology of internal organs.

Aswar *et al*. (2012) conducted an 8 week study on a standardised phytoestrogen-rich fraction of *Cissus*. No signs of toxicity were observed during the 8week study in rats. In an acute study in mice, the LD_{50} of the extract was determined to be more than 2000 mg/kg body weight. A detailed sub-chronic toxicity study was conducted in rats that were given a *Cissus* extract (CQR-300) at 0, 100, 1000 and 2500mg/kg body weight per day for 90 days by gavage (Kothari *et al*., 2011). The no-observed-adverse-effect-level was 2500mg/kg. No significant treatment-related changes were observed with respect to clinical chemistries, haematology, urinalysis, organ weights, body weights or food consumption at any dosage level. Functional observation tests and ophthalmological examination were also unremarkable. Furthermore, no genotoxicity was observed based on mutagenicity studies using the Ames, *in vitro* chromosomal aberration and *in vivo* micronucleus assays. This is clearly the most detailed safety assessment to date involving *Cissus* extract.

An acute toxicity was conducted in mice that were given ethanol extract of CQat 100, 1500, 2500 and 5000 mg/kg body weight for 24 h using Pihan *et al*. (1987) method with slight modification. There was no mortality observed in all the groups of mice that were given CQ orally after 24 h of treatment. Therefore, the LD_{50} value of CQ was estimated to be above 5000 mg/kg body weight. This results show that ethanol extract of CQ is relatively safe (Enechi *et al*., 2013).

2.6.3.5 Reproductive potential activity

A considerable emphasis has been made on natural food sources as preventive and therapeutic agents for the treatment of reproductive toxicity. Green leafy vegetables have greater antioxidant potential, which have been well studied and proved through free-radical scavenging activities (Singh *et al*., 2002). Plant extracts are rich source of phenolic compounds and are commonly found in edible plants. They have been reported to have multiple biological effects, including antioxidant activity (Wanasundara and Shahidi, 1996). Several studies have reported the potential effect of herbs and fruits in recovering environmental toxicants induced rigorous damages in vital organs such as liver, kidney and testes via antioxidants activity (Olagunju *et al*., 2009;Jalali-e-Emam *et al*., 2011; Saafi *et al*., 2011). Studies showed that a number of plant products including phytosteroids and polyphenolic substances (e.g. flavonoids, alkaloids and tannins) exert antioxidant actions (Yokozawa *et al*., 1998; Marja *et al*., 1999; Liu and Ng, 2000; Woyengo *et al*., 2009). In the same way, CQ which is medicinally important, prominent edible item and traditionally can be used for treating infertility in Siddha medicine (Kokilavani *et al*., 2014). The users of an herbal dietary supplement (Super Cissus RX$^{®}$) have reported the supplement to improve male sexual performance with an improved erectile function, a decrease in premature ejaculation, low libido and other symptoms of sexual dysfunction (Anon, 2016).

2.7 Antioxidant Properties of Medicinal Plants

Phytochemicals are the secondary metabolites produced by plants that are responsible for the smell, colour and flavour of fruits, vegetables, plant foods. Plant extracts are also rich sources of phenolic compounds containing these phytochemicals. They have been reported to have multiple biological effects, including antioxidant activity (Wanasundara and Shahidi, 1996). They contribute towards the antioxidative effect of medicinal plants, may be grouped into various classes, i.e. tannins, cardiac glycosides, flavonoids, alkaloids, saponins and others. Such phytochemicals, vitamins and other nutrients may be collectively called antioxidants(Mongalo, 2013). The concept of biological antioxidant refers to any compound that, when present at a lower concentration compared to that of an oxidisable substrate, is able to either delay or prevent the oxidation of the substrate (Godic *et al*., 2014), or are often referred to as free-radical scavengers, are molecules that can delay or prevent an antioxidative reaction catalysed by free radicals (Biapa *et al*, 2007). As such, antioxidants may also be broadly defined as any substance that delays, prevents or removes oxidative damage to target molecules (Halliwell and Gutteridege, 2007) as a result of oxidative stress which is initiated whenever there is an imbalance between the pro-oxidants and antioxidants. Increasing the intake of antioxidants can neutralise the free radicals and protect the body from cell damage. The antioxidative effect may be mainly due to the presence of phenolic components, such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Agbor *et al*., 2007).

Oxidative damage is one result of such an imbalance and includes oxidative modification of cellular macromolecules, cell death by apoptosis or necrosis, as well as structural tissue damage (Lykkesfeldt and Svendsen, 2007). Aerobic metabolism is associated with the generation of pro-oxidant molecules called reactive oxygen species that include the hydroxyl radicals, superoxide anion, hydrogen peroxide, and nitric oxide. There is a complex interaction of the pro-oxidants and antioxidants, resulting in the maintenance of intracellular homeostasis (Gupta *et al*., 2008).

Under physiological conditions, the body usually has sufficient antioxidant reserves to cope with the production of free-radicals (Miller *et al*., 1993; Castillo *et al*., 2001), which are produced continuously during metabolism and may increase as a result of pathological and other circumstances (Roth, 2000). However, when free-radical generation exceeds the body's antioxidant production capacity, oxidative stress develops (Castillo *et al*., 2004).

It is generally accepted that foods, beverages and natural health products rich in antioxidants can reduce the risk of developing diseases and promote health. Besides the prevention of a variety of diseases (Lugasi *et al*., 2003), antioxidants may play a role in protection of the nitrergic neurotransmitter (Colpaert and Lefebbvre, 2002), organ preservation and transplantation (Salehi *et al*., 2006), treatment of male infertility (Kefer *et al,* 2009), stimulation of mutagenic response (Corwin and Shloss, 1980), andrology and assisted reproductive technology (Sikka, 2004) and the control of lead pollution and enhancement of growth of specific biota in rivers (El-Shebly, 2009).

2.7.1 Role of antioxidants in male animal reproduction

Most literatures focused on the relationship among free radicals, oxidative stress, and the role of antioxidants in human reproduction (Chatterjee and Chatterjee 2009), but few articles attempted to review these aspects in animal reproduction. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are normal prooxidant molecules in aerobic metabolism (Zhong and Zhou, 2013).The three major types of ROS are superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl (OH), while nitric oxide (NO) is a main type of RNS (Dong *et al*., 2001). The production of free-radicals is a double-edged sword in reproduction system (Silva *et al*., 2010). In humans, the physiological concentrations of free-radicals are required to mediate normal processes of capacitation, hyperactivation, acrosome reaction, fertilisation, and embryo development (Rhee 2006; Desai *et al*., 2009; Gonçalves *et al*., 2010). However, above-physiological levels of free-radicals can result in oxidative stress which leads to sperm or ovum damage, deformity, endometriosis, pre-eclampsia, miscarriage, intrauterine growth retardation, and infertility (Agarwal *et al*., 2005; Bansal and Bilaspuri, 2010).

In the male animals, a physiological level of ROS plays important roles in their reproductive systems (Agarwal *et al*., 2008), by moderately elevated concentrations of ROS induce sperm immobilisation via depletion of intracellular ATP and subsequent decrease in the phosphorylation of axonemal, but over physiological levels of ROS induce lipid peroxidation and result in sperm cell death (Misro *et al*., 2004). Spermatozoa membranes are vulnerable to free-radical induced damage because they are not only rich in polyunsaturated fatty acids (Maneesh and Jayalekshmi, 2006), but also contains low concentration of antioxidant enzymes (Sawyer *et al*., 2001; Saleh and Agarwal, 2002; Maneesh and Jayalekshmi, 2006). Furthermore, spermatogenesis in testes is an extremely active replicative process to generate sperm at a high rate. This high rate of cell division is accompanied with high production of free-radicals due to high amounts of mitochondrial oxygen consumption by germinal epithelium (Aitken and Roman, 2008).

An imbalance of free-radical generation and detoxification therefore, cause oxidative stress and damage to cellular lipids, proteins, amino acids, sugars. Others are nucleic acids, and mid-pieces in sperm and testicular tissues which lead to subsequent poor semen qualities. Poor semen qualities account for more than 80% failure of fertilisation and embryogenesis, miscarriage, and infertility in male animals (Gadea and Matas, 2000; Rabbani *et al*., 2010; Enciso *et al*., 2011).

Besides normal generation via spermatogenesis *in vivo*, ROS are also generated during *in vitro* storage, such as semen cryopreservation, which is an important procedure in livestock industry, especially for cattle, sheep, and goats (Bucak *et al*., 2008, 2009). During semen collection and freezing/thawing procedures, semen is exposed to cold shock and atmospheric oxygen, which in turn increases the susceptibility to lipid peroxidation (Bucak *et al*., 2011). In addition, many environmental factors, such as extremely high ambient temperature, chemicals, medicines, and other toxins, induce ROS production in reproduction system. Das *et al*. (2002) found that cyclophosphamide increased testicular lipid peroxidation levels and decreased the activity of catalase in rats. The detrimental effects of heat stress on reproduction system of rats (Ikeda *et al*., 1999), rabbits (Marai *et al*., 2002), bulls (Nichi *et al*., 2006), rams (Marai *et al*., 2009), and chickens (Ayo *et al*., 2011) have been confirmed. Furthermore, it has ascertained that oxidative stress is a possible cause of the heat stress (Hanafi *et al*., 2010).

Exogenous antioxidants play a key role in the delicate equilibrium between oxidation and antioxidation, but they are double-edged swords in cellular redox state of living organisms (Zhong and Zhou, 2013). The pro-oxidant or antioxidant activities intimately depend on their concentrations. In most circumstances, physiological doses of antioxidants exert beneficial effects; while overdoses of them exhibit detrimental effects (Kawanishi *et al*., 2005; Bouayed and Bohn 2010).

Though some synthetic antioxidants, such as vitamin C and vitamin E, have been used to protect ovum and embryo against oxidative stress, disputes still exist indeed because a series of adverse effects in animal reproduction were observed (Nayyar and Jindal, 2010). For example, Olson and Seidel (2000) reported that 100 μmol L^{-1} vitamin E markedly improved bovine blastocyst development, whereas Sudano *et al*. (2010) found that the addition of 200 μmol L^{-1} of vitamin E had a deleterious effect on bovine embryo development *in vitro*. Both the above studies found that lower than 50 μmol L^{-1} of vitamin C lacked efficiency to improve embryo production.

Recently, the alternative strategy of using natural plants or their extracts as antioxidants in animals has been confirmed to be effective and utilised extensively.Phytochemicals present in the plants are reported to have antioxidants properties that will prevent the oxidative chain reaction initiated by the free-radicals and counteract the damaging effects of ROS produced within the organism from molecular oxygen (Zhong and Zhou, 2013).

The most effective constituents responsible for antioxidative properties of plants are phenolic compounds, including flavonoids, hydrolysable tannins, phenolic acids, and pholoc terpenes (Gupta and Sharma 2006; Ogunlesi *et al*., 2009; Carlsen *et al*., 2010). The antioxidant activities of phenolic compounds are due to their structure and particularly ability to donate a hydrogen ion to the peroxy radical generated as a result of lipid peroxidation (Kashima 1999; Bisby *et al.*, 2008). In the case of *Cissus quadrangularis*, the presence of vitamin C, flavonoids, phytosterols, tannins and other phytochemicals tend to confer it with the antioxidant property.

To evaluate reproduction status of male animals, many factors must be considered, including spermatogenesis, semen functions, sperm quality, and fertility. Spermatogenesis depends on intratesticular and extratesticular hormonal regulatory processes and functions of the intertubular microvasculature (Holstein *et al.*, 2003). Semen parameters such as sperm count and concentration, viability, mobility, and morphology are indicators to evaluate semen functions (Huynh *et al.*, 2000; Rodriguez-Martinez, 2003, 2006). Infertility is not only a major public health problem in humans, but also the case in animals due to extensive feeding system and application of synthetic feed additives (Zhong and Zhou, 2013).

Oxidative stress is a main underlying cause which can interfere with spermatogenesis, reduce sperm quality and production, and even cause infertility (Boonsorn *et al.*, 2010). Since elevated ROS generation causes damage to the spermatozoa DNA, results in increased apoptosis of cells, and therefore, leads to a low fertility rate (Kaur and Bansal, 2003). The application of exogenous plant derived antioxidant is likely to improve health status of male animals (Agarwal and Prabakaran, 2005; Nantia *et al.*, 2009). Numbers of flavonoid-containing plants are known to have antioxidant, androgenic, and anti-infertility activities and have been extensively used against animal reproductive diseases (Middleton *et al.*, 2000; Dobrzyñska *et al.*, 2004; Purdy *et al.*, 2004). In addition to natural herbaceous plants, some fruit and vegetable extracts with antioxidant properties display beneficial effects in animal reproduction system. Nevertheless, some plants, despite containing antioxidant substances, display detrimental effects and, therefore, cause defects and reproductive failure in male animals (Knight and Walter, 2004). The dose-dependent manner of antioxidants may explain such dual functions (Moskaug *et al.*, 2005;Na and Surh, 2008).

In addition to above *in vivo* processes, semen cryopreservation *in vitro* is an important procedure which allows specific advantages to livestock industry. Freezing/thawing procedure of sperm is routinely performed in ruminants breeding industries for artificial insemination (Bucak *et al*., 2008). High ROS production in these procedures induces oxidative stress which in turn causes low quality of seminal material or death of sperm cells, which becomes a major obstacle of successful cryopreservation (Janice *et al*., 2000). Study showed that the conception rates and percentage of fertilised ova with frozen-thawed ram semen were approximately 20% less than that with fresh semen (Maxwell *et al*., 1993). Storey *et al*. (1998) demonstrated that *in vitro* fertilisation rates with cryopreservation epididymal mouse sperm were, at best, 62% that of unfrozen controls. To overcome these disadvantages, antioxidant treatment may be a feasible strategy to improve cryopreservation techniques (Anghel *et al*., 2010).

Rhodiola sacra aqueous extract from *Rhodiola rosea* (Crassulaceae) roots, a genus of Chinese herb, has been used as an antioxidant (Ohsugi *et al*., 1999). Zhao *et al*. (2009) indicated that *R. sacra* aqueous extract improved biochemical and sperm characteristics in cryopreserved boar sperm. Rosemary (*Rosmarinus officinalis*) is a perennial herb with antioxidant properties due to bioactive substances, such as diterpenes, triterpenes, flavonoids, and polyphenols, as well as sesquiterpenes (Samotyja and Matecka 2010). Malo *et al*. (2011a) reported that rosemary extract supplementation in freezing medium improved boar sperm mobility and fertility after cryopreservation. *Ferula hermonis* is a wonderful antioxidant, aphrodisiac herb for both sexes (Hanafi *et al*., 2010). Malo *et al*. (2011b) demonstrated that *F. hermonis* extract added in freezing extender increased sperm mobility and viability and decreased lipoxidation during boar sperm cryopreservation. Trehalose is seaweed extract known to protect the sperm membrane structure against oxidative and cold shock damage during the freezing/thawing process. Trehalose supplementation in freezing medium could improve sperm quality of rams (Bucak *et al*., 2007), bulls (Hu *et al*., 2010), and rabbit bucks (Reddy *et al*., 2010).

