STUDIES ON SOME ANTIFERTILITY INDICES OF AQUEOUS EXTRACT OF PAWPAW (*Carica papaya)* SEEDS AND ITS EFFECTS ON SOME ACROSOMAL ENZYMES OF MALE WISTER ALBINO RATS

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (PhD) DEGREE IN BIOCHEMISTRY

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OCTOBER, 2011

CERTIFICATION

This dissertation titled "**Studies on some Antifertility indices of Aqueous Extract of Pawpaw (*Carica papaya)* Seeds and its Effects on some Acrosomal Enzymes of Male Wister Albino Rats"** by Onaolapo, Mary Aderoju Olalonpe meets the regulations governing the award of the degree of Doctor of Philosophy (PhD) in Biochemistry Department, Ahmadu Bello University, Zaria, Nigeria, and it is approved for its contribution to knowledge and literary presentation.

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DECLARATION

I hereby declare that this dissertation titled "**Studies on some Antifertility indices of Aqueous Extract of Pawpaw (*Carica papaya)* Seeds and its Effects on some Acrosomal Enzymes of Male Wister Albino Rats"** is a record of my own research work and has not been submitted for the award of a degree in any other university. The information derived from the literature has been duly acknowledged in the text and a list of reference provided.

_____	_____	_____
Name of Student	**Signature**	**Date**

DEDICATION

This work is dedicated to the Lord God Almighty, my Father, Friend, Teacher and Counsellor, who makes ALL things possible.

Quote for thought

"So then it is not of him that willeth nor of him that runneth but of God that showeth mercy" Romans 9:16

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ABSTRACT

The antifertility properties of *Carica papaya* seeds were investigated on the serum biochemistry, haematological profile and the testicular function of 90 male wister albino rats. Treatment of male albino rats orally with the crude and the chromatographic chloroform fraction of the aqueous extract of *Carica papaya* seeds led to reduced sperm counts, low percentage live sperm and reduction in percentage normal sperm. These effects were more pronounced with the animals treated with 200mg/kg body weight for both crude and the partially purified extract. In all these investigations, the weights of the animals, haematology and serum clinical chemistry and were not adversely affected. It was also observed that there was an unaltered serum testosterone level. The present study has added to earlier findings on the crude and partially purified seed extract by testing the effects on sialic acid and acrosomal enzymes namely acrosin and hyaluronidase levels. The 200mg/kg body weight dosage showed pronounced reductions for sialic acid, hyaluronidase and acrosin values after three weeks and one week treatment with the crude aqueous and partially purified extract of *Carica papaya* seeds respectively. Therefore it may be concluded that the aqueous extract of *Carica papaya* seeds possesses a great potential as a male antifertility agent. However, the extract may not be absolutely safe because it brought about increased percentage of sperm defects, caused cellular infiltration in testes, epididymis, liver and kidney.

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CHAPTER ONE

Introduction

1.1 Global Population Control

Globally, population has become an issue of concern, because rising human population throughout the world and more particularly in the developing and under developed nations has detrimental effects on the life supporting system on earth. Procreation is more than a physiological phenomenon; it is a major characteristic of man and ensures the continuity of life. It is conditioned by behavioural, social and cultural factors and has profound economic and political repercussions. In Nigeria and the rest of Africa today, there is a cycle of poverty and illiteracy as a result of over population and economic depression (Udoh *et al.,* 2005, Gupta and Sharma, 2006). Recently the United Nation said the Africa's economy is not growing fast enough to meet targets to reduce poverty in the world poorest continent. The paper further said the per-capital income may expand to 2.7 per cent in 2011 and 2.8per cent in 2012, but it is still lower than the three percent "minimum rate of growth to make a substantial dent in poverty" (United Nations, 2011). Population growth rate is usually influenced by the interplay of the three main demographic processes of fertility, mortality and migration. The relatively low mortality of about 13 to 14 per 1000 (crude death rate) and a declining infant mortality rate, as well as increasing expectancy in the population, tend towards higher survival

chances that may swell the size of future population. The reality of this scenario might not necessarily be with the absolute size of the population but more importantly with the implications of the growth rate for the future size of the population, and the ability of the economy to grow commensurately with and therefore, cope with the increase in population size (Ugochukwu, 2010).

Fertility regulation, comprising contraception and management of infertility, forms an important component of reproductive health (Allag and Rangari, 2002). Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception among females, similar progress and possibilities on males are still slow and limited. With recent progress towards a better understanding of male reproductive physiology, there is a need to develop new contraceptive modalities for male (Chaudhury, 1993, Gupta and Sharma, 2006).

Recently, efforts are being made to explore the hidden wealth of medicinal plants for contraceptive use. With the exciting prospects of gene therapy, herbal medicine remains one of the common forms of therapy, available to much of world's population, to maintain health and treat diseases. One of such medicinal plants is *Carica papaya* commonly called pawpaw, a tropical and subtropical plant of the family Caricaceae

1.2 Carica papaya

Cultivated *Carica papaya* 'is a fast growing tree-like herbaceous plant in the family *Caricaceae* with four genera in the world. The genus *Carica linn* is the most common of which *Carica papaya linn* is the most widely cultivated and best known species, (Krishna, *et al.,* 2008). *Carica papaya* is widely believed to have originated from the lowlands of Eastern Central America (Nakasone and Paull, 1998). Presently, Carica *papaya* is grown in all tropical countries and many sub-tropical regions of the world (Garet, 1995). The plant is known as *Ibepe* in Yoruba, *Gwanda* in Hausa and *Okwuru Beke* in Ibo. *Carica papaya* is soft-wooded perennial plant that has a life span of about 5-10 years, although commercial plantations are usually replanted sooner. *Carica papaya* is an erect, usually unbranched tree or shrub with a crown and large palmate leaves emerging from the apex of the trunk (Duke, 1978). The soft, hollow, cylindrical trunk ranges from 30cm in diameter at the base to about 5cm in diameter at the crown. Under normal conditions, *Carica papaya* trees can reach 8 – 10 meters in height, though in cultivation, suitable heights for easy harvesting is of the desired target (Garret, 1995, Fig 1.1).

Carica papaya is an interesting tree in that some are either male (staminate) or female (Pistillate), whereas others have both male and female flowers on same plant. When sexes are separate, fruits are produced on the female through cross-pollination. *Papaya* is reported to tolerate drought, high pH, insects, laterites,

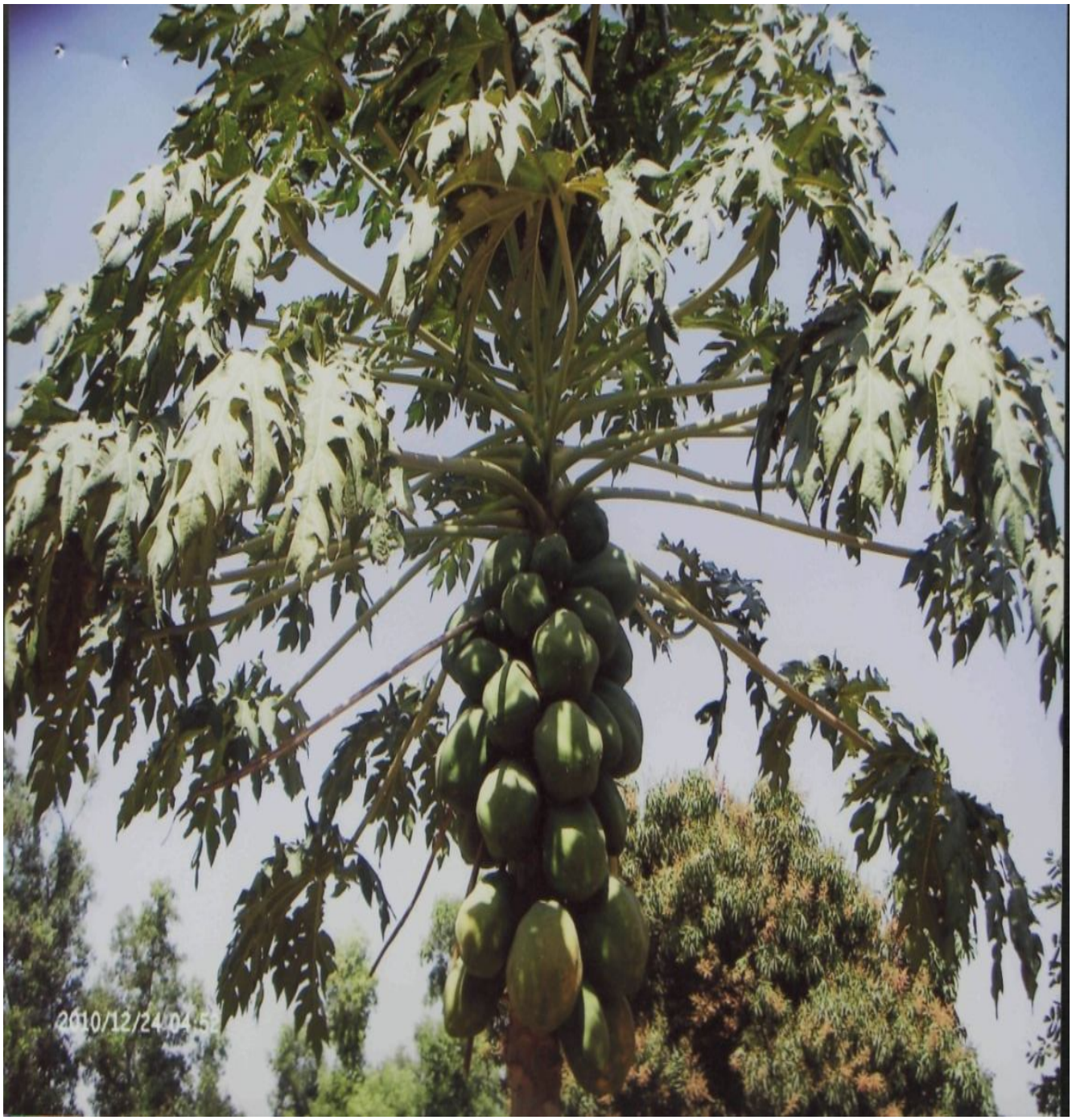


Figure 1.1 *Carica papaya* plant with fruits

myobacteria; slope and virus (Duke, 1978). Ripe papaya fruits have smooth, thin yellow-orange coloured skin of about 1.5 to 4cm thick, usually sweet and juicy. Maturated fruits contain numerous grey-black spherical seeds about 5mm in diameter each enclosed in a gelatinous membrane (Duke, 1978; Nakasone and Paull, 1998). The fruit bearing trees are usually less than 18 months old (Krishna *et al.,* 2008). Economically, *Carica papaya* is the most important species within the *Caricaceae,* being widely cultivated for consumption as either fresh drinks, Jams, and Candies, or as dried and crystallized fruit. The green fruit, leaves and flowers are also cooked as vegetable (Nakasone and Paull, 1998).

1.2.1 Some Chemical Consituents of Carica papaya

Carica papaya belongs to a group of plant species called laticiferous plants. These plants contain specialized cells (laticifens), dispersed throughout most of the plant tissues which secrete a substance known as 'latex' (El Moussaoui *et al*., 2001). Latex is a milk-like liquid containing complex mixture of chemical compounds with diverse chemical activities. According to Nakasone and Paull (1998); El Moussaoui *et al.,* (2001) and Krishna *et al.,* (2008), the leaves and fruits of *Carica papaya* are biochemically complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications. However only the unripe green fruits contain the milky latex. One of the most important chemical compositions of the latex is papain, a proteolytic enzyme employed in the textile industry for

Table 1.1: Some Chemical Constituents of *Carica papaya*

Parts of the plant	Constituents
Fruits	Protein fat, fibre, carbohydrate, minerals, calcium, phosphorous, iron, vitamin C, thiamine, riboflavin, niacin, carotene, amino acid , citric and malic acids (green fruits), volatile compounds: linalool, benzyl isothiocyanate, cis and trans 2, 6-dimethy-3, 6 epoxy-7 octen-2 ol, Alkaloid, a carpaine, benzyl β – D glucoside, 2- phenylethyl β-D-glucoside, 4-hydroxy-phdroxy-pheny -2 ethyl-p-D glucoside and four isomeric malonated benzyl-p-D/glucosides.
Juice	N-butyric, n-hexanoic and n-octanoic acids, lipids; myristic, palmitic, stearic, llinoleic, linolenic and cis-vaccenic and oleic acids
Seed	Fatty acids, crude protein, crude fibre, papaya oil, carpaine, benzylisocyanate, benzylglucosinolate, glucotropaclin, benzylthiourea, hentriacontane, p-sitosterol, caricin and an enzyme myrosin.
Root	Carposide and an enzyme myrosin
Leaves	Alkaloids carpin, pseudocarpain and dehydrocapaine 1 and II, choline, carposide, vitamin C and E
Bark	β- sitosterol, glucose, fructose, surose, galactose and xylitol
Latex	Proteolytic enzymes, papain and chemopapain, glutamine cyclotransferase, chymopapains A, B, and C, peptidase A and B and lysozmes.