Thus, it will be of utmost benefit to knowledge into the research of the dual functions (positive and negative effects) of *Cissus quadrangularis* on male animal reproduction due to the antioxidant activity it possess. Though the exact mechanisms of double-edged actions of plant derived antioxidants in animal reproduction system are unclear, four possible reasons may explain them. The first main reason is dose-dependent manner (Zhong and Zhou, 2013). The second underlying reason may be attributed to that the individual isolated compounds lose the chances of synergistic interactions with other substances (Zielinska *et al*., 2007; Bouayed and Bohn, 2010). The third reason of the same antioxidative substances displaying slightly different pharmacological efficiency may be attributed to plant characteristic and animal species tested (Zhong and Zhou, 2013). The toxicity therefore, appears to be quite variable, with animal susceptibility being dependent on the quantity and type of plant extracts. Snakeweed contains steroids, terpenoids, and saponins and is referred as teratogens. Cattle on a good plane of nutrition can consume up to 30% dried snakeweed without apparent detrimental effect; however, the fresh and to a lesser extent the dried snakeweed may cause abortions in cattle, sheep, and goats at any stage of gestation (Martinez *et al*., 1993). The last possible reason may be due to that they may have different chemical structures, for instance, the pharmacological function of phenolic compounds with different positions of hydroxyl groups attached to the benzene rings may vary (Zhong and Zhou, 2013). In this regards, further studies are needed to study the characteristic of individual antioxidants and the synergistic reactions with other nutrients in plants or animals' diets.

2.8 Rabbit Production in Nigeria

Rabbit (*Oryctolagus cuniculus*) is a micro-livestock species, appearing to be one of the cheapest and fastest means of producing high-quality animal protein and fast-growing livestock (Vietmeyer, 1985). Rabbits possess a number of features that might be of advantage in the small holder subsistence type integrated farming in developing countries. Rabbit production provides the impoverished urban population and rural dwellers with opportunities to earn additional income on a sustainable basis (Shehu *et al*., 2016) and provide an excellent source of protein for human consumption and may play a significant role in solving the problem of meat shortage in developing countries (Abdel-Azeem *et al*., 2007). The meat of rabbits is characterised by a high protein and low fat and cholesterol content and it is considered as a delicacy and a healthy food product (Dalle-Zotte, 2000). Rabbits have a number of other characteristics that might be advantageous to subsistence farming system, such as their small body size with a relatively short gestation period average of 30-31 days (Ortiz-Hernandez and Rubio-Luzano, 2001). High prolificacy and early attainment of puberty make rabbit an ideal animal for meat production in developing tropics.

Rabbit production is being encouraged in Nigeria as a means of improving the daily protein intake of individuals (Ekpenyong and Bioaku, 1986). Exotic rabbits are assuming prominence in an effort to alleviate the supply-demand of animal protein in Nigeria. The extent to which such efforts succeed will depend on how well local and other management practices can be put in place to ensure optimum performances (Berepubo *et al*., 1995; Ukachukwu, 1997; Owen *et al*., 2008; Owen *et al*., 2009).

Rabbitary, the science and occupation of raising rabbits for food, can be regarded as a new breed of animal farming in Nigeria with its potentialities, opportunities and challenges. The potentialities of rabbit rearing are that the cooked meat has a high nutritional value with high protein (56%), low fat (9%), and low in cholesterol, sodium and calories (8%) and contain 28% phosphorus, 13% iron, 16% zinc, 14% riboflavin, 6% thiamin, 35% B12 and 48% niacin – making it ideal meat for hypertensive patients. Rabbitary also requires comparatively low level of capital set up, a little space, and is well-adapted to domestic rearing (Owen and Amakiri, 2010).

Despite the challenges of non-readily available market when the farmers are ready to sell their stock, low knowledge of rabbit genetics/production techniques and inadequate knowledge and information about advantages of eating rabbit meat; the prolific nature of rabbits coupled with its short gestation period and generation interval, makes the animal of choice for multiplication, and serve as a short way of increasing animal protein intake. Rabbit production, thus have enormous potentials in alleviating the problem of animal protein supply in developing countries (Owen and Amakiri, 2010).

In Nigeria, low animal protein intake has remained a major nutritional problem, especially for the low income and non-wage earners (Amaefule and Obioha, 2005; Akinola, 2009). There is therefore an urgent need to develop rabbit (*Oryctolagus cuniculus*) production as a cheap source of animal protein to bridge the wide gap, existing between animal protein supply and consumption. Rabbit farming is a new area in animal farming and is adapted to both rural and urban centres, tropical and temperate regions of the world alike. The domestic rabbit is as efficient as other farm animals in converting feed to meat for human consumption. It has since been identified as an economic livestock for small-scale rural farmers/dwellers, capable of producing about 47kg of meat, enough to solely meet the animal protein requirements of a medium size family (Abdulmalik, 1994; Hassan and Owolabi, 1996).

Rabbit's skin also has some commercial values. They may be dressed, dyed and made into fur garment and slippers. Even though most domestic rabbits are raised for meat production while some are for laboratory and biological purposes (Lebas *et al*., 1997). Bolaji (2005) reported that rabbit manure is high in nitrogen and phosphorus and useful in improving soil fertility. The prolific nature of rabbits coupled with its short gestation period and generation interval, makes the animal of choice for multiplication and serve as a short way of increasing animal protein intake (Egbo *et al*., 2001; Ironkwe, 2004). Rabbit production, thus has enormous potentials in alleviating the problem of animal protein supply in developing countries (Ezea, 2004).

In spite of the exceptional attributes and advantages of keeping rabbits, its production in Nigeria is still comparatively rudimentary (Onifade *et al*., 1999) and befaced with many challenges, resulting in gross shortage of meat to meet up the growing demands (Nworgu, 2007). One of the major problems of rabbit production in Nigeria is high cost of concentrates, feeds and feeding and relatively smaller weight gain, especially during the cold-dry and hot-dry season period, non-readily available market when the farmers are ready to sell their stock and inadequate knowledge and information about the advantages of eating rabbit meat (Nworgu, 2006). Forages sometimes are also the limiting factor in successful rabbit production, especially conventional forages such as groundnut hay in which there is competition between the rabbit and ruminant animals.

In view of the factors, it becomes necessary to find out alternative forages for the rabbit. Rabbit production under tropical conditions is affected adversely by environmental stress, mainly from the effect of high ambient temperatures and high relative humidity, low wind speed and indirect solar radiation (Fadare, 2015). However, the knowledge of rabbit genetics and production techniques still lag behind, when compared with other species (Sogunle *et al*., 2009).

2.8.1 Reproductive anatomy

The reproductive system consists of the testes (2), epididymis (2), ampoules (2), *vas deferens* (2), urethra, penis, preputial glands (2) and the accessory glands. It presents a peculiarity in the external genitalia, which is common in marsupials and rabbits, a well-developed scrotum located cranial to the penis and the urogenital opening (Capello and Lennox, 2006). The scrotum has few hairs (Donnelly, 2004), and it is formed by the *tunica vaginalis*, *tunica dartos* and cremaster muscle. Its main function is to keep the testicles away from the abdominal cavity, so that the right testicular temperature is maintained between 0.5 and 4°C below body temperature, as required for normal spermatogenesis (Alvariño, 1993). The scrotum and abdomen have communication through the inguinal ring, which conveys the excretory duct (*vas deferens*) that comes from the epididymis. During periods of sexual inactivity or stress the testicles return to the abdominal cavity through the inguinal ring, and may go down again by the action of the cremaster muscle (Alvariño, 1993; Capello and Lennox, 2006). They are positioned cranially to the penis (Brewer, 2006; Capello and Lennox, 2006), located in the scrotum, each one on one side of the inguinal line, positioned almost horizontally (Holtz and Foote, 1978a). Rabbit testicles are similar to those of cats, but can move freely from the scrotum to the abdomen through an opening in the inguinal canal (Brewer, 2006). Soft tissue herniation and strangulation of the bowel is prevented by a large fat mass associated with the epididymis, which lies in the inguinal canal when the testis is in the scrotum (Donnelly, 2004). The position of the testicles depends on many factors, including body position, body temperature, reproductive activity, repletion of the gastro-intestinal tract, amount of abdominal fat (Capello and Lennox, 2006) and stress (Richardson, 2003). According to Fraser (1988), the appearance and testis weight depend on the location. For example, testes located in the scrotum are heavier, firm in texture and red in colouring. Abdominal testes are light, reddish-brown and limp.

Dorso-medial to the end portion of the testis, a set of efferent tubules pierces the *tunica albuginea* and enters the initial segment of the head of the epididymis. The functional part of the epididymis consists of a single duct. It originates in the efferent ducts; it is highly curled over the head, body and tail of the epididymis, and connects straight to the *vas deferens* (Holtz and Foote, 1978a). The tail of the epididymis is U-shaped. The rabbit is one of the species in which sperm stored in the cauda epididymis exhibit vigorous motility even in their own fluid (Turner and Reich, 1985). The *vas deferens* extends dorsocranial the body of the epididymis through the inguinal canal and enters in the abdominal cavity (Holtz and Foote, 1978a). The final portion of the *vas deferens* forms a loop around the ureter and at this point becomes fusiform. Although the thickness of the diameter does not differ from the rest of the *vas deferens*, this segment is generally called ampulla (*ampulla vas deferens*).

The glands of the rabbit reproductive tract differ in number, location, size and proportion, among other aspects, like those in other mammals (Vásquez and Del Sol, 2009). This set of glands consists of a vesicular gland, bulbo-urethral gland and a complex formed by the prostate, pro prostate and paraprostate (Holtz and Foote, 1978a; Vásquez and Del Sol, 2009). According to Hafez (1995), they contribute to the greater part of the volume of ejaculate. The vesicular gland is located between the prostate gland complex (a very muscular bag with a glandular lining) and the two ampoules which are side by side. Thus, the vesicular gland is variable in length and sometimes becomes temporarily enlarged, depending on the amount of fluid therein. This fluid fluctuates from slightly viscous to gel consistency (Holtz and Foote, 1978a). This gland contributes 45.6% of the ejaculate volume of rabbits (Del Niño Jesus *et al*., 1997).

Some authors have reported that the rabbit prostatic complex, which is located on the dorsal side near the urethra and the bladder, consists of the seminal vesicle, vesicular gland and prostate gland (Seki and Suzuki, 1989). Others say that the prostatic complex is formed by the vesicle gland, coagulation gland, dorsal ventral lobe of the prostate and bulbo-urethral glands (Cockle *et al*., 1989).However, to standardise the terms and facilitate understanding, the terminology was proposed by Holtz and Foote (1978a) and adopted by Vasquez and Del Sol (2002) and Dimitrov and Stamatova (2011). The names used were adopted according to embryological origin and morphology of the glands. The coagulation gland is called the pro-prostate, the dorsal lobe and ventral prostate paraprostate. These authors affirm that the prostate complex consists of three lobes: pro-prostate, prostate and paraprostate (two units). It is noteworthy that the different elements of the rabbit prostate gland have anatomical, histological and immune-histochemical varieties, suggesting that each part of the gland plays a specific role in reproduction (Dimitrov, 2010).

The prostate is located caudally to the vesicular gland and cranially to the prostate and the latter is located cranially to the bulbo-urethral glands (Vásquez and Del Sol, 2002). This gland is not derived from the Wollfian duct, nor is the source gel mass (erroneously called the coagulation gland). Nor is it a lobe of the prostate, but an independent glandular unit that has a separated duct system. The accumulation of white granular secretion in the pro prostate gland gives a whitish appearance and makes the compartmentalisation visible observed from the outside (Holtz and Foote, 1978a). The prostate gland is yellowish-white in color and is located between the pro prostate and bulbo-urethral glands (Vásquez and Del Sol, 2002). It shares the same connective tissue capsule as the pro-prostate, and only a small layer of tissue separates these two glands (Holtz and Foote, 1978a). The paraprostate glands are small and were named this way because they are located on both sides of the prostate. In other words, the right and left sides are located ventrally and sideways to the pro-prostate (Dimitrov and Stamatova, 2011). They have an irregular embossed surface and are hammer-shaped (Vásquez and Del Sol, 2002).

The rabbit bulbo-urethral gland is a small mass of glandular tissue that is surrounded by a capsule and widely covered by skeletal bulb glandular muscle that separates it into lobules. This gland originates in the urethral wall, as distinct from other species. It is fairly small in the rabbit, but relatively larger than that of man (Vásquez and Del Sol, 2001). The penis is the copulatory organ. An unusual feature of the rabbit is the absence of the glans in the penis (Brewer, 2006), but the body of the penis is cylindrical, 40-50 mm long and the diameters decrease at its end. During rest from sex, it lies in the foreskin, which is located ventrally to the anus (Alvariño, 1993; Brewer, 2006) and caudally to the testicles (Capello and Lennox, 2006). The rabbit preputial glands are imperceptible and are embedded in the dermis around the preputial orifice and it is believed that they are increased sebaceous glands (Holtz and Foote, 1978a).

2.8.2 Puberty and sexual maturity

Rabbits are well known for their ability to reproduce quickly. Puberty occurs between 4-6 months, and in smaller breeds it occurs earlier than in larger breeds (Harcourt-Brown, 2002). In rabbits, sexual maturity varies with age (125-150 days), breed, lineage, food and environmental factors such as photoperiod, temperature and seasonality. According Macari and Machado (1978), puberty in rabbits precedes the appearance of sperm in the ejaculate, so that puberty and sexual maturity are different phases. Skinner (1967) affirms that at 63 days of age, rabbit testes descend into the scrotum. Other studies revealed that although the rabbit is pubertal in 4 months, the testes are not in the scrotum yet, the descent is observed into the scrotum only at six months of age (Fraser, 1988). However, sexual maturity is defined as the moment at which the daily spermatozoa production ceases to increase, which is reached at 32 weeks in New Zealand White rabbits (Amann and Lambíase Jr., 1967; Lebas *et al*., 1997). Studies revealed that this species reaches sexual maturity at 18 weeks of age (Chubb *et al*., 1978; Frame *et al*., 1994). For Skinner (1967), rabbits are pubertal when their testicles become androgenically active and accessory glands begin to produce fructose and citric acid and the animal assumes a characteristically male behaviour. In this context, in rabbits, sperm appear closer to the end than the onset of puberty.

2.8.3 Spermatogenesis

Spermatogenesis starts between 42 and 63 days of age, but sperm do not appear in ejaculated sperm before 119 days (Skinner, 1967). It is known that spermatogenesis is a process that depends on the low temperature of the scrotum. Thus, temperatures higher than that of the scrotum (e.g., abdominal temperature) may block spermatogenesis (Hua *et al*., 2000). The total estimated duration of spermatogenesis in rabbits depends on the point chosen as the beginning of spermatogenesis. If it is judged that spermatogenesis begins with the first part of a series of division of spermatogonia leading to the production of primary spermatocytes, then about four cycles of the seminiferous epithelium (4 x 10.9 = 43.6 days) are required. However, it is assumed that spermatogenesis begins with the formation of spermatogonia stem and that the lifetime of such stem cells is a cycle of seminiferous epithelium, then spermatogenesis extends approximately 4.75 cycles and 51.8 days (Swierstra and Foote, 1965). Morton (1988), considering that one cycle lasts 10.8 days and not 10.9 as suggested by Swierstra and Foote (1963), who stated that spermatogenesis in this species, lasts about 48 days. Swierstra and Foote (1963) demonstrate that the cycle of seminiferous epithelium of rabbits is divided into eight stages, using as criterion the shape of the spermatids core, the location of spermatids and spermatozoa in relation to the basement membrane, the presence of meiotic figures and release of spermatozoa in the lumen (Swierstra and Foote, 1963). Campos *et al*. (2014) also reported that during spermatogenesis there is considerable loss of spermatogenic cells in the rabbit. There are about 20% fewer spermatids than expected from the theoretical considerations. A smaller number of spermatids were also observed by Morton (1988), and Zhang *et al*. (2002). Swierstra and Foote (1963) reported that most of this loss occurs during and immediately after the two maturation divisions. However, recent studies have demonstrated the presence of round spermatids in the epididymis (sloughing of spermatids), in other words, they leave the testes before maturation (Zhang *et al*., 2002). Its also been suggested that the age of the animal and season contribute to the sloughing of spermatids, which may occur more frequently after puberty or when spermatogenesis begins to occur in an active form (Zhang *et al*., 2002).