Source: Bruneton (1999)

degumming silk and softening wool, in the cosmetic industry as ingredients in soaps and shampoo making, in chill-proffing beer, tenderizing meat, production of chewing gums and medically in the treatment of gangrenous wounds (Garret, 1995).

1.2.2 Medicinal Activity of Carica papaya

In traditional medicine, *Carica papaya* leaf, fruits and root extracts have been reported to possess medicinal properties (Akah *et al.,* 1997; Eno *et al.,*, 2000). Crushed *papaya* seed have been confirmed to have anti-helminthic action due to the presence of benzyl isothiocyanate (BITC), which have also been shown to have effect on vascular contraction (Eno *et al.,* 2000). Other studies have shown possible and anti-hypertensive activities for fruit extracts (Eno *et al.,* 2000). Whole and extract of *Carica papaya roots have* purgative effects (Akah *et al.,* 1997). Seeds have been the subject of evaluation for anti-fertility properties in male rabbits, rats and monkeys (Pathak *et al.,* 2000, Lohiya *et al.,* 2002). Also the petroleum ether extract of the rind of raw papaya fruit is said to exhibit significant anti malarial activity (Bhat and Surolia, 2007). Dried *Carica papaya* seeds have been found to be effective against human intestinal parasites (Okeniyi *et al.,* 2011). Blood pressure depression was recorded in renal and DOGA-induced hypertension in rats, after treatment with the fruit juice of *Carica papaya* Linn (Eno *et al.,* 2000)

Table 1.2: Medicinal uses of *Carica papaya* plant

Parts of the plant	Medicinal Uses/Disease conditions used to treat
Latex	Helminthosis dyspepsia, diarrhea, pain of burns and topical use, bleeding haemorrhoids, stomach ache, whooping cough.
Ripe fruits	Laxative, diuretic, dried fruits reduces enlarged spleen and liver, used in snake bite to remove poison, abortifacient, counter irritant, as paste in the treatment of ringworm and psoriasis, anti-fertility agents in males.
Seeds	Carminative, emmenagogue, vermifuge, abortifacient, counter irritant, as paste in the treatment of ringworm and psoriasis, anti-fertility agents in males.
Seeds juice	Bleeding piles and enlarged liver and spleen
Root	Abortifacient diuretics, checking irregular bleeding from the uterus, piles, anti-fungal activity
Leaves	Jaundice (fine paste), urinary complaints and gonorrhea (infusion), dressing wounds (fresh leaves), antibacterial activity, vermifuge, in colic, fever, beriberi, abortion (infusion), asthma (smoke).
Flowers	Jaundice, emmenagogue, febrifuge and pectoral properties.
Stem bark	Jaundice, anti-haemolytic activity, STD, sore teeth (inner bark), anti-fungal activity.

Source: Kirtikar and Basu (1998); Arya (2005)

1.2.3 Nutritional value

Carica papaya is a common man's fruit, which is relatively cheap and of a high nutritive value. It is low in calories and rich in natural vitamins and minerals (Krishna *et al.,* 2008). *Carica papaya* is the first among fruits for vitamins C, vitamin A, riboflavin, calcium, thiamine, iron, niacin, potassium and fibre (Nakasone and Paul, 1998; Krishna, *et al.,* 2008).

According to Durowaye (2005), pawpaw is made up of about 86.5 percent water, 9.5 percent carbohydrates, 0.5 percent protein, while the rest is mineral salts, especially calcium, phosphorous, tartaric and citric acids. Hence green papaya is used as vegetable, salads, spices, juices and confectionaries. Many people in the tropic love the fruit for its luscious taste (Bhattacharjie, 2001).

Regular consumption of papaya fruit ensures a good supply of vitamins A and C which are essential for good health, eyesight and prevention of early age blindness in children. The presence of papain and pepsin in unripe fruit makes it an excellent aid to digestion, particularly of protein food, whether at acid, alkaline or neutral medium (Krishna, et al 2008). Thus in dyspeptic patients, papain may help greatly in digesting proteins. The black seeds (Fig 1.2) are edible and have a sharp, spicy taste. They are sometimes ground up and used as a substitute for black pepper (The Wealth of India, 1992). The fermented papaya fruit is a promising nutraceutical as an antioxidant. It improves the antioxidant defence in elderly patients even without any overt antioxidant deficiency state (Marotta *et al.,* 2007).



Figure 1.2 Dried *Carica papaya* seeds

Table 1.3: Constituent Nutritive Value of 100g of Papaya Fruits

Constituents	Ripe papaya	Green papaya
Protein (g)	0.6	0.7
Fat (g)	0.1	0.2
Minerals (g)	0.5	0.5
Fibre (g)	0.8	0.9
Carbohydrate (g)	7.2	5.7
Energy (k cal)	32	27
Total carotene (µm)	2,740	0
Beta carotene (µm)	888	0

Source: The wealth of India, (1992)

The fruits, seeds, roots, leaves, bark and the latex of the plant contain several chemicals and substances (Tables 1.1 – 1.3) which make C*arica papaya* very versatile and effective in treating several diseases in traditional medicine among many communities of the world (Akah *et al.,* 1997, Eno *et al.,* 2000; Arya 2005).

1.3 Medical Indications for Male Infertility

In animals, spermatozoa is the male gamete produced in the epididymis through spermatogenesis, during a biological process of gradual transformation of germ cells into spermatozoa over an extended period of time in the seminiferous tubule of the testis (Hess, 1990). The epididymis consists of three compartments: the caput, the corpus and the cauda epididymides (Ogunlade *et al.,* 2006). The storage and maturation of spermatozoa takes place in the cauda epididymides (Satchell, 1989). It has been established (Brooks *et al*., 1974; Casilla and Erickson, 1975; Milkowski *et al.,* 1976; Michelle DeRosa *et al.,* 2005) that in the epididymal spermatozoa, carnitine facilitates the transfer of long chain fatty acids across the mitochondria membrane for p-oxidation, thereby regulating the level of acetyl-coA. Acetylated carnitine is probably the main source of intracellulary bound acetate carried by the epididymal spermatozoa into the ejaculated semen. It is highly probable that carnitine and acetylated carnitine are metabolized by spermatozoa in the same way as elsewhere in the body, especially as in the liver to metabolic energy (Wolf, 1965). Johansen and Bohmer (1976), reported that

human intracellular level of acetylated carnitine is positively correlated with sperm motility. The intracellular free L-carnitine accumulated by spermatozoa might participate in a buffering role, trapping excess mitochondrial aceyl-CoA as L-acetylcarnitine (Casillas and Erickson, 1975; Milkowski *et al.,* 1976). This system protects the activity of pyruvate dehydrogenase, a key enzyme for mitrochondrial respiration which is inhibited by excess acetyl CoA. In short, the epididymis provides a mechanism for sperm quality control. It has been reported that the number of sperm leaving the epididymis via the vas deferens is not lower than the number entering it from the testis (Lino *et al.,* 1967; Amann and Lambiase, 1969; Amann *et al.,* 1974). During the transit to the proximal epididymis, sperms undergo a number of biochemical changes which are important to confer upon them the ability to fertilize a mature ovum (Cooper, 1995).

Sperm output also depends on the secretions from various sex glands and epididymal epithelium into the semen which serve to nourish and activate the sperm cells. During ejaculation, spermatozoa are transported through the ductus deferens, ampullae, pelvic urethra and penis. Sperm from the ampullae (rich in fructose) are mixed with accessory secretions in the pelvic urethra (Amann and Lambiase, 1969 and Cooper, 1995). The relative contribution of the accessory organs to the seminal fluid differs between species. In the bull, the vesicular gland (rich in fructose) contributes about half of the ejaculate. In the rabbit, there are two

organs with a common duct, the glandula seminalis and the glandula vesicularies which are thought to be homologous to the seminal vesicles of other animals. Substances found in the secretions of the accessory glands include fructose, citric acid, ergothionine, inositol and prostaglandins (Lino *et al.,* 1967 and Milkowski, *ete al.,* 1976). The sperm-rich fraction contains most of the ergothionine which comes from ampullae. The relative resistance of rabbit and human ejaculates to cold shock is due to their cholesterol content and higher degree of maturation of the phospholipids of these species in contrast to those of the ram and bull. Thus lipids, especially cholesterol phospholipids are involved in cellular action and functions of sperm cells (Michelle DeRosa *et al.,* 2005).

The age and weight of male animals at puberty varies among different breeds and are directly under the control of hormones such as follicle stimulating hormones (FSH), estrogens, prostaglandins, testosterone and progesterone (Karg et al; 1976, Rekwot et at., 2000). According to Karg *et al.,* (1976), there is a very close association between onset of spermatogenic activity and increased testosterone concentrations in bulls, noting that very low levels of testosterone before puberty rose sharply at the onset (age or time) at which the generative organs of reproduction become functional and reproduction may occur. It was also concluded that concentrations of serum testosterone increased linearly with advancing age in bulls (Lunstra *et al.,* 1978).

Generally, infertility in male animals is diagnosed in terms of low sperm concentration, poor motility and high sperm defects (Schaller and Glander, 2000). Therefore there has been increasing efforts to device suitable biochemical parameters such as the levels of fructose, marker enzymes like alpha glycosidase and fructosidase for determination of infertility in male animals (Dandekar and Hankunar, 1997). Moreover, the activity of various enzymes on spermatozoa determine sperm quality since enzymes are key molecules for cellular functions for energy production, membrane transport and metabolism (Schaller and Glander, 2000). There has been indication that regulation of sperm motility may depend upon communication between protein kinases, protein phosphatase and their modulator (Schaller and Glander, 2000). According to Verma and Chinoy (2001) and Hess (1990), one of such modulators is sialic acid which is secreted in the epididymal epithelium and coats spermatozoa as they pass through the epididymis.

Thus coat of sialic acid on the membrane of maturing spermatozoa confers certain antigens and that affect the development of spermatozoa fertility capacity. Testicular sperm is infertile and must undergo maturational changes in the epididymis to acquire fertilizing capacity. Both the capacities for fertility and motility are acquired in the corpus epididymides (Hess, 1990). Sperm is produced in the testes continuously or during a breeding season, while the accessory glands add secretions to the seminal fluid as this passes from the vas deferens into the

urethra. The Leydig cells of the interstitial cells in the testes produce steroid (testosterone).

1.3.1 Plant Chemicals that Inhibit Fertility

Much information concerning the mechanisms by which chemicals or plant parts or their extracts may inhibit fertility has been obtained from studies carried out in commonly used laboratory species such as the rat, mouse, guinea pig and rabbit (Farnsworth *et al.,* 1975). The reproductive cycles of these species differ among themselves and as such can explain the varying effects sometimes exhibited by a given anti-fertility substance when tested in different species. The anti-fertility agents interrupt reproductive functions at some point (Udoh and Kehinde, 1991; Pathak *et al.,* 2000). In females, these areas or points may include hypothalamus, anterior pituitary, ovary, oviduct, uterus and vagina, whereas in males, it may involve the testes, epididymis, the spermatozoa. A given compound or plant extract may exert its antifertility effects in more than one of these areas by different pharmacological mechanisms. Conversely, different compounds or extracts may act in the same way to inhibit fertility but in some cases by different mechanisms. There are however other antifertility substances that affect other stages e.g. implantational and post-implantational mechanism (Farnsworth *et al.,* 1975; Verma and Chiony, 2002; Ekanem and Okoronkwo, 2003).