In this species, multi-nucleated spermatids are often found (giant spermatids), but this incidence may be increased by stress or environmental management (Morton, 1988; Tsunenari and Kast, 1992; Barakat, 2007). Multi-nucleated spermatids are easily recognizable because they are spherical in shape and have from two to four small, rounds, pyknotic cores with dense chromatin (Morton *et al*., 1986; Tsunenari and Kast, 1992). The testis is the main source of testosterone in rabbits (Castro *et al*., 2002). It is also the main androgen produced during sexual maturation (Chubb *et al*., 1978). Although their essential function is the maintenance of normal spermatogenesis, serum testosterone levels above the base-line level do not appear to influence the efficiency of spermatogenesis (Castro *et al*., 2002).

2.8.4 Sperm production

The testicles continue to grow and increase sperm production until six months of age (Morton, 1988). Spermatozoa can already be present in the *cauda epididymis* at around 15 weeks of age (Chubb *et al*., 1978). Campos *et al*. (2014) also observed that daily spermatozoa production increases from 15 to 52 weeks of age. Other studies reported that there is positive correlation between the gonadal reserve and testicular weight (Orgebin-Crist, 1968) and body of the rabbit (Ewuola and Egbunike, 2010). However, daily exposure to a continuous 14 h light period negatively affects gonadal reserves (Orgebin-Crist, 1968). According to Campos *et al*. (2014), under normal conditions, the average yield is 147.4×10^6 per day and 1g of testis produces 26.5×10^6 spermatozoa per day. However, if the animal is subjected to a rate of two weekly collections, the daily release of spermatozoa in the ejaculate is consistently lower than the testicular production, indicating that approximately 50% of the spermatozoa produced are reabsorbed (Holtz and Foote, 1972). Different daily production of spermatozoa has been observed: $148 \pm 11\times10^6$ spermatozoa per day (Amann and Lambíase Jr, 1967), 187×10^6 per day (Holtz and Foote, 1972) and 210×10^6 per day (Amann and Lambíase Jr, 1969). It is noteworthy that the rhythm of semen collection does not affect daily spermatozoa production (Amann, 1966). A study showed that in this species, the spermatozoa reserve is smaller in the left testis and left epididymis than in the right ones (Ewuola and Egbunike, 2010).

2.8.5 Semen characteristics

The semen is a mix of spermatozoa produced by the testes and seminal plasma secreted by the epididymis and different accessory glands, which are combined at the time of ejaculation (El-Azim and El-kamash, 2011). However, rabbit semen consists of two main parts, a fluid and a gelatinous portion (Mukherjee *et al*., 1951).

2.8.5.1 Gel plug

The gel plug or gelatinous mass from rabbit semen originates in the vesicular gland (Holtz and Foote, 1978a; Del Niño *et al.*, 1997) and it is androgen-dependent (Parson, 1950; Bell and Mitchell, 1984). It consists of a significant amount of estrogenic substances and citric acid, and small amount of fructose (Parson, 1950; Mukherjee *et al*., 1951; Holtz and Foote, 1978b). When animals subjected to two daily semen collections the gelatinous mass can be found in 75.4% of first ejaculates, but only 4.8% in the second ejaculate (Amann, 1966; Amann and Lambíase Jr, 1967; Holtz and Foote, 1978b). A large number of spermatozoa can be entrapped and the gelatinous mass seminal inactive, but after dilution in saline solution and incubated at 37°C, the gelatinous mass dissolves releasing the spermatozoa, which in turn become highly active (Mukherjee *et al*., 1951). It is believed that this occurs because during ejaculation the two semen fractions are in a fluid state (Mukherjee *et al*., 1951). Later studies showed that the consistency of the fluid contained in the vesicular gland varies from slightly viscous to gelled (Holtz and Foote, 1978a). Although it is common in rabbit semen, no function was found for the gel apart from preventing the loss of retrograde spermatozoa in rodents (Quesenberry *et al*., 2004). This function has also been suggested by Mukherjee *et al*. (1951), who speculated that after ejaculation, the gel fills the vaginal lumen as buffer coagulation. Thus, IRRG Guidelines (2005) recommends removing the gel immediately after collection and before evaluating rabbit semen.

2.8.5.2 Seminal plasma

It represents the fluid portion of the semen and its presence positively affects the survival and parameters of spermatozoa motility in rabbits (Castellini *et al*., 2000, Hagen *et al*., 2002). Seminal plasma contains constituents such as carbohydrates, lipids, proteins and minerals (Holtz and Foote, 1978b; Müller and Kirchner, 1978, Castellini *et al*., 2006b; Zaniboni *et al*., 2004), which are important for sperm metabolism. Fructose and glucose is one of the main energetic constituent in semen, as well as substrates for the sperm cell metabolism (Mann, 1946; Arruda-Alencar, 2011). Previous studies have found that the initial fructose concentration in the seminal plasma is usually higher in the summer (245.9mg/dL), while the testosterone level is high in the spring (137.0mg/dL), while the testosterone is low level (Okab, 2007). Thus, the concentration of fructose in seminal plasma reflects the testosterone activity and semen quality (Mann and Parsons, 1947; Okab, 2007). However, the sugar concentration in rabbit semen is well below that found in ruminants (Anand, 1973; Mendoza *et al.*, 1989; Roca *et al.*, 1993). Another sugar identified in rabbit seminal plasma was glucose (Arruda-Alencar, 2011), that despite being present in low concentrations (13.8 to 22mg/dL) was considered an effective constituent of seminal plasma in this species. Rabbit seminal plasma contains Na^+, K^+, Ca^{2+}, Mg^{2+}, Se^{2+} and Zn^{2+} (Holtz and Foote, 1978b; Castellini *et al.*, 2007), and some trace elements such as Cu^{2+}, Fe^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+} and Ni^{2+} (Lukáč *et al.*, 2009). The $Na^+$and K^+ were found in similar concentrations (1:1), but the mineral concentrations cited are much smaller than those found in other species (Blackshaw and Salisbury, 1957; Holtz and Foote, 1978b). Thus, it contributes little to maintaining the semen osmotic pressure, so it seems that the organic components are the main constituents responsible for this maintenance (Holtz and Foote, 1978b). Seminal Na^+ reduces significantly in the case of removal of the seminal vesicle gland (Del Niño Jesus *et al.*, 1997).

A previous study on the effect of different Ca^{2+}, K^+ and Cl^- concentrations in diluting of washed and unwashed semen demonstrated that washing does not significantly affect spermatozoa motility in rabbits (Blackshaw, 1953). Oliveira *et al.* (2004) affirmed that the inclusion of Zn^{2+} in the breeding diet can affect the amount of spermatozoa. Rabbit seminal plasma also contains several drops and vesicles (prostatic secretory granules) of different sizes and origins, which play different roles that are partially unknown (Castellini *et al.*, 2006a). Mourvaki *et al.* (2010) suggested that the prostatic secretory granules may represent a source of protection for spermatozoa against oxidative stress *in vitro* by supplying the spermatozoa with endogenous alpha-tocopherol.

2.8.5.3 Sperm motility

Motility is a common feature of spermatozoa in the entire animal kingdom, and is the percentage of spermatozoa moving steadily in a straight line (Chrenek *et al*., 2007). Moreover, for species with internal fertilisation, motility is important for the transport of spermatozoa in the reproductive tract and oocyte penetration (Holt and Van Look, 2004). For the determination of motility, drops of a suspension of semen should be placed on slide and covered with cover slide and observed on microscope slides cover glasses (Emmes, 1947; IRRG Guidelines, 2005). The subjective estimation of the evaluation of motility and sperm morphology are the two most widely used laboratory tests to evaluate semen in rabbit season insemination (Lavara *et al*., 2008a). These characteristics result in potential spermatozoa fertility, because there are some correlations between seminal parameters with motility, indicating the relationship between morphometric parameters and semen quality of rabbits (Hagen *et al*. 2002; Lavara *et al*., 2008b).

Until recently, the sperm quality was evaluated based on the subjective evaluation of parameters such as mass and individual motility, as well as subjective parameters such as the concentration and morphology (Verstegen *et al*., 2002). Campos *et al*. (2014) affirmed that subjective estimates of semen parameters are affected by many factors, including changes in the training and experience of the evaluators. Computerised spermatozoa analysis was developed for an objective assessment of spermatozoa motility. This system includes a phase contrast microscope equipped with a heating plate, connected to a high resolution video camera and a computer (IRRG Guidelines, 2005). However, this system requires large investment. In goats, the motility evaluation method was superior to the conventional computer (Cavalcante *et al*., 2005). Roca *et al*. (2000) rated the progressive motility of spermatozoa in rabbits using an arbitrary scale 0-5 (0, 1, 2, 3, 4 or 5, D 0-10, 10-25, 25-50, 50-70, 70 -90 or 90-100%, respectively, showing progressive spermatozoa motility). The age at which 50% of sperm cells in the ejaculate have motility is 117 days (Bell and Mitchell, 1984).

2.8.5.4 Spermatozoa morphology

In rabbit, the characteristics of semen, in particular morphological, feature from medium to high heritability (Lavara *et al*., 2008c), and can be evaluated with optical microscopy procedures using different staining techniques (IRRG Guidelines, 2005). In resume, the rabbit sperm morphometric measurements were: total length from 46 to 55µm (Cummins and Woodall, 1985; Eddy, 2006); head length from 7.8 to 8.6µm (Bedford, 1963; Gravance and Davis, 1995); middle piece is 8.5µm with mitochondria arranged and about 41 turns (Eddy, 2006); the main piece measures about 38µm long (Cummins and Woodall, 1985). The head is shaped like a spatula and the acrosome does not extend beyond the core, and it also has a small equatorial region (Phillips, 1977; Eddy, 2006). Kuzminsky *et al*. (1996) developed an illustrated guide to various abnormalities of rabbit semen. The average values observed by quantitative optical microscopy (x400) were: 18.2% total abnormalities, head abnormalities 2.9%, tail abnormalities 13.6% and 1.7% broken spermatozoa.

Campos *et al*. (2014) suggested counting only the curly tails to speed up the process of semen analysis, because these are the most representative of abnormalities in the tail and are easily observed under an optical microscope, even when viewed under low magnification. They also affirm that for an acceptable ejaculate the concentration of spermatozoa with curly tails should not exceed 17-18% of 200 cells observed. It is worth noting that rabbit spermatozoa are very sensitive to high ambient temperatures, and abnormal spermatozoa can indicate a heat stress condition suffered by the animal (Finzi *et al*., 1995). It also asserted that this condition may be easily observed by the increase in the number of curly tailed spermatozoa, since this abnormality has 80% correlation with total morphological abnormalities. Among other abnormalities Branham (1969) found that the presence of the intermediate piece of the protoplasmic drop is associated with low rabbit spermatozoa displacement speed. The rabbit spermatozoa acrosome is evident as a swelling in the anterior margin of the head (Gould *et al*., 1971). The lines demarcating the equatorial segment are much closer together than in the hamster.

2.8.5.5 Semen volume and sperm concentration

The ejaculate volume and sperm concentration in rabbits may range from 0.3 to 0.6mL and 150-500x10^6 sperm/mL, respectively (Adams and Singh, 1981; Lebas *et al*., 1997). However, seminal characteristics can vary among different breeds: Rex (0.54 ± 0.03 mL, 415.10 ± 10.11x10^6 spermatozoa/mL), New Zealand White (0.54 ± 0.04 mL; 416.72 ± 9.16 x 10^6 sptz/mL), California (0.62 ± 0.03 mL, 454.11 ±11.40 x10^6 sptz/mL) and Baladi Red (0.56 ± 0.04 mL, 423.23 ± 12.11x10^6 sptz/mL) (Amann, 1966; Hassanien and Baiomy, 2011). Other factors that may vary these parameters include diet (Kamel and Attia, 2011), collection frequency (Amann, 1966, Castellini *et al*., 2006c), age (Theau-Clement *et al*., 2009), ejaculate sequences and ambient temperature (Finzi *et al*., 1995).

It is noteworthy that the semen volume seems to be more affected by temperature than the sperm concentration (Garcia-Tomás *et al*., 2008, Roca *et al*., 2005). Lebas et *al*. (1997) affirmed that when sexual stimulation was performed without copulation, 1-2 min before copulation, the sperm concentration increases. Previous studies have shown that this type of stimulation increases the number of spermatozoa in the *vas deferens* and consequently in the ejaculate (Prins and Zaneveld, 1979). According to Campos *et al*. (2014), this is because, in rabbits, during sexual stimulation sperm are moved from the epididymis into the *vas deferens*, where they are quickly removed during ejaculation. The *vas deferens* is then restored to the gradient sperm maintained during sexual rest. Thus, the *vas deferens* is an active organ during sexual inactivity (Prins and Zaneveld, 1979).

2.8.5.6 Colour and aspect of semen

Some studies have reported that rabbit semen is white and the intensity depends on sperm concentration (Bilbao, 1996; Alvarez *et al*., 2006). There are some ratings for the colour of rabbit semen. For Bilbao (1996), the semen is often pearly white and ivory, but grey semen is considered of poor quality. Alvarez *et al*. (2006) reported that milky-white semen is the best and predominant in the rabbit and represents normal semen with good quality. Yellowish semen is often contaminated with urine that is normally obtained when the temperature is too high in artificial vagina (Chang, 1959). Several studies have associated colour and appearance as a single parameter (Scapinello *et al*., 1997; Matavelli, 2008; El-Azim El-kamash, 2011). Normal semen is white, homogeneous and opalescent (IRRG Guidelines, 2005). According to Matavelli (2008), the ejaculate is mostly milky-white, but the best quality is found in creamy-white semen. Arrebola and Fernandez (2011) reported that pearly-white semen is good quality and other colours are classified as poor. Likewise, a uniform appearance is the most desirable.

2.9 Effect of Heat Stress on Rabbit Buck Reproduction

Thermal environmental conditions of high ambient temperature and high relative humidity, characteristic of the hot-dry season in Nigeria, where the temperature humidity index (THI) exceeds a value of 30°C considered as a major cause of severe heat stress (Nizza *et al*., 2003 and Dzenda *et al*., 2011). The negative effects of high ambient temperatures on reproductive traits of rabbit bucks are well known, and several works using different breeds reported their susceptibility to heat stress that produces drastic changes in their biological functions that can lead to impairment of production and reproduction (Marai *et al*., 2002; Theau-Clément *et al*., 2009).

Rabbits, as a homoeothermic animal, can regulate the heat input and output of their bodies using physical, morphological, biochemical, and behavioural processes to maintain a constant body temperature (Marai and Habeeb, 1994). They are very sensitive to high environmental temperature, where the dense fur and lack of sweat glands make heat loss very difficult above the zone of thermal neutrality. Excess heat is dissipated mainly by expired air (evaporation of water), stretching out to increase body surface, and vasodilatation of ear veins (Harkness, 1988). Johnson *et al*. (1957) reported that short hair and larger ears helped the cooling process in New Zealand White rabbits.

The impact of heat stress on rabbits has been reported by Ondruska *et al*. (2011) that total and daily feed intake, feed conversion ratio, and total and daily gain in body weight for growing rabbits are affected negatively by elevated temperature. According to Marai *et al*. (2002), animals with higher heat load make effort to dissipate body heat resulting in increased body temperature, as well as increase in consumption of water and a decline in feed intake. Selection of suitable breed of rabbit to particular environment conditions is very much essential for successful rabbit production (Kumaresan *et al*., 2011).