In males during spermatogensis, pre and leptotene spermatocytes differentiate from type B spermatogonia and spermatogonia residing near the basal lamina translocate across the blood-testis barrier in the inter-sertoli compartment of the seminiferous epithelium, entering the adlumina compartment for further development (Kretser and Karr, 1988). This timely movement of developing germ cells across the epithelium, coupled with the continual attachment of germ cells onto Sertoli cells throughout their development are conceivably essential for the completion of spermatogenesis. The cellular events of germ cell movement and germ cell attachment are also potential targets for fertility control.

1.4 Statement of Research Problem

In all the continents of the world, highly effective, acceptable and reversible methods of contraceptives have been developed and are in use for females. Many of such methods are considered safe and reversible. There is therefore the need to develop complimentary, safe, easily reversible and accessible contraceptive methods for males.

1.5 Research Justification

According to Sofowora (1993), eighty percent of the resources of poor African population are channeled towards traditional medicines, which are mainly plants for their health care needs. Plants have provided the basis for traditional treatment for different types of diseases and still offer enormous potential sources of new chemotherapeutic agents in orthodox medicine. Plant derived compounds, which are mainly secondary matabolites such as alkaloids, flavonoids, terpenes, and coumarins, are believed to be bioactive and produce definite physiological effects such as analgesics, antimalarial, antiplasmodic and antifertility actions in animals and human (Sofowora, 1993; Rates, 2001). Several potential approaches for induction of infertility have been investigated over a long period; these include hormonal, chemical and immunological approaches. Two compounds from plant origin have been identified to possess anti-fertility activity in males and have reached clinical evaluation stage. They are gossypol from cotton seeds (Lohiya *et al.,* 1990, Waites *et al.,* 1998) and triptolide from *Tripterygium Wilford***,** (Hecht, 1999), but both compounds are toxic, inducing renal toxicity, hypokalemia, antispermatogenesis action and irreversibility of the contraceptive-induced infertility.

The seeds of *Carica papaya* have shown various degrees of contraceptive effects in many laboratory animals like mice, rats, rabbit, langur monkey etc (Lohiya and

Goyal, 1992; Lohiya *et al.,* 2002, Udo and Kehinde, 1999; Pathak *et al.,* 2000; Verma and Chinoy, 2001 and 2002; Manivannan *et al.,* 2004). These studies have shown that the extract of *Carica papaya* seed is an effective antifertility agent in male reproductive system and that the antifertility action is post-testicular. Pathak *et al.,* (2000) and Lohiya *et al.,* (2002), showed that the physiology and hormonal status in the treated animals at various dosages is normal. This research is set out to investigate the antifertility effects of aqueous extract of *Carica papaya* seeds on serum biochemistry, clinical chemistry, haematological profiles, liveweight changes, testosterone production and testicular function of male wister albino rats and its effects on some acrosomal enzymes.

1.6 Research Aim and Objectives

The general aim of this research is to investigate some antifertility indices of aqueous extract of pawpaw (*Carica papaya*) seeds and its effects on some acrosomal enzymes.

The specific objectives of this research are:

i. To identify the phytochemical constituent (qualitative and quantitative) of both the crude and partially purified extract of *Carica papaya* seeds

ii. To assess the antifertility indices of both the crude and partially purified extract of *Carica papaya* seeds.

iii. To examine the toxicity profiles of extract of *Carica papaya* seeds.