In general, reproductive performance of male rabbits is enhanced under long day conditions and disturbed with high temperature (Roca *et al*., 2005). Season is one of the important factors that have influenced the variation in semen quality and fertility (Mathevon *et al*., 1998). Humid hot season is not desirable for the production of highly motile and fertile semen (Perumal *et al*., 2015). The severity of damage to sperm cells subjected to heat stress varies with the intensity, frequency and duration of heat exposure (Collins and Lacy, 1969; Paul *et al*., 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Areaand Meteorological Data

The study was carried out at the rabbitary pen of the animal house of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The institution is located in Samaru, a suburb of Zaria Nigeria; situated on latitude 11^0 12" N and longitude 07^0 37" E, and located in the Northern Guinea Savannah zone of Nigeria.The period of the study was during the hot-dry season from March to June. The climatic data were collected from the Meteorological Unit, Institute for Agricultural Research, Samaru-Zariafor a period of 10months from September,2015 to July, 2016. The mean daily air temperature and relative humidity were calculated as the average of two recordings (10 am and 04 pm)using wet- and dry-bulb thermometersand according to the manufacturer’s manual attached. The temperature-humidity index (THI) was calculated using the following formula for rabbits(Marai *et al.*,2002):

THI: $db^0C - [(0.31 - 0.31(RH))(db^0C - 14.4)]$

Where db ^{0}C is dry-bulb temperature and RH is the relative humidity, expressed in percentage.

3.2 Experimental Animals

Twenty four (24), apparently, healthy, domestic rabbit bucks (*Oryctolagus cuniculus*) 8 ± 2.0 months old with average body weight of 2.2 ± 0.5 kg were used for the study. The bucks were screened and treated with broad-spectrum medication (ivermectin, penicillin-streptomycin and oxytetracycline) against haemoparasites, helminths and other microorganisms before the commencement of the experiment. Water and feed were provided *ad libitum*. The bucks were housed in standard rabbit cages, one buck per cage.

3.3Plant Extract Sample

Cissus quadrangularis extract (USP Labs Super Cissusherbal supplements) as obtained from Amazon (an online retailing outlet in the United Kingdom and United States of America).

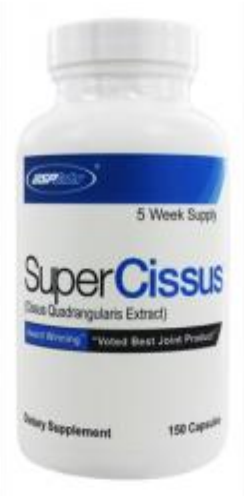

Plate II: Super Cissus ®(produced by USP labs, Dallas, Texas 75220, USA) - a supplement of *Cissus quadrangularis* extractused for the research.

3.4 Experimental Design

After 56 days of acclimatisation, the rabbit bucks were randomly assigned into four groups of six each, designated as group A, B, C and D. The bucks in all groups were fed *ad libitum* on a diet with composition and proximate analysis as shown in Table 3.1 and Table 3.2, respectively. The period of feeding lasted for 134 days. In the course of feeding, blood samples were collected forth-nightly, and semen samples once a week for evaluation. At the end of feeding, two bucks from each group were humanely euthanised and the testes were harvested for histological examination.

Table 3.1: Dietary composition (%)

Diet make-up	**(%)**
Maize	30.16
Maize offal	35.32
Groundnut cake	28.12
Bone meal	4
Palm oil	1
Vitamin premix	0.5
Salt	0.5
Methionine	0.4
Total	**100**

Table 3.2: Proximate analysis/chemical composition of experimental diet

Feed composition	A (%)	B (%)
Carbohydrate	41.96	40.96
Protein	12.48	12.88
Lipid	29.75	29.75
Fibre	10.06	10.06
Ash	12.47	12.87
Moisture	3.34	3.54

3.5 Measurement of Live Weight Changes

Live-weights of the bucks in each of the groups were evaluated weekly for 18 weeks using a measuring weight scale (Camry mechanical dial spring scale 10kg-South El Monte, California 91746, USA). This was carried out weekly in the morning before feed intake throughout the research period, and changes in weights were recorded in kilograms (kg).

3.6 Semen Collection and Evaluation

3.6.1. Assembling of the artificial vagina

The bucks were trained for semen collection for 6 weeks during the acclimatisation period of 2 months. Semen collection was done using a specially designed artificial vaginafor rabbits as shown (Plate III).

The artificial vagina (AV) was assembled as follows: a short plastic cylinder was obtained; a latex condom was used as a liner, whose end was cut off to allow both ends opened. A rubber band was used to fix the liner on the cylinder at one end, then glycerol was administered into the space between the cylinder and the rubber liner and the other end of the cylinder was fixed with another rubber band to assemble the AV. The assembled AV was placed in a beaker of warm water at 40°C, the warm water caused expansion of the glycerol within the liner and also provided the necessary pressure and temperature. Traces of water were cleaned from the AV, a short test tube was attached at one end of the AV and the other end lubricated with non-perfumed petroleum jelly for easy penetration.

To collect the semen from the bucks, it was ensured that the collector was properly gloved and a rabbit doe was introduced to the buck's cage to tease the buck. The buck was monitored closely as it attempted to mount the doe. The AV was placed gently in the direction of the vulva, so that the buck directed the penis into the AV for penetration and eventual ejaculation.

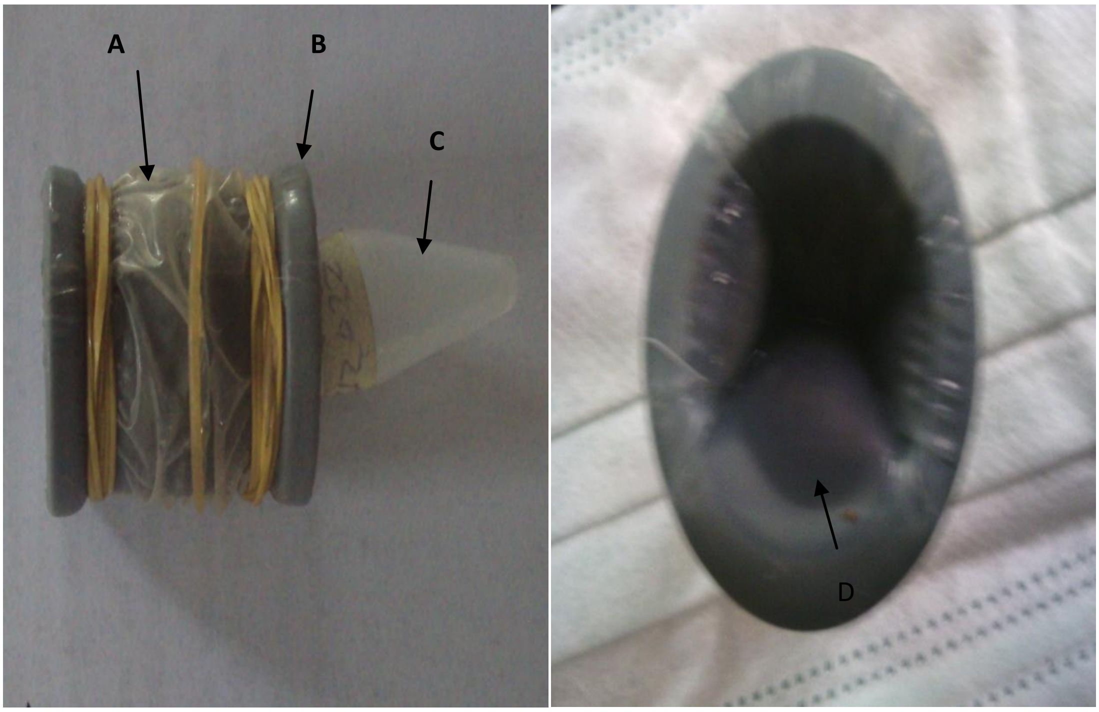

Plate III: Specially designed Artificial Vagina (AV) for rabbits.

A. rubber liner fixed on both ends by rubber bands. **B.** Plastic cylinder. **C**. Short test tube for collection of ejaculates. D. Expanded glycerol within the liner, narrowing the lumen of the AV.

3.6.2 Semen evaluation

The semen ejaculate obtained was evaluated as described by Zemjanis (1970), as follows; visual or gross evaluation of the ejaculate soon after collection for volume, colour and pH while microscopic examination was evaluated for individual motility, concentration, percentage live spermatozoa and abnormalities.

3.6.2.1. Volume: The volume was read directly from the calibrated tube used for the collection.

3.6.2.2. Colour: The colour was evaluated as milky, creamy or watery and designated 1, 2 or 3 for scoring the semen as described by Zemjanis (1970).

3.6.2.3. Semen pH: This was determined by dipping a litmus paper into the ejaculate and corresponding colour changes were checked on a pH colour chart and recorded.

3.6.2.4. Gross motility: This was examined as soon as possible after collection. A drop of the semen sample was placed on a pre-warmed glass slide, then covered with a slip and examined at ×10 magnification.

3.6.2.5. Spermatozoa concentration: This was determined using Neubauer haemocytometer as described by Azawi and Ismaeel (2012). Micropipette was used to aspirate 25 µL of semen and diluted with 5 mL of 3 % NaCl in a test tube, dilution factor of 5000. Outside of the pipette was wiped to remove any adhering semen. A cover slip was placed on the haemocytometer and two drops of the diluted semen was placed under the cover slip on each side of the haemocytometer. The haemocytometer then carefully placed in a pre-wetted chamber and the lid closed and left for 5 minutes. It was then examined using a microscope at ×40 magnification and the sperm cells were counted in five Thomasquares of the chamber (i.e. four corner and the center squares). The semen concentration was calculated as follows:

Concentration (sperm cells/mL) = Number of sperm cells counted in the twenty five small squares × dilution factor × 10^4

3.6.2.6. Percentage live sperm cells: This was determined as described by Esteso *et al.* (2006). A thin smear of the semen was made on a clean grease free slide and stained with Eosin-Negrosin stain. This technique was based on the principle that Eosin-Negrosin penetrates and stains dead sperm cells, while live sperm cells repel the stain. Dead spermatozoa stained pinkish or reddish, while live spermatozoa remained colourless. One hundred stained and unstained sperm cells were counted when the slides were dried, using light microscopy at ×40 magnification and percentage of each estimated.

3.6.2.7. Sperm abnormalities: They were determined by making a thin smear of the semen sample, on clean grease-free glass slide and stained with Eosin-Negrosin. One hundred sperm cells were counted per slide using hand counter under light microscopy at × 40 magnifications. Five cell types were recorded: normal cell, free head, free tail, coiled tail and bent tail.

3.7 Determination of Gonadal Sperm/Spermatid Reserves

Gonadal sperm/spermatid reserves were determined as described by Rekwot *et al*. (1994), with slight modifications. Two bucks from each group were humanely slaughtered and the testes removed, the length, weight and volume of each testis were determined using a measuring tape, digital weight balance and water displacement method, respectively and the values recorded. The *tunica albuginea* was carefully removed with a scalpel blade from each testis. The testicular spermatozoa number determined by homogenisation (Igboeli and Rakha, 1971; Egbunike *et al*., 1976). Each testis was homogenised in 25 mL of physiological saline solution using a mortar and pestle, antibiotics (Streptomycin sulphate 1mg/mL and Penicilin G 100 IU/mL) was added to the solution. The homogenate volume was measured after rinsing the mortar with 10mL of physiological saline solution and added to the effluent. Thereafter, 2.5 mL of the homogenate was transferred into a conical flask and further diluted with 40 mL of normal saline solution. The diluted testicular homogenate sample was stored overnight at 5°C and filtered through gauze and the filtrate volume measured. Spermatozoa/spermatids concentration was determined using haemocytometer according to the method of Kwari and Waziri (2001).

3.8 Determination of Epididymal Sperm Reserves

This was done as described by Olukole *et al*. (2010). The epididymis was carefully removed from the testis with scalpel blade and the length and weight of the head, body and tail portion were determined using a measuring tape and digital weighing balance. These portions were minced separately in 20 mL of normal saline with a sharp scissors and stored for 24 h at 5°C. The products were then filtered through gauze and the volume measured. Then 1mL of epididymal filtrate was then diluted with 2 mL of normal saline and the concentration of the sperm reserves was determined using Neubauer haemocytometer under a light microscope.

3.9 Blood Sampling for Haematological Examination.

Four millilitres (4 mL) of blood samples were collected forthnightly through the marginal ear veno-puncture using 25 gauge hypodermic needle and shared into ethylenediamine tetra acetic acid (EDTA) impregnated sample bottles for haematology and plain sample bottles for serum extraction for testosterone and antioxidant analysis.

3.10 Biochemical Assay

3.10.1 Malondialdehyde (MDA)

This was done according to the method described by Okhawa *et al.* (1979) with slight modification by Atawodi *et al.* (2011). Exactly 2mL of 15% trichloroacetic (TCA) acid was measured into a test tube, 2mL of thiobabitutric (TBA) acid was added and 100µL of serum homogenate was added. The mixture was incubated at 80^0C for 30 minutes in a water bath and allowed to cool for some time, followed by centrifugation at 805 x g for 10 minutes. The clear supernatant was collected and the absorbance determined at 535nm in a spectrophotometer (Spectrumlab 23A).

TBA’s concentrations were expressed in nmol/mg protein calculated as follows:

$$\text{Concentration nmol/mg protein} = \frac{\text{Absorbance of sample}}{1.5\text{x}10^{-5-}\text{x protein conc. (mg)}}$$

3.10.2 Catalase (CAT)

This was measured using Aebi’s method (1974). 10µ of serum was added to a test tube containing 2.8 mL of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1mL of freshly prepared 30mM hydrogen peroxide (H_2O_2) and the decomposition rate of H_2O_2 was measured at 240nm for 5minutes on a spectrophotometer. A molar extinction coefficient of $0.041\text{nM}^{-1}\text{-cm}^{-1}$ was used to calculate the catalase activity.

3.10.3 Superoxide dismutase (SOD)

Superoxide dismutase was done by the method of Fridovich (1989). Exactly 100µL of serum was added to 2.5mL of 50mM carbonate buffer. The reaction was started with the addition of 0.3mL of 0.3mM adrenaline. The reference mixture contained 2.5mL of 50mM carbonate buffer, 0.3mL 0.3mM and 0.2mL of distilled water. The absorbance was measured over 30 seconds up to 150 seconds at 480nm.

Calculations:

Increase in absorbance per minute = $(A_2\text{-}A_1)/2.5$

Percentage inhibition = 100-(increase in absorbance for sample/increase in absorbance of blank) x 100)

3.10.4 Glutathione concentration (GPx)

This concentration measurement was done according to Ellman (1959) as described by Rajagopalan *et al.*(2004). 150µL of serum was added to 10% TCA and centrifuge at 1500g for 5minute. 1mL of the supernatant was treated with 0.5mL of Ellman’s reagent (5,5-

dithiobisnitrobenzoic acid -DTNB) and 3mL of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm. The quantity of glutathione concentration was deduced.

3.11 Testosterone Assay and Procedure

Once the serum was thawed, testosterone (17-Hydroxy-4-androstene-3-one) was assayed using the Testosterone AccuBind^{TM} Microplate Test System kit (Monobind Inc, USA, Product Code 3725-300) in the Hormone Laboratory, Biotechnology Research Program of National Animal Production Research Institute (NAPRI), Zaria. The test kit manufacturer instruction was followed.

Before proceeding with the assay, serum references of testosterone, samples and reagents, were brought to room temperature (25± 2°C). The procedure was carried out as follows:

i. A total of 10 µL (0.010 mL) of each serum reference for testosterone (0.0, 0.1, 0.5, 01.0, 2.5, 5 and 12 ng/mL) were added and coated in duplicate wells A1 through B12 (A1-A12, B1-B2).
ii. Pipettte 10µL (0.010 mL) of respective serum samples into wells B3 through H12 (B3-B12, C1-C12, D1-D12, E1-E12, F1- F12, G1-G12, H1-H12)
iii. Add 50µL (0.050 mL) of the working testosterone enzyme reagents to all the wells.
iv. The microplates were gently swirl for 30 seconds to mix,
v. Add 50µL of testosterone biotin reagents were added to all the wells.
vi. The microplates were gently swirl again for 30 seconds to mix and then covered and incubated for 60 minutes at room temperature.
vii. The contents were discarded following incubation in a sink by decanting and the plates were blot dried with absorbanct tissue paper. These were washed three times by tapping and blot drying on a tissue paper following addition of 350 µL of wash buffer in each well before each wash.
viii. Add 100µL of working substrate solutions to the entire well in the same order and then incubated at room temperature for 15 minutes.

ix. Finally, 50 µL of stop solutions were added toall the wells and gently mix for 15 seconds.

x. The absorbance of each well was read at 450nm (wavelength) in ELx800 microplate reader within 20 minutes of adding the stop solution.

xi. Absorbance from the micro reader was subjected to MasterPlex® ReaderFit ELISA analysis software from which absorbance for each duplicate serum reference versus corresponding testosterone concentration in ng/mL was plotted. A best-fit curve was automatically generated. Testosterone concentration in ng/mL for each unknown sample was interpolated from best-fit curve.

3.12 Histological Examination

At the termination of the experiment, two bucks from each group were humanely sacrificed, the testes sample was taken in Bouin's solution for histology. These were refrigerated at 4°C for seven days for fixation, thereafter, the tissues were processed and infiltrated with molten paraffin wax as described by Spencer *et al*. (2013). The testes were dehydrated then embedded in paraffin blocks using automatic embedding station (Leica E. G. 1160 Germany). Then sectioned at 5 microns thickness using a rotatory microtome (Leica RTRM 2125, Germany)as described by Spencer *et al*. (2013). Each section was dewaxed and stained with Haematoxylin and Eosin (H&E) using standard staining procedures according to Jones *et al*. (2008). Finally, each slide was examined under the light microscope at ×250, photomicrographed and interpreted using High Definition (HD) Leica DM750 (Germany) with photo attachment.

3.13 Data Analysis

Data collected were expressed as mean ± standard error of mean (SEM).Repeated measure one-way analysis of variance (ANOVA) was used to test for differences between groups, followed by Tukey's multiple comparison Test (using GraphPad Prism version 5.0).Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Pre-Treatment Parameters

The mean ± SEM of the body weight, semen colours, ejaculate volume, pH, gross spermatozoa motility and concentration of the bucks pre-treatment are presented in Table 4.1.

Table 4.1: Mean (± SEM) values of semen characteristics and live weight of the rabbit bucks pre-treatment

Parameters	**Groups**			
	A	**B**	**C**	**D**
Body Weight (kg)	2.20 ± 0.39	2.33 ± 0.24	2.20 ± 0.22	2.27 ± 0.22
Colour	1	1	1	1
Ph	7.63 ± 0.46	7.62 ± 0.43	7.34 ± 0.30	7.40 ± .030
Volume (mL)	1.03 ± 0.57	0.76 ± 0.18	0.75 ± 0.19	0.90 ± 0.27
Motility (%)	64.58 ± 8.81	67.60 ± 7.34	64.00 ± 7.64	67.72 ± 8.05
Concentration (×10^6/mL)	124.78 ± 34.04	140.33 ± 36.12	123.87 ± 33.36	145.35 ± 26.55
Live spermatozoa (%)	75.86 ± 2.03	75.62 ± 2.02	76.28 ± 2.03	76.50 ± 2.03
Abnormal sperm cells (%)	23.26 ± 1.93	23.42 ± 2.07	25.55 ± 2.15	24.95 ± 1.71

(*P* □ 0.05) Colour: milky = 1,n = 6

A = Control
B = 125mg/mL
C = 250mg/mL
D = 500mg/mL

4.2 Live Weight Changes

Table 4.2 shows the mean (± SEM) live weight values (kg) of the rabbit bucks following treatment with *Cissus quadrangularis* extract for 12 weeks. Values obtained in the treated groups did not differ significantly(P □ 0.05) from those ofthe control.

Table 4.2: Mean (± SEM) values of live weight (kg) of rabbit bucks administered with graded doses of *Cissus quadrangularis*supplement.

Weeks	Group A	Group B	Group C	Group D
1	2.30 ± 0.14	2.33 ± 0.23	2.33 ± 0.22	2.33 ± 0.22
2	2.29 ± 0.15	2.35 ± 0.24	2.35 ± 0.22	2.35 ± 0.22
3	2.30 ± 0.17	2.37 ± 0.24	2.37 ± 0.22	2.37 ± 0.22
4	2.30 ± 0.16	2.38 ± 0.24	2.39 ± 0.21	2.40 ± 0.24
5	2.31 ± 0.17	2.40 ± 0.24	2.41 ± 0.23	2.43 ± 0.24
6	2.32 ± 0.16	2.42 ± 0.24	2.43 ± 0.23	2.45 ± 0.25
7	2.35 ± 0.18	2.45 ± 0.25	2.46 ± 0.24	2.48 ± 0.23
8	2.39 ± 0.17	2.47 ± 0.24	2.48 ± 0.23	2.49 ± 0.23
9	2.41 ± 0.18	2.49 ± 0.24	2.51 ± 0.23	2.53 ± 0.22
10	2.43 ± 0.17	2.51 ± 0.25	2.52 ± 0.24	2.49 ± 0.24
11	2.44 ± 0.18	2.52 ± 0.25	2.54 ± 0.27	2.55 ± 0.21
12	2.46 ± 0.17	2.53 ± 0.24	2.55 ± 0.46	2.57 ± 0.22
Overall mean± SEM	2.36 ± 0.17	2.44 ± 0.24	2.45 ± 0.25	2.45 ± 0.23

P □ 0.05

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

4.3 Semen Characteristics

The values (mean ± SEM) of semen volume, motility, concentration, percentage live and sperm abnormalities for the four groups (A, B, C, and D) are shown inTables 4.3-4.9. No significant(*P* □ 0.05) differences were observed between the control group and the treated bucks.

Table 4.3: Mean (± SEM) values of semen volume (mL) of rabbit bucks administered graded doses of*Cissus quadrangularis* supplement.

Weeks	Group A	Group B	Group C	Group D
1	0.32 ± 0.24	0.37 ± 0.15	0.95 ± 0.38	0.80 ± 0.36
2	0.17 ± 0.10	0.75 ± 0.27	0.57 ± 0.25	0.77 ± 0.46
3	0.50 ± 0.32	0.87 ± 0.31	0.40 ± 0.13	0.55 ± 0.37
4	0.50 ± 0.23	0.80 ± 0.08	0.60 ± 0.23	0.58 ± 0.22
5	0.65 ± 0.28	0.75 ± 0.20	0.68 ± 0.40	0.67 ± 0.29
6	0.43 ± 0.23	0.50 ± 0.14	0.58 ± 0.25	0.72 ± 0.30
7	0.63 ± 0.29	1.00 ± 0.24	0.62 ± 0.30	0.83 ± 0.47
8	0.52 ± 0.15	0.87 ± 0.17	0.70 ± 0.23	0.88 ± 0.45
9	0.68 ± 0.20	0.60 ± 0.11	0.48 ± 0.31	0.62 ± 0.39
10	1.07 ± 0.51	0.97 ± 0.19	0.65 ± 0.29	0.52 ± 0.15
11	0.78 ± 0.25	0.83 ± 0.21	0.67 ± 0.24	0.73 ± 0.27
12	0.97 ± 0.37	0.88 ± 0.11	0.75 ± 0.40	0.75 ± 0.19
Overall mean ± SEM	0.60 ± 0.26	0.77± 0.18	0.64 ± 0.28	0.70 ± 0.33

P **□ 0.05**

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

Table 4.4: Mean (± SEM) values of semen motility (%) of rabbit bucks administered graded doses of *Cissus quadrangularis* supplement.

Weeks	Group A	Group B	Group C	Group D
1	47.50 ± 14.65	55.00 ± 11.76	37.50 ± 16.97	54.17 ± 13.81
2	24.17 ± 11.50	48.33 ± 14.36	34.17 ± 16.12	38.33 ± 14.87
3	23.33 ± 14.81	35.81 ± 16.50	40.00 ± 17.94	17.50 ± 12.50
4	38.65 ± 17.83	58.46 ± 15.95	43.33 ± 13.08	42.50 ± 15.53
5	33.00 ± 11.03	60.03 ± 12.96	33.91 ± 14.24	51.67 ± 13.21
6	34.17 ± 12.74	55.17 ± 11.67	55.00 ± 17.61	45.83 ± 15.35
7	50.00 ± 16.18	68.50 ± 13.98	44.17 ± 19.98	64.17 ± 13.99
8	55.83 ± 15.30	59.18 ± 11.99	68.33 ± 14.36	48.33 ± 17.83
9	50.38 ± 13.87	61.33 ± 14.11	58.24 ± 14.12	58.33 ± 13.64
10	65.18 ± 14.11	74.67 ± 11.67	59.17 ± 13.99	60.12 ± 12.52
11	57.50 ± 14.07	80.35 ± 13.12	49.76 ± 16.09	67.50 ± 14.01
12	66.67 ± 13.40	88.33 ± 12.40	70.83 ± 18.23	50.55 ± 16.43
Overall mean ± SEM	45.53 ± 14.12	62.10 ± 13.37	49.53 ± 16.06	49.92 ± 14.47

P □ **0.05**

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

Table 4.5: Mean (± SEM) values of semen concentration (× 10^6/mL) of rabbit bucks administered graded doses of *Cissus quadrangularis* supplement.

Weeks	Group A	Group B	Group C	Group D
1	70.33 ± 46.63	123.30 ± 41.61	78.17 ± 39.17	105.20 ± 48.06
2	63.33 ± 34.08	156.00 ± 47.42	69.17 ± 37.20	51.67 ± 35.43
3	28.67 ± 25.06	110.50 ± 45.04	84.17 ± 42.42	36.17 ± 23.54
4	40.83 ± 23.50	88.83 ± 31.40	69.33 ± 33.87	77.83 ± 36.59
5	50.17 ± 17.34	72.33 ± 21.40	79.50 ± 23.38	79.67 ± 25.12
6	71.50 ± 32.05	103.00 ± 39.48	136.00 ± 45.50	113.00 ± 18.35
7	84.33 ± 35.37	95.67 ± 23.34	102.50 ± 46.32	105.50 ± 39.01
8	81.83 ± 27.54	103.80 ± 24.34	99.80 ± 25.98	93.67 ± 30.85
9	76.50 ± 21.68	81.50 ± 22.87	79.00 ± 30.22	69.17 ± 25.81
10	112.30 ± 24.94	120.50 ± 30.43	106.70 ± 27.28	86.83 ± 26.44
11	108.50 ± 28.40	136.50 ± 17.04	122.80 ± 28.41	81.00 ± 29.03
12	114.70 ± 16.72	153.70 ± 42.56	126.50 ± 27.72	74.50 ± 26.45
Overall mean ± SEM	75.25 ± 27.78	112.14 ± 32.24	96.14 ± 33.96	81.18 ± 30.39

P **□ 0.05**

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

Table 4.6: Mean (± SEM) values of percentage live spermatozoa of rabbit bucks administered graded doses of *Cissus quadrangularis* supplement.

Weeks	Group A	Group B	Group C	Group D
1	30.83 ± 15.62	52.50 ± 11.74	49.17 ± 17.63	56.67 ± 14.06
2	32.50 ± 14.59	63.33 ± 13.33	48.00 ± 15.86	48.33 ± 15.58
3	21.67 ± 13.76	61.67 ± 13.02	40.00 ± 18.07	23.33 ± 14.81
4	10.00 ± 10.00	50.83 ± 16.40	36.67 ± 16.72	22.50 ± 14.24
5	42.50 ± 13.52	47.50 ± 15.59	44.17 ± 14.52	34.17± 15.51
6	22.50 ± 14.24	64.17 ± 13.57	49.17 ± 15.62	41.67 ± 18.69
7	52.50 ± 17.02	70.00 ± 5.16	66.67 ± 13.58	41.67 ± 18.87
8	52.50 ± 17.26	70.00 ± 14.61	65.83 ± 14.17	52.50 ± 17.11
9	46.67 ± 15.20	52.50 ± 17.40	49.17 ± 15.94	40.83 ± 18.55
10	61.67 ± 12.63	72.50 ± 4.43	42.50 ± 13.52	35.83 ± 16.15
11	51.67 ± 16.57	75.00 ± 3.65	66.67 ± 14.36	40.83 ± 18.28
12	51.67 ± 14.87	77.50 ± 5.28	62.50 ± 13.40	47.50 ± 15.26
Overall mean ± SEM	39.72 ± 14.61	63.13 ± 11.18	51.71 ± 15.28	40.49 ± 16.40

P □ 0.05

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

4.4Spermatozoa Morphological Abnormalities

Cissus quadrangularis supplementadministered to the rabbit bucks had no significant effects across the treatment groups B, C and D on the sperm morphology($P > 0.05$). However, treated group B (125 mg/mL/kg body weight) had higher number of normal spermatozoa than the other groups (Table 4.7).

Table 4.7: Mean (± SEM) spermatozoa morphological abnormalities (%) of rabbit bucks

administered graded doses of *Cissus quadrangularis* supplement for 12 weeks.

Parameters	Group A	Group B	Group C	Group D	*P*– value
Normal Cells	47.89 ± 4.88	70.99 ± 3.53	55.90 ± 4.38	47.44 ± 3.15	□ 0.05
Free Heads	2.76 ± 0.37	2.69 ± 0.32	2.86 ± 0.59	4.31 ± 0.28	□ 0.05
Free Tails	2.20 ± 0.39	2.57 ± 0.50	2.50 ± 0.32	3.86 ± 0.21	□ 0.05
Coiled Tails	1.79 ± 0.54	2.01 ± 0.53	1.71 ± 0.28	2.26 ± 0.39	□ 0.05
Bent Tails	2.13 ± 0.37	2.03 ± 0.35	2.38 ± 0.39	3.26 ± 0.41	□ 0.05

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

4.5 Gonadal and Extragonadal Weight, Length and Spermatids/Sperm Reserves

The gonadal weight, length and sperm reserves of rabbit bucks administered the various graded doses of *Cissus quadrangularis* supplementare shownTable 4.8.There was no significant (*P*> 0.05) difference in gonadal weight and length and sperm reserves between the different groups.

The mean (± SEM) values for epididymal weight, length and sperm reserves of rabbit bucks groups administered with *Cissus quadrangularis* supplement at graded doses of 125, 250 and 500 mg/mL, respectively (Table 4.9). No significant (*P*> 0.05) difference was observed in epididymal weight and length between the groups.

Table 4.8: Mean (± SEM) values of gonadal weight, length and sperm reserves of rabbit

bucks administered graded doses of *Cissus quadrangularis* supplement.