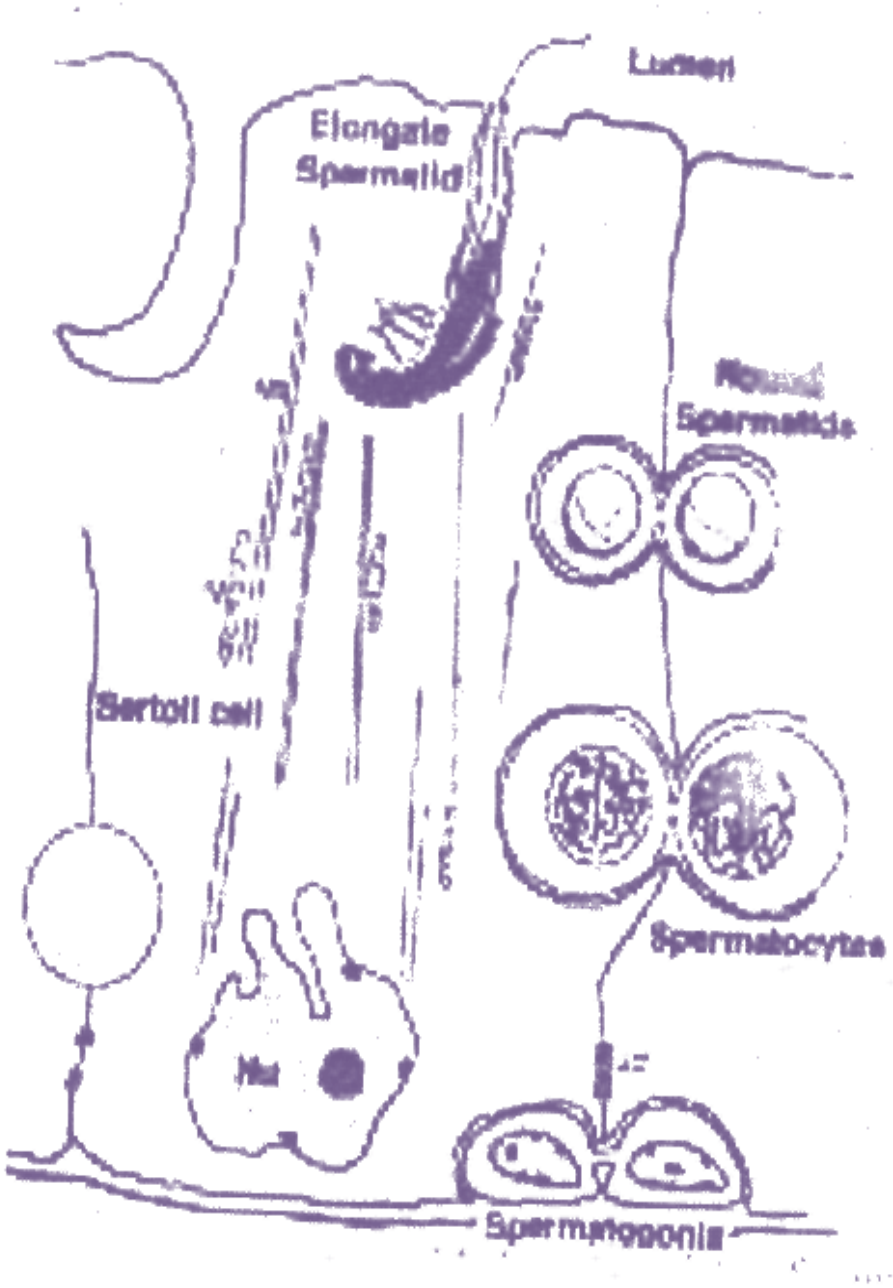
CHAPTER TWO

Literature Review

2.1 Spermatogenesis

Spermatogenesis is the biological process of gradual transformation of germ cells into spermatozoa over an extended period of time within the the seminiferous tubules of the testis. This process involves cellular proliferation of repeated mitotic divisions, duplication of chromosomes, genetic recombination through cross-over, reduction-division by meiotic divisions to produce sertoli haploid spermatids, and finally terminal differentiation of the spermatids into spermatozoa. Thus spermatogenesis can be divided into three phases: proliferation, reduction-division (or meiosis) and differentiation. These phases are associated with specific germ cell types, spermatogonia, spermatocytes, and spermatids, respectively (Starpe, 1994).

Spermatogenesis occurs within the extensive seminiferous tubular structures of the testis. Seminiferous tubules are lined by the seminiferous epithelium and contain a fluid filled lumen into which fully formed spermatozoa are released. The seminiferous epithelium consists of two basic cell types; somatic and germinal cells (Fig. 2.1), the germs cells are found at different levels from the base of the tubule to the lumen and are surrounded by cytoplasm of the somatic cell and the sertoli cell (Fig. 2.2). The sertoli cell cytoplasm extends the



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Figure 2.1: The Seminiferous Epithelium consisting of somatic cells, the Sertoli cells.

Source: Rex (1999)

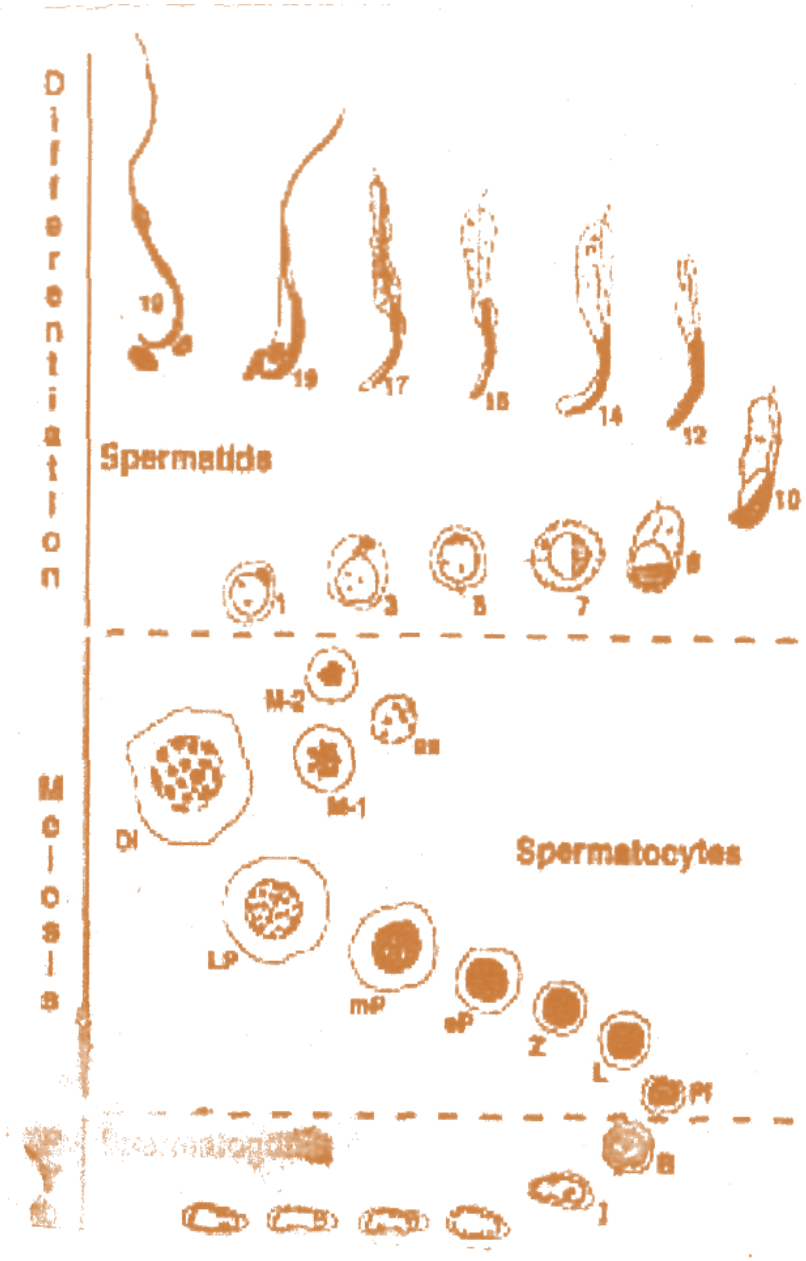


Figure 2.2: Germ Cell Development in Rat Spermatogenesis

Source: Rex (1999)

entire height of the epithelium because the cell serves to nurture the germ cells through their cycles of development. As the germ cells divide and develop into different types of cells, they move from the basement unto the region through tight junctional complexes or adjacent Sertoli cells until they reside in the adluminal compartment. The sertoli-sertoli cell junctions form the blood-testis barrier, which helps to protect the developing germ cells from potentially harmful blood borne chemicals (Clermont, 1972). The germ cells develop as a syncytium or clonal unit connected to one another by intercellular bridges after cell division (Fig. 2.3). This unique process of incomplete division ensures synchrony of germ cell development on large areas of the seminiferous tubule containing vast numbers of cells at the same level of development, (Rex, 1999).

2.1.1 Phases of Spermatogenesis

2.1.1.1 Proliferation

This is the first stage of the development and consists of spermatogonia which are the most immature cells and are located along the base of the seminiferous epithelium (Fig 2.1). They proliferate by mitotic division and multiply rapidly to continually replenish the germinal epithelium. Spermatogonia are capable of self-replication and thus also produce stem cells that proliferate along the base as well as committed cells that are on a one-way tract to produce spermatozoa.