Parameters	Group A	Group B	Group C	Group D
Weight (g)				
Right Testis	2.33 ± 0.34	2.93 ± 0.28	2.88 ± 0.07	2.39 ± 1.39
Left Testis	2.36 ± 0.31	3.01 ± 0.11	2.85 ± 0.79	2.72 ± 0.05
Length (cm)				
Right Testis	3.10 ± 0.10	1.50 ± 1.50	3.50 ± 0.30	3.10 ± 0.10
Left Testis	3.40 ± 0.20	3.50 ± 0.30	3.30 ± 0.50	3.50 ± 0.10
Gonadal Sperm Reserve (× 10^6)				
Right Testis	7.00 ± 2.00	13.00 ± 9.00	10.00 ± 6.00	9.00 ± 2.00
Left Testis	3.50 ± 0.50	12.00 ± 5.00	7.00 ± 5.00	4.50 ± 1.50

$P > 0.05$

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 2

Table 4.9: Mean (± SEM) values of epididymal weight, length and epididymal sperm reservesof rabbit buck groups administered with graded doses of *Cissus quadrangularis* supplement.

Parameters	Groups	Caput		Corpus		Cauda	
		Right	Left	Right	Left	Right	Left
Weight(g)	A	0.28 ± 0.06	0.20 ± 0.02	0.03 ± 0.03	0.00 ± 0.00	0.55 ± 0.08	0.56 ± 0.08
	B	0.12 ± 0.12	0.32 ± 0.03	0.00 ± 0.00	0.02 ± 0.02	0.27 ± 0.27	0.67 ± 0.09
	C	0.30 ± 0.11	0.36 ± 0.10	0.07 ± 0.03	0.04 ± 0.01	0.53 ± 0.15	0.57 ± 0.20
	D	0.39 ± 0.02	0.39 ± 0.06	0.00 ± 0.00	0.03 ± 0.03	0.45 ± 0.05	0.41 ± 0.04
Length(cm)	A	1.15 ± 0.05	1.05 ± 0.05	3.80 ± 1.20	3.05 ± 0.95	1.90 ± 0.10	1.90 ± 0.10
	B	1.25 ± 1.25	1.60 ± 0.30	1.30 ± 1.30	2.10 ± 0.20	1.00 ± 1.00	2.15 ± 0.05
	C	1.80 ± 0.70	1.60 ± 0.20	3.85 ± 0.45	3.70 ± 0.30	1.90 ± 0.00	1.80 ± 0.40
	D	1.70 ± 0.30	2.00 ± 0.00	2.60 ± 0.40	3.00 ± 0.60	1.75 ± 0.25	1.95 ± 0.05
ESR(x 10^6)	A	4.50 ± 4.50	5.00 ± 5.00	0.00 ± 0.00	0.00 ± 0.00	99.50 ± 2.50	87.50 ± 18.50
	B	9.50 ± 9.50	13.00 ± 6.00	2.50 ± 2.50	0.00 ± 0.00	116.00 ± 49.00	140.00 ± 51.00
	C	4.50 ± 4.50	6.00 ± 2.00	0.00 ± 0.00	2.00 ± 2.00	60.50 ± 60.50	96.00 ± 33.00
	D	2.00 ± 2.00	9.00 ± 5.00	0.00 ± 0.00	3.50 ± 1.50	44.50 ± 24.50	87.50 ± 21.50

$P > 0.05$, ESR - epididymal sperm reserves

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 2

4.6 Haematological Parameters

The mean (± SEM) of packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) count and haemoglobin concentration are presented in Tables 4.10-4.13.

The mean (± SEM) values for PCV of rabbit bucks in the various groups, A, B, C and D were not significantly different ($P > 0.05$) (Table 4.10). Similarly, the mean (± SEM) values for RBC counts of rabbit bucks in groups A, B, C and D were not significantly different ($P > 0.05$) however group B (125 mg/mL) had significantly ($P < 0.05$) lower RBC than the control (group A) at week 4 post-treatment (Table 4.11). The mean (±SEM) values for WBC counts of rabbit bucks in groups A, B, C and D were not significantly different (P □ 0.05) with the exception of bucks in group B (125 mg/mL) and D (500 mg/mL) that had significantly lower ($P < 0.05$) WBC than the control (group A) at week 10 post-treatment (Table 4.12). The mean (± SEM) values for haemoglobin concentration of rabbit bucks in groups A, B, C and D were not significantly (P □ 0.05) different. Exceptions were observed at week 10 when the haemoglobin concentration values for the control group, group B (125 mg/mL)and C(250 mg/mL) were significant ($P < 0.05$) (Table 4.13).

Table 4.10: Mean (± SEM) values of packed cell volume(%) of rabbit bucks administered with graded doses of *Cissus quadrangularis* supplement.

Weeks	Group A	Group B	Group C	Group D
0	38.47 ± 0.72	39.37 ± 1.23	40.27 ± 0.72	38.58 ± 1.16
2	39.28 ± 0.91	40.35 ± 0.72	39.62 ± 0.84	38.08 ± 0.76
4	30.67 ± 2.09	40.70 ± 3.14	35.40 ± 2.16	39.05 ± 2.27
6	34.43 ± 1.80	43.53 ± 2.98	40.40 ± 3.53	41.23 ± 2.55
8	34.07 ± 2.34	35.10 ± 2.60	39.92 ± 1.87	36.38 ± 2.72
10	31.38 ± 2.43	37.43 ± 3.17	37.10 ± 5.58	37.55 ± 4.40
12	35.03 ± 2.68	37.52 ± 2.69	40.28 ± 3.57	37.22 ± 3.42
Overall mean ± SEM	34.76 ± 1.85	39.14 ± 2.36	38.10 ± 2.61	38.30 ± 2.47

P □ 0.05

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

Table 4.11: Mean (± SEM) values of red blood cell (×10^{12}/L) of rabbit bucks administered with graded doses of *Cissus quadrangularis* supplement.

Weeks	Group A	Group B	Group C	Group D
0	5.70 ± 0.19	5.80 ± 0.09	5.70 ± 0.09	5.63 ± 0.09
2	5.53 ± 0.28	5.50 ± 0.04	5.64 ± 0.08	5.61 ± 0.09
4	4.55 ± 0.30^{a}	5.88 ± 0.45^{b}	5.15 ± 0.36	5.79 ± 0.31^{b}
6	4.64 ± 0.25	5.69 ± 0.47	5.37 ± 0.39	5.60 ± 0.30
8	4.86 ± 0.43	5.33 ± 0.54	5.85 ± 0.23	5.44 ± 0.47
10	4.87 ± 0.26^{a}	5.64 ± 0.44^{b}	5.92 ± 0.23^{b}	5.98 ± 0.37^{b}
12	5.09 ± 0.42	5.49 ± 0.40	5.97 ± 0.52	5.85 ± 0.35
Overall mean ± SEM	5.03 ± 0.30	5.62 ± 0.35	5.66 ± 0.27	5.70 ± 0.28

P □ 0.05,$^{a, b}$= means with different superscript on the same row are significantly different.

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

Table 4.12: Mean (± SEM) values of white blood cell (WBC) count ($\times 10^9$/L) of rabbit bucks administered with graded doses of *Cissus quadrangularis* supplement.

Weeks	Group A	Group B	Group C	Group D
0	8.13 ± 0.22	9.85 ± 0.86	9.63 ± 0.92	8.65 ± 0.65
2	7.77 ± 0.41	8.35 ± 1.10	9.43 ± 0.66	8.20 ± 0.44
4	6.22 ± 0.37	8.35 ± 0.69	6.80 ± 0.99	5.22 ± 0.57
6	5.78 ± 0.69	5.60 ± 0.57	5.90 ± 1.08	4.22 ± 0.44
8	7.53 ± 0.77	6.75 ± 0.76	8.23 ± 0.85	6.85 ± 0.96
10	5.22 ± 0.57^{a}	14.53 ± 9.57^{b}	7.15 ± 1.57	5.35 ± 0.85^{c}
12	5.98 ± 0.67	6.22 ± 0.89	6.67 ± 0.94	5.98 ± 1.16
Overall mean ± SEM	6.66 ± 0.53	8.52 ± 2.06	7.69 ± 1.00	6.35 ± 0.72

P □ 0.05;$^{a, b,c}$= means with different superscripts on the same row are significantly different.

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

Table 4.13: Mean (± SEM) values of haemoglobin concentration (g/dL) of rabbit bucks administered with graded doses of *Cissus quadrangularis* supplement.

Weeks	**Group A**	**Group B**	**Group C**	**Group D**
0	13.02 ± 0.24	13.73 ± 0.19	13.05 ± 0.18	12.52 ± 0.30
2	12.97 ± 0.39	14.08 ± 0.21	13.60 ± 0.21	12.85 ± 0.21
4	10.78 ± 0.60	13.22 ± 0.78	11.87 ± 0.70	13.05 ± 0.64
6	10.83 ± 0.48	13.12 ± 0.70	12.03 ± 1.02	13.03 ± 0.41
8	10.27 ± 0.90	10.98 ± 0.84	11.82 ± 0.84	11.30 ± 0.85
10	9.75 ± 0.84^{a}	14.20 ± 2.46^{b}	10.55 ± 1.47^{c}	11.52 ± 1.25
12	10.62 ± 0.87	11.50 ± 0.78	12.52 ± 1.23	11.10 ± 0.94
Overall mean ± SEM	11.18 ± 0.62	12.98 ± 0.85	12.21 ± 0.81	12.20 ± 0.66

P □ 0.05; $^{a, b, c}$= means with different superscripts on the same row are significantly different.

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

4.7 Testosterone Levels

The testosterone values of the bucks in the various groups are shown in Table 4.14. A significant (P< 0.05) difference in values was only observed between the treated bucks in group D (500 mg/mL) and untreated control (group A) at week 6 and across all the treated groups at week 12.

Table 4.14: Mean (± SEM) of testosterone values (ng/dL) of rabbit bucks administered with graded doses of *Cissus quadrangularis* supplement.

Weeks	Group A	Group B	Group C	Group D	P - value
0	1.11 ± 0.33	1.20 ± 0.32	1.18 ± 0.25	1.02 ± 0.24	□ 0.05
6	0.67 ± 0.12^{a}	0.89 ± 0.27	0.99 ± 0.22	1.64 ± 0.19^{b}	< 0.05
12	0.64 ± 0.16^{a}	1.15 ± 0.24^{b}	1.19 ± 0.22^{b}	1.58 ± 0.11^{c}	< 0.01
Overall mean ± SEM	0.81 ± 0.20	1.08 ± 0.28	1.12 ± 0.23	1.41 ± 0.18	

$^{a, b, c}$= means with different superscripts on the same row are significantly different.

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

4.8.Antioxidant Enzymes Assay

The results of the assay of antioxidant enzymes are shown in Table 4.15. Significant ($P < 0.05$) differences in the levels of MDAconcentrations were observed in the control (group A,187.9 ± 26.24) and the treatment groups (B, 218.7 ± 14.28; C,272.6 ± 9.97). Superoxide dismutase (SOD) activities were also significantly ($P < 0.05$) different between group A (5.79 ± 0.51) and the treatment group D (11.18 ± 0.52). However, catalase and glutathione peroxidase activitiesin the treated groups (B, C and D) were not significantly ($P > 0.05$) different from that of the control (group A).

Table 4.15: Antioxidant biomarkers of rabbit bucks administered with graded doses of *Cissus quadrangularis* supplement.

BIOMARKERS	GROUP A	GROUP B	GROUP C	GROUP D	***P* - value**
MDA (nmol/mL)	187.9 ± 26.24^{a}	218.7 ±14.28^{b}	272.6 ± 9.97^{c}	255.5 ± 4.26	< 0.01
GPx (µM)	9.46 ± 0.49	9.59 ± 0.49	9.78 ± 1.10	9.95 ± 0.64	□ 0.05
Catalase (U/mg)	1.50 ± 0.14	1.68 ± 0.17	1.81 ± 0.20	2.03 ± 0.11	□ 0.05
SOD (U/mL)	5.79 ± 0.51^{a}	7.81 ± 1.03	8.15 ± 1.27	11.18 ± 0.52^{b}	< 0.01

MDA= Malondialdehyde, GPx= Glutathione peroxidase, SOD= Superoxide dismutase;
$^{a, b, c}$=means with different superscripts on the same row are significantly different.

A = Control
B = 125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

4.9. Testicular Histological Changes

The testicular histological changes observed in the control (group A) and treated groups B (125 mg/mL), C (250 mg/mL) and D (500 mg/mL), respectively are shown in Plates III-VI. The control had normal architecture with their lumen filled with spermatogenic cells, and spermatozoa at various stages of maturation (Plate III). While mild vacuolations and few prominent Leydig cells were observed in the group treated with the CQ supplement at 125 mg/mL (Plate IV). Marked vacuolations and testicular degeneration and loss of seminiferous tubules normal architecture are observed in the testicles of treated group administered with 250 mg/mL of CQ supplement (Plate V). Absence of spermatogenic cells with hyperplasia of Leydig cells was observed in the seminiferous tubules of bucks treated, with 500 mg/mLof CQ supplement(Plate VI).

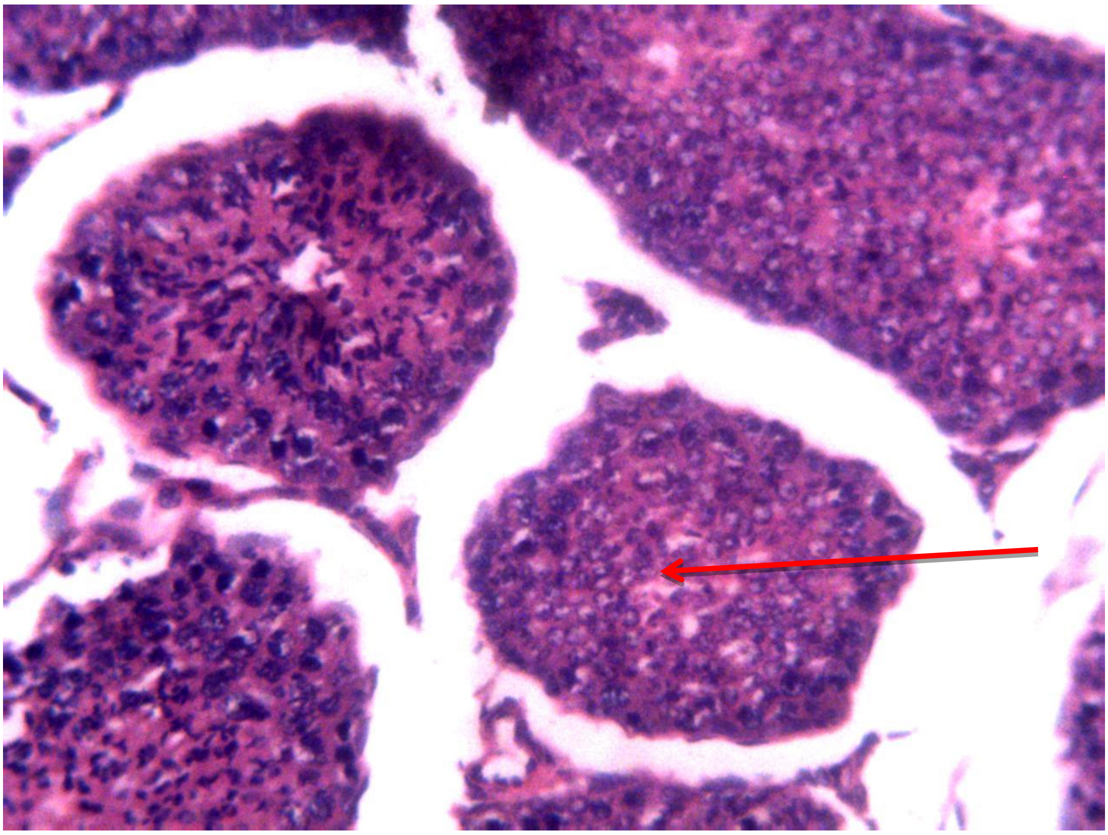

Plate III: Photomicrograph of the testis of rabbit buck (Control group A). Showing the seminiferous tubules filled with spermatogenic cells showing mature spermatozoa (red arrow). (H & E x 250).

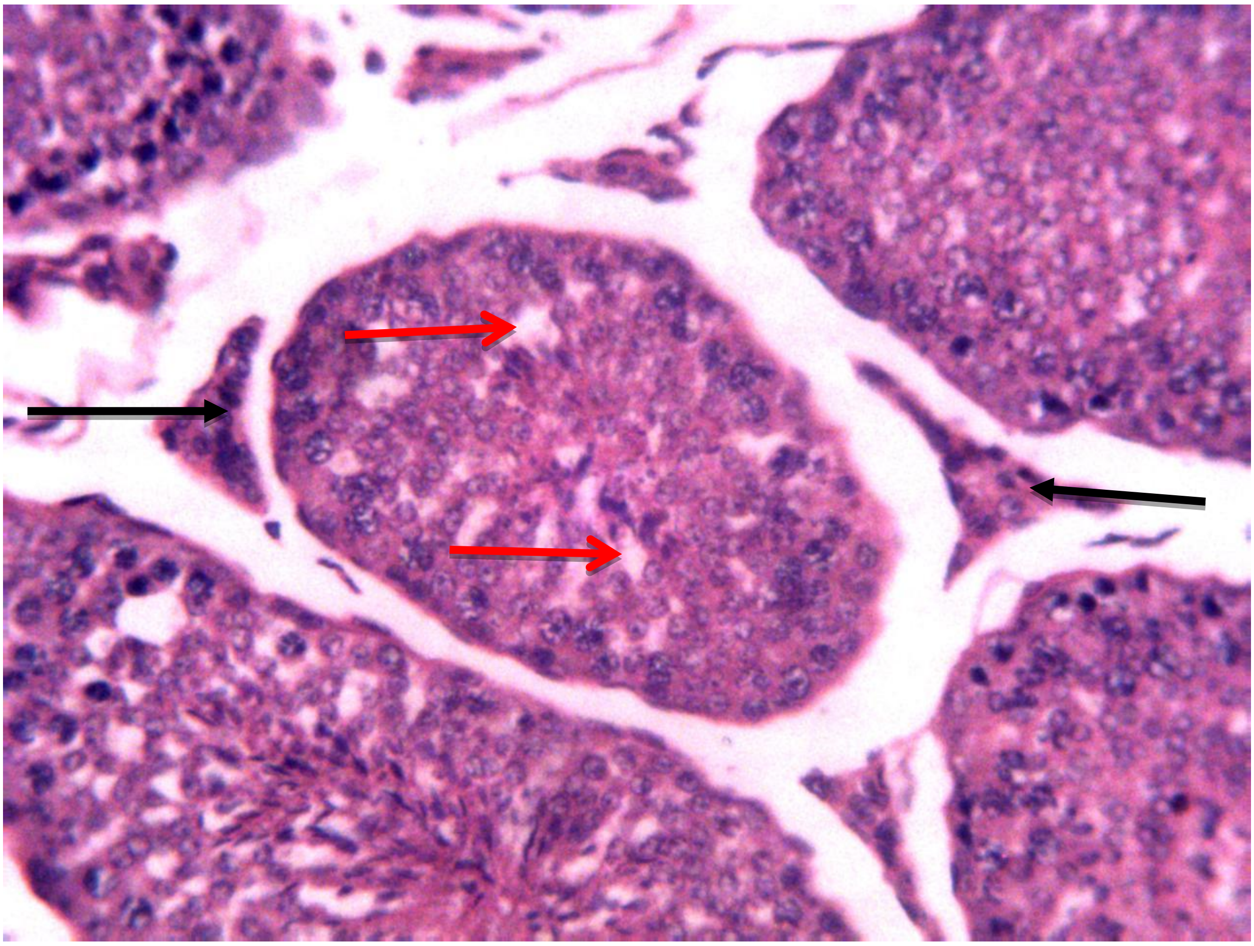

Plate IV: Photomicrograph of the testis of rabbit buck (Group B) showing mild loss of spermatogenic cells (vacuolations) (red arrow) inthe seminiferous tubules and prominent Leydig cells (black arrow). (H & E x 250).

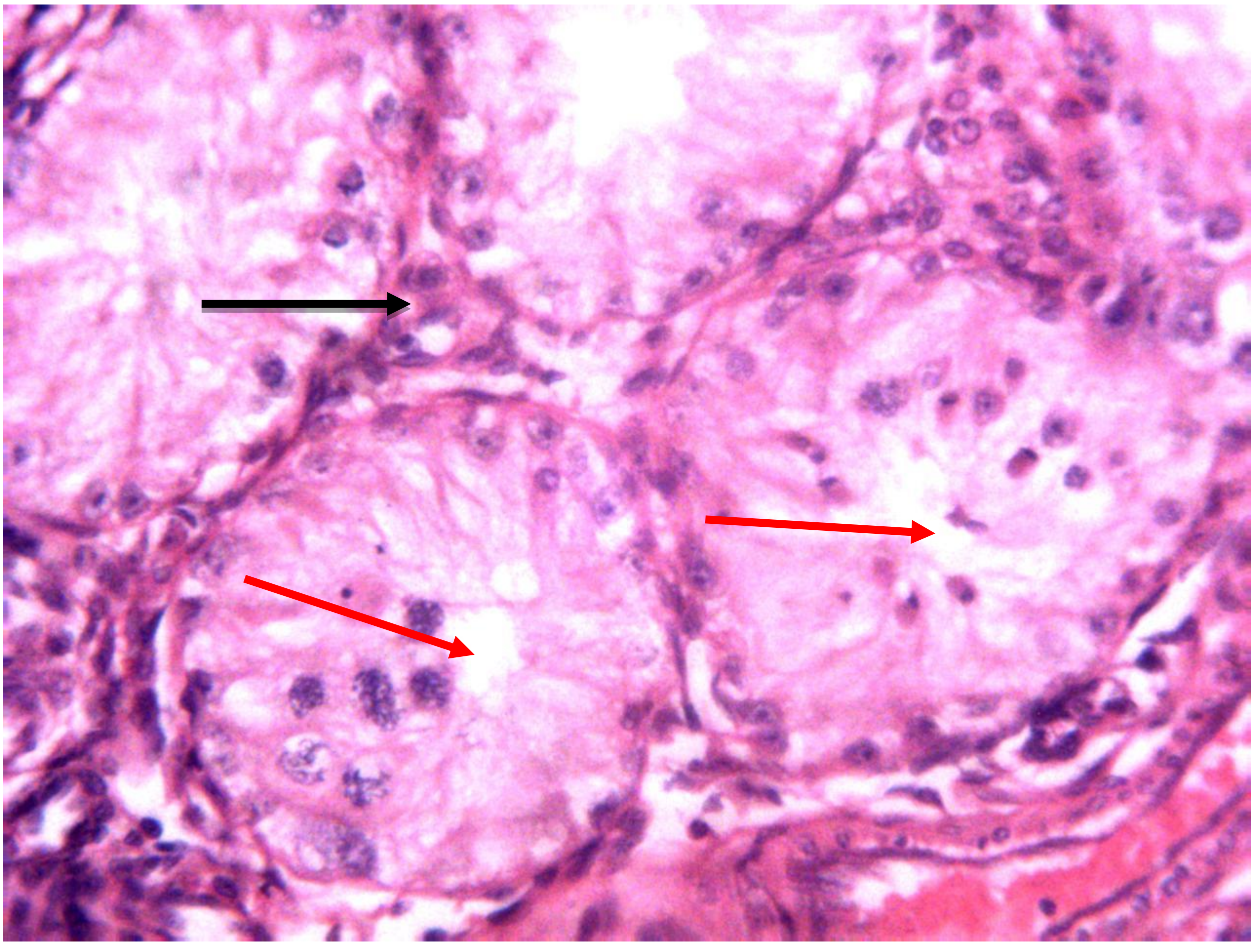

Plate V: Photomicrograph of the testis of rabbit buck (Group C) showing marked depletion of spermatogenic cells (red arrow) in the seminiferous tubules with hyperplastic Leydig cells (black arrow). (H & E x 250).

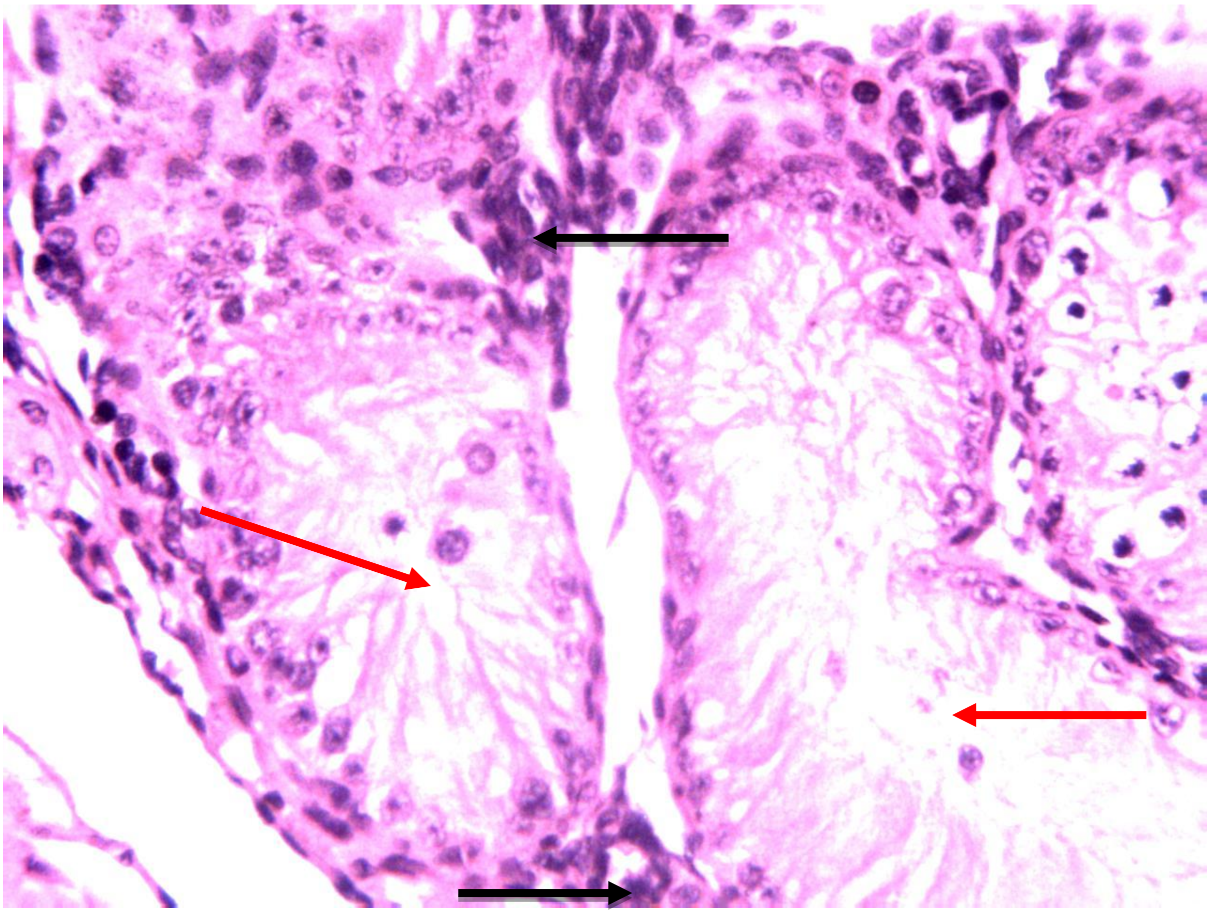

Plate VI: Photomicrograph of the testis of rabbit buck (Group D) showing loss of cellular architecture of the seminiferous tubules, absence of spermatogenic cells (red arrow) and accompanying Leydig cell hyperplasia (black arrow). (H & E x 250).

CHAPTER 5

5.0 DISCUSSION

The semen volume values for all the groups were not of significantly different ($P > 0.05$), although the semen volume for all the treated groups were higher than the control up to week 8.These may be attributed to antioxidant property of the CQ supplement as reported by Jainu and Devi (2006), Kokilavani*et al*. (2014) and Atram (2015) that antioxidants contained in CQ could play a role in spermatogenesis and leading to an increase in sperm count.However, the values were lower than those of the control from week 9 to the end of the experiment.This may be attributed to the cytotoxic effects of phytosterol and other phytotoxic constituents of CQ extract. This finding agrees with the work of Meyer *et al*. (1998), Adcox *et al*. (2001) and Maguire *et al*. (2003), who reported the cytotoxic effect of the secondary metabolite of the CQ extract on testicular parenchyma.

Similarly, semen motility valuesfor all the groups were not significantly different ($P> 0.05$), though were higher than the control values.This could be due to the antioxidant activity of the phytoconstituents of the CQ supplement. This agrees with Daader *et al*. (1997) and Seleem *et al*. (2007), who reported reduction in semen motility and semen quality in New Zealand rabbit bucks due to the influence of high environmental temperatures which affect adversely spermatogenesis. However, the finding of this study contradicts that of Kokilavani *et al*. (2014) who obtained significant semen motility from the effect of CQ extract against quinalphos-induced male reproductive toxicity in mice. This may be due to antioxidant mediated effect of CQ extract and a controlled environmental temperature at 23 ± 1ºC, which is suitable for spermatogenesis.

There was no significant difference ($P > 0.05$)in the sperm concentration values between the control and experimental groups, however higher in the treated groups. This may be due to the ameliorative effect of antioxidants contained in the CQ supplementin hot-dry season as reported by Kokilavani *et al*. (2014) and Atram (2015). Also this varies with the result obtained by Kokilavani *et al*. (2014), who had a significant sperm cell concentration by the effect of CQ extract and *Commelina benghalensis* against quinalphos-induced male reproductive toxicity on mice, apparently due to high antioxidant activity of CQ extract and a favourable environmental temperature (at 23 ± 1ºC), which are suitable for spermatogenesis.

The percentage live spermatozoa values for all the groups (control and treatment) were not significantly different ($P > 0.05$), but with higher values in the treated than the control, which could be due to the protective effect of CQ extract. This agrees with the work of Lin *et al*. (2007) and Atram (2015). This contradicts the findings of Kokilavani *et al*. (2014), who observed a three-fold decrease in percentage viable sperm cells by the effect of CQ on quinalphos-induced male reproductive toxicity on mice. Treatments with CQ extracts and *Commelina benghalensis* had ameliorative and protective effects on the testis resulting in a restored and significant viability of sperm cells by the plant extracts, with CQ extract found to be more potent than *Commelina benghalensis*. The percentage abnormal spermatozoa valuesfor all the groups were not significantly different,but were higher than the control values. This may be attributed to the cytotoxic effects of phytosterol and other phytotoxic constituents of CQ extract, and the result agrees with the work of Meyer *et al*. (1998), Adcox *et al*. (2001) and Maguire *et al*. (2003), who reported the cytotoxic effect of the secondary metabolite of the CQ extract on testicular parenchyma.

This may also be related to the possible influence of high ambient temperature on spermatogenesis. However, the result contradicts the findings by Kokilavani *et al*. (2014), who observed lower percentage abnormal spermatozoa in micetreated with CQ extracts and *Commelina benghalensis*, apparently due to ameliorative and protective effects of the extracts.