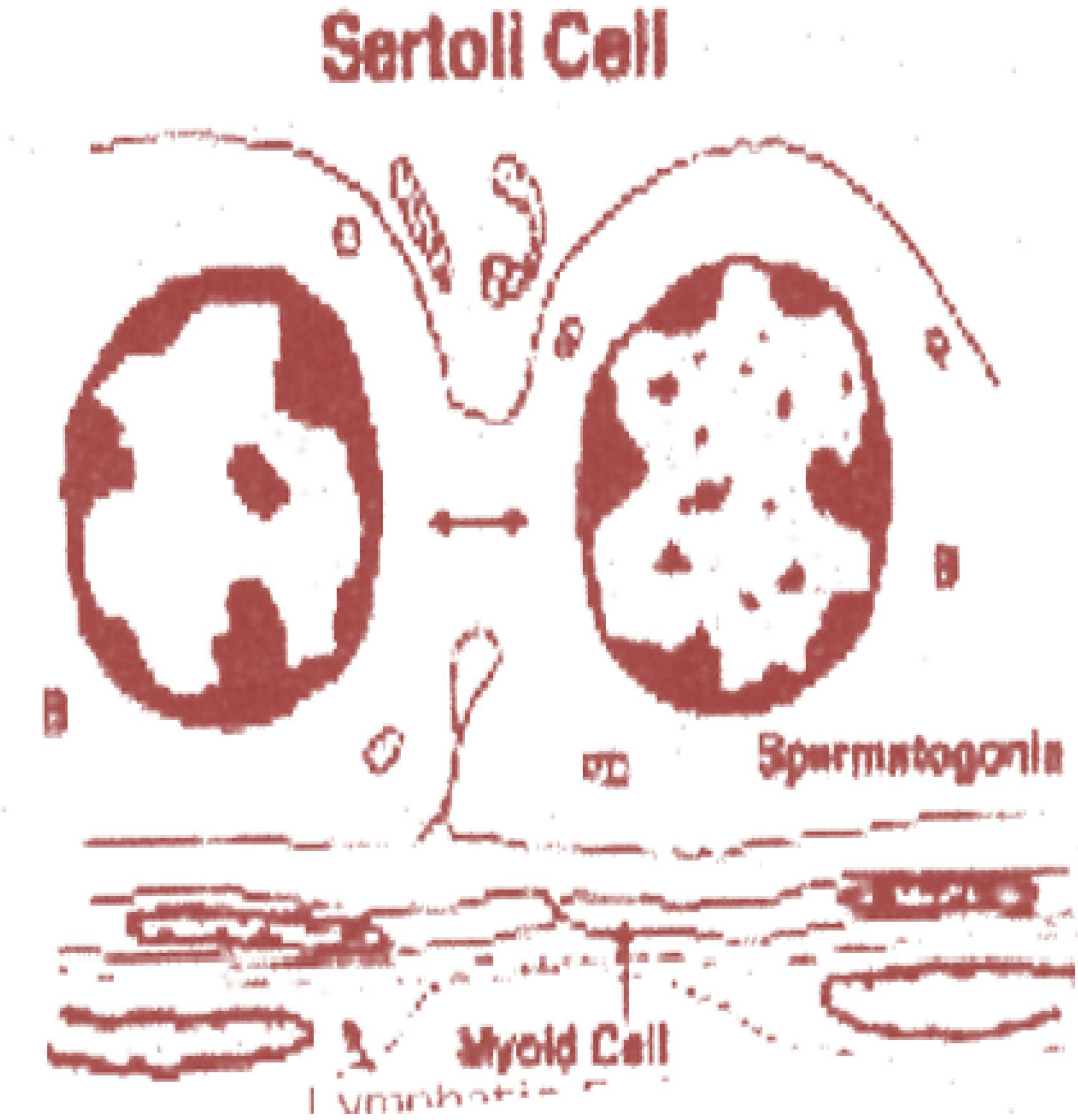


Figure 2.3: Two B-Type spermatogonia are connected through the inner cellular bridge (double arrow)

Source: Rex (1999)

In most species, the B, spermatogonia is the last to divide by mitosis. Its division produces the first cell of the second phase, the preleptotene spermatocyte, which migrates upwards away from the base of the seminiferous tubule to cross through the sertoli-sertoli junction (Roosen - Runge, 1962; Dym and Faweett, 1971).

2.1.1.2 Meiosis

This is referred to as reduction-division and it involves numerous types of spermatocytes that range in size from cells smaller than a red blood cell (preleptotene) to very large cells (pachytene) that occupy portions of every cross section of seminiferous tubules. Reduction -division is a biological mechanism by which a single germ cell can increase its DNA content, divide twice to produce four individual germ cells containing a single strand of each chromosome or half the number of chromosomes normally found in cells of the body. The process of meiosis is extended over a long period of time. Therefore, spermatocytes are found in every stage of spermatogenesis; in some stages two different types of spermatocytes may be observed. During meiosis, the changes that take place in the chromosomes are easily recovered, (Hess, 1990).

DNA synthesis occurs in preleptotene spermatocytes. Prophase of the first meiotic division may last for nearly 3 weeks, during which time the chromosomes first unravel as thin unpaired filaments (leptotene), Homologous chromosomes

become paired in the zygotene cell, forming the synaptonemal complex. Pachytene spermatocytes begin as small cells but their nucleus enlarges greatly as the chromosomes become shorter and thicker. Genetic recombination occurs through cross-over between paired chromosomes. Pachytene cells also exhibits an increase in RNA and protein synthesis in preparation for the next phase. Diplotene spermatocytes separate the synaptonemal complexes and the chromosomes condense. Both meiotic divisions occur rapidly, thus limiting these cells to one stage. Small secondary spermatocytes (2N) are produced by meiosis I which then rapidly divide again by meiosis II, with unique metaphase formations by the chromatin. Meiosis II produces very small haploid (N) cells called round-spermatids that enter the next phase called differentiation (Rex, 1999).