This study has, therefore, shown that *Cissus quadrangularis* supplement had ameliorative effects on semen quality parameters during the experimental period. This may be in agreement with the guidelines of International Rabbit Reproduction Group (2005), which states that a minimum period of 10 weeks is recommended to determine the effect of certain exogenous factors in rabbits since the duration of spermatogenesis is about 7-8 weeks.

Results show that all the haematological parameters studied comprising of PCV, haemoglobin, RBC, WBC were not significantly different from those of control rabbit bucks and were all within the normal range as reported byOlabanji *et al.* (2007) and Research Animal Resource(2009) for rabbits. This agrees with the result obtained by Kothari *et al.* (2011), who evaluated the effects of *Cissus quadrangularis* on rats and observed no significant changes in haematological parameters. Similar observations were made in rats with no effect on haematological parameters following the administration of CQ extract (Shamina and Kokilavani, 2015). This study has, therefore, shown that *Cissus quadrangularis* supplement had no detrimental effects on haematological parameters during the experimental period.

Thetestosterone levelswere significantly higherin rabbit bucks in the treated group D (500mg/mL) at week 6 compared to control group. There was also an increase in the serum testosterone level at the end of the study in the treated groups as compared to that of the control. This may be due to the effect of some phytoconstituents contained in the CQ supplement(for example, phytosterol), which has been reported to increase circulating serum testosterone concentration of male voles following a two-week exposure (Nieminen *et al*., 2003).The presence of phytosterol as a flavonoid in the CQ supplement may play a role in altering androgen levels which may also be responsible for the enhanced male sexual behavior experienced by traditional users of the plant.This result agrees with those ofChiericato *et al*. (1995) and El-Hanoun *et al*. (2007, 2014), who recorded that testosteronesignificantly decreased in male rabbits exposed to heat stress as observed in rabbit bucks of the control group.High level of male intra-testicular testosterone is critically required for normal spermatogenesis, development and maintenance of morphology, and normal physiology of seminiferous tubules (Sharpe *et al.*, 1992).

In this study, the mean MDAconcentration was significantly lower in groups administered with 125 mg/mL and 250 mg/mL of CQ supplement than in the control group. The SOD was also significantly higher in rabbit bucks in the group administered with 500 mg/mL than that of the control group. The increase in SOD activitiesindicates the response of the primary antioxidant system to increased ROS generated from heat stress and the cytoprotective ability of the CQ supplement as reported by Jainu and Devi(2005a),Boots *et al.*(2008) and Kokilavani *et al*. (2014). Further, MDA concentrationin the testis indicates enhanced lipid peroxidation owing to tissue injury and malfunctions of antioxidant defence mechanism, caused by possible heat stress, and resulting in a decreased spermatogenesis. This findingindicates that heat stress initiated its adverse reproductive effects by the formation of ROS, as evident from the high levels of MDA and antioxidant enzymes in the blood serum (Leonard *et al*., 2003).

Histological results revealed matured spermatozoa within the seminiferous tubules in the control were observed in this study, while the treated groups of 125mg/mL and 250mg/mL showed the presence of mild to marked vacuolations and prominent Leydig cells. These changes are consistent with testicular degeneration as reported by Foster and Ladds (2007). The changes were more severe in rabbit bucks administered with the highest dose of500mg/mL.

It is unclear specifically which chemical constituents and their mechanism of action that are responsible for these effects produced by CQ supplement, but the observed effect may be attributed to the presence of phytosterol andpossibletoxicantscontained in CQ extract, such as oxalate, tannin, phytate and saponins content (Mishra *et al*., 2010).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

From the findings of this work, the following conclusions were made:

i. Administration of 125 mg/mL of CQ extract showed higher testicular function (Group B bucks had better sperm concentration ($153.70 \pm 42.56 \times 10^6$/mL) than the sperm concentration $126.5 \pm 27.72 \times 10^6$/mL, $114.7 \pm 16.72 \times 10^6$/mL and $74.5 \pm 26.45 \times 10^6$/mL for group A, C and D respectively.

ii. Testosterone values were increased in the CQ treated groups (B = 1.15 ± 0.24 ng/dL, C = 1.19 ± 0.22 ng/dL and D = 1.58 ± 0.11 ng/dL), which may be responsible for its use as a libido enhancer by the natives.

iii. The concentration of MDA decreased significantly ($P < 0.05$) in the group administered with 125 mg/mLof CQ extract (218.7 ± 14.28) compared to 250 mg/mL (272.6 ± 9.97) and 500 mg/mL (255.5 ± 4.26) treated groups.

iv. The activity of SOD activity was significantly ($P < 0.05$) higher in the 500 mg/mL CQ treated group (11.18 ± 0.52), compared to the control (5.79 ± 0.51).

v. The extract of CQ has no effect on haematological parameters in rabbit bucks, but it induced testicular degeneration in the treated groups.

6.2 Recommendation

i. 125mg/mL of CQ extract is recommended for male animal dietary supplementation.

ii. Further studies should be carried out to assess the possible use of *Cissus quadrangularis* plant to improve male fertility.

iii. Further studies should be carried out to determine the mechanism of toxicity of the extract on testicular parenchyma.

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APPENDICES

APPENDIX I: Mean (± SEM) values of semen colour of rabbit bucks administered *Cissusquadrangularis* supplement.

WEEKS	GROUP A	GROUP B	GROUP C	GROUP D
1	1.00 ± 0.45	2.00 ± 0.00	1.33 ± 0.42	1.67 ± 0.33
2	1.00 ± 0.45	1.83 ± 0.17	1.17 ± 0.40	1.17 ± 0.31
3	0.83 ± 0.40	1.67 ± 0.21	1.17 ± 0.40	1.33 ± 0.42
4	1.33 ± 0.42	2.00 ± 0.00	1.67 ± 0.33	1.5. ± 0.34
5	1.33 ± 0.42	2.00 ± 0.00	1.17 ± 0.40	1.33 ± 0.33
6	1.33 ± 0.42	1.83 ± 0.17	1.33 ± 0.42	1.33 ± 0.42
7	1.67 ± 0.33	2.00 ± 0.00	1.17 ± 0.40	1.33 ± 0.33
8	1.50 ± 0.34	2.00 ± 0.00	1.50 ± 0.34	1.50 ± 0.34
9	1.67 ± 0.33	2.00 ± 0.00	1.67± 0.33	1.50 ± 0.34
10	1.50 ± 0.34	2.00 ± 0.00	1.50 ± 0.34	1.67 ± 0.33
11	1.67 ± 0.33	1.83 ± 0.17	1.17 ± 0.40	1.67 ± 0.33
12	2.00 ± 0.00	2.00 ± 0.00	1.67 ± 0.33	1.83 ± 0.17

***P*□ 0.05**

A = Control
B =125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

APPENDIX II: Mean (± SEM) values of semen pH of rabbit bucks administered *Cissus quadrangularis* supplement.

WEEKS	GROUP A	GROUP B	GROUP C	GROUP D
1	4.17 ± 1.89	7.33 ± 0.33	6.50 ± 1.36	6.17 ± 1.28
2	3.83 ± 1.74	6.83 ± 0.17	4.67 ± 1.48	6.17 ± 1.28
3	3.17 ± 1.42	7.17 ± 0.40	4.50 ± 1.43	4.67 ± 1.48
4	5.00 ± 1.61	7.00 ± 0.00	5.83 ± 1.25	5.67 ± 1.15
5	4.67 ± 1.48	7.33 ± 0.33	4.67 ± 1.48	6.17 ± 1.28
6	5.00 ± 1.61	7.67 ± 0.42	4.83 ± 1.58	5.00 ± 1.61
7	6.17 ± 1.28	6.83 ± 0.17	4.67 ± 1.48	6.17 ± 1.28
8	6.50 ± 1.36	7.67 ± 0.42	6.50 ± 1.36	6.50 ± 1.36
9	6.17 ± 1.28	7.00 ± 0.00	6.17 ± 1.28	6.50 ± 1.36
10	6.17 ± 1.28	8.00 ± 0.45	6.50 ± 1.36	6.17 ± 1.28
11	5.83 ± 1.17	7.00 ± 0.00	5.00 ± 1.61	5.83 ± 1.17
12	7.33 ± 0.33	7.00 ± 0.00	6.17 ± 1.28	7.67 ± 0.42

P □ 0.05

A = Control
B =125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

APPENDIX III: Mean (± SEM) values of the reaction time of rabbit bucks administered *Cissus quadrangularis* supplement.

WEEKS	GROUP A	GROUP B	GROUP C	GROUP D
1	1.33 ± 0.62	3.67 ± 0.72	3.67 ± 0.96	3.50 ± 0.76
2	4.33 ± 1.98	4.00 ± 0.68	4.33 ± 1.52	4.67 ± 1.15
3	3.67 ± 1.82	5.83 ± 1.78	2.50 ± 1.06	4.00 ± 1.55
4	4.83 ± 1.80	6.67 ± 0.92	4.50 ± 1.23	4.67 ± 2.17
5	3.67 ± 1.31	5.33 ± 1.20	2.67 ± 1.09	5.00 ± 1.59
6	3.83 ± 1.52	5.33 ± 0.61	3.33 ± 1.52	3.50 ± 1.29
7	5.00 ± 1.63	3.83 ± 0.48	2.83 ± 1.11	5.33 ± 1.41
8	4.17 ± 1.11	4.00 ± 0.58	5.50 ± 1.38	5.33 ± 1.17
9	3.50 ± 0.81	4.00 ± 0.26	4.50 ± 1.50	3.17 ± 0.83
10	5.67 ± 1.48	6.17 ± 1.08	6.33 ± 2.11	3.67 ± 0.92
11	3.17 ± 0.65	6.17 ± 1.49	3.50 ± 1.20	3.67 ± 0.76
12	4.00 ± 0.52	5.00 ± 0.26	3.17 ± 0.83	5.83 ± 2.87

P □ **0.05**

A = Control
B =125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

APPENDIX IV: Mean (± SEM) values of semen death ratio of rabbit bucks administered *Cissus quadrangularis* supplement.

WEEKS	GROUP A	GROUP B	GROUP C	GROUP D
1	19.17 ± 11.29	30.83 ± 8.11	10.83 ± 5.23	26.67 ± 9.89
2	19.17 ± 8.80	20.00 ± 5.77	15.00 ± 7.19	18.33 ± 6.54
3	11.67 ± 7.49	21.67 ± 6.01	10.00 ± 5.16	10.00 ± 6.46
4	10.00 ± 10.00	20.83 ± 9.95	12.50 ± 8.14	13.33 ± 6.79
5	24.17 ± 7.79	19.17 ± 7.35	15.83 ± 7.57	22.50 ± 8.14
6	10.83 ± 6.88	25.00 ± 3.65	8.33 ± 4.01	17.50 ± 5.74
7	14.17 ± 5.83	30.00 ± 5.16	8.33 ± 4.77	16.67 ± 4.22
8	14.17 ± 6.51	13.33 ± 4.94	17.50 ± 6.29	14.17 ± 6.11
9	20.00 ± 7.30	17.50 ± 6.55	14.17 ± 6.88	15.83 ± 11.29
10	21.67 ± 5.11	27.50 ± 4.43	24.17 ± 7.79	14.17 ± 6.64
11	15.00 ± 5.48	22.50 ± 5.28	9.17 ± 4.17	16.67 ± 6.28
12	15.00 ± 6.58	19.17 ± 5.83	19.17 ± 6.64	20.83 ± 6.38

P□ 0.05

A = Control
B =125 mg/mL
C = 250 mg/mL
D = 500 mg/mL
n = 6

APENDIX 5

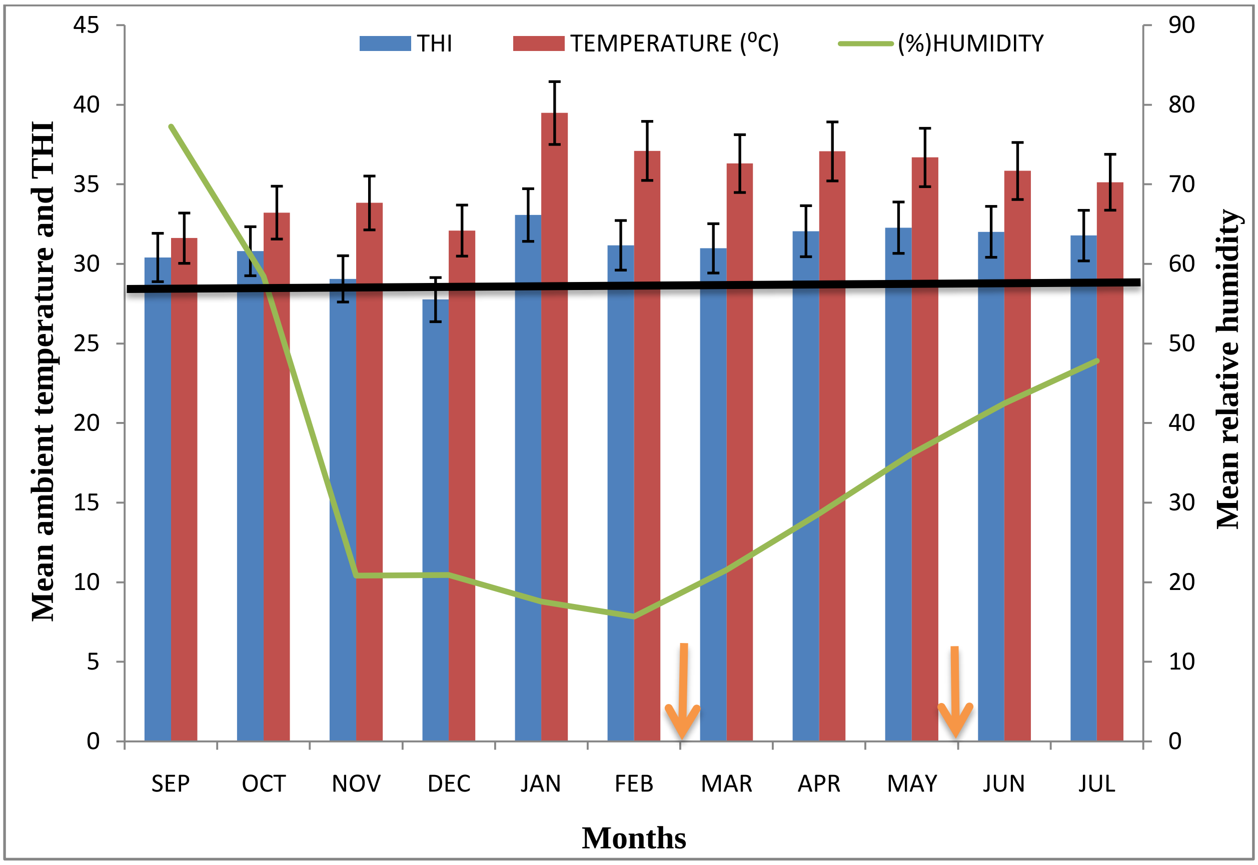

Figure 1: Shows mean temperature-humidity index (THI), ambient temperature (°C) and relative humidity (%) of Samaru-Zaria environment during the acclimatisation and experimental period.Below the black line - indicates the comfort zone for rabbits and above the black line – indicates heat stress. Orange arrows indicate the duration of the research.

NOTE:

THI formula for rabbits

THI = db°C – [(0.31 – 0.31(RH)) (db°C – 14.4)]

Where db°C = the temperature and RH = relative humidity / 100 (Marai *et al*. 2002).

Data source: Meteorological Unit, Institute for Agricultural Research, Samaru-Zaria.