2.1.1.3 Differentiation

The haploid germ cells undergo a prolonged phase of terminal differentiation known as spermiogenesis. The cells undergo dramatic changes that include the following three major modifications:

The nucleus elongates and chromatin condenses into a very dark staining structure having unique shapes that are species-specific (Fig 2.4).

The golgi apparatus produces a lysosomal - like granule that elaborate over the nucleus to form the future acrosome, which contains the hydrolytic enzymes required for sperm-egg interaction and fertilization.

The cell forms a long tail lined with mitochondria in the proximal region and it loses excess cytoplasm, which is discarded first as the cytoplasmic lobe that eventually is phagocytize by the Sertoli cell as the residual body. Recognizable changes in the differentiation of a spermatid are called "steps" of spermiogenesis. In rats, the first step is the small round step I spermatid produced by meiosis II. Step I occurs in the first stage of the cycle. In all species, the late elongated spermatids, steps 15 - 19 in the rat, overlap with the younger round spermatic, (Hess, 1990). Thus in some stages, two generations of spermatids are present in the same tubule cross section.

2.2. Semen Evaluation

When investigating male infertility, there are a number of test and factors that must be examined and evaluated. A semen analysis evaluates certain characteristics of the semen and the sperm contained in the semen. This is the most common test performed for male infertility and it assesses how well sperm has been produced and matured as well as how it interacts with the seminal fluid (semen quality).

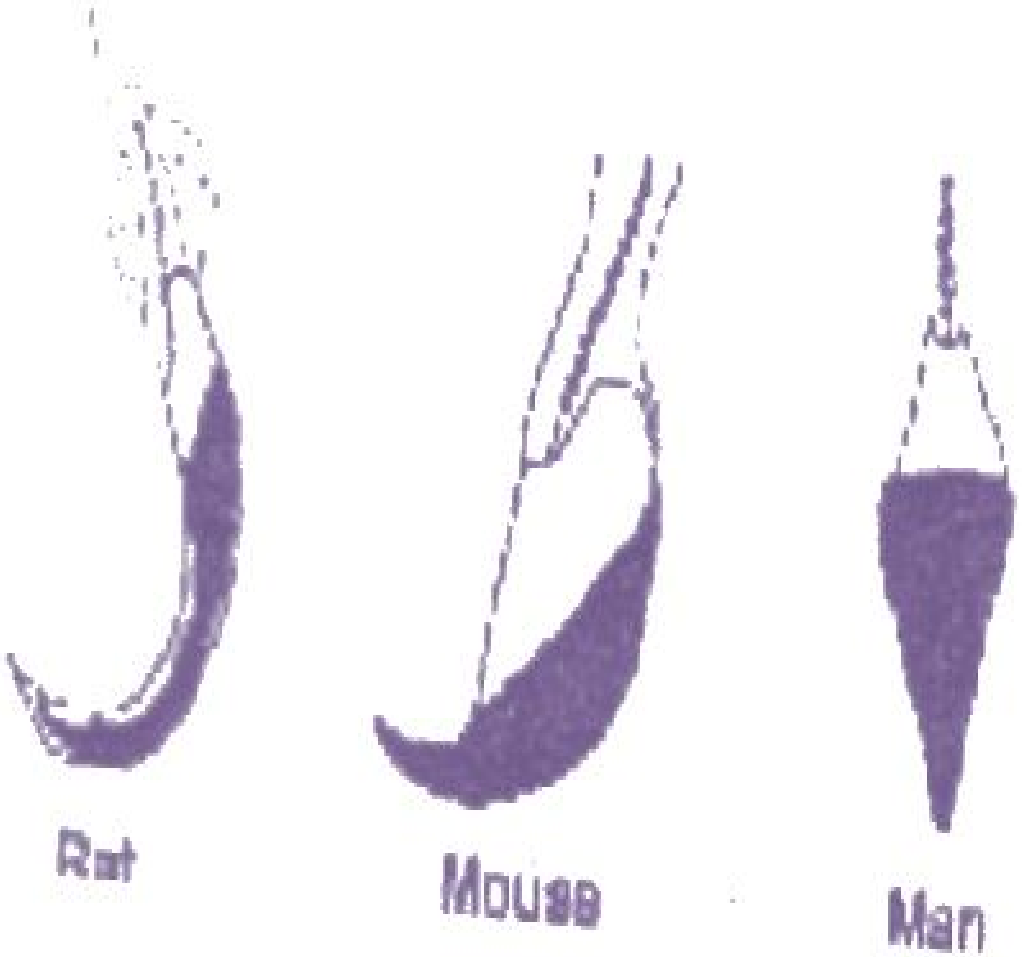


Figure 2.4: heads of newly released sperm from three species illustrating the variation achieved through differentiation of the haploid spermatic. The black areas represent portions of the nucleus covered by the acrosome.

Source: Hess (1990)

Practically, seminal fluid is classified according to their features:

i. Aspermia - absence of semen.

ii. Azoospermia - absence of sperm in ejaculate.

iii. Oligospermia - low number of sperm in the ejaculate, usually less than 20 million per ml.

iv. Asthenozoospermia - poor sperm motility consisting of less than 50% fast forward and slow forward movement

v. Teratozospermia - sperm carrying more morphological defects than usual, less than 4% normal morphological forms (Ellington, 2004).

2.2.1 Sperm count

This is the concentration of how many million sperm an animal has per millimeter of semen. For man, an average of 60 million/ml is expected, while a count of below 20 million/per millimeter is regarded as infertile.

2.2.2 Sperm Motility

Sperm motility or mobility is an assessment of how well an animal's sperm move. Hess (1990) defines normal motility in man as 60% of observed sperm, or at least 8 million per millimeter, showing good forward movement. The implication is that a man can have a total number' of sperm far over the limit of 20 million sperm cells per millimeter, but still have bad quality sperm because too few of them are

motile. However if the sperm count is very high, then a motility of less than 60% might not matter, because the fraction might still be more than 8 million per millimeter. Otherwise, a man can have a sperm count far less than 20 million per milimeter and still have good motility, if more than 60% of the observed sperm cells show good forward movement. There is a motility grade, where the motility of sperm are divided into four different grades (Ellington, 2004), according to their quality.

a. Grade 4: These are sperm with progressive motility. These are the strong sperm cells that swim fast in a straight line and it is sometimes referred to as "motility a"
b. Grade 3: These are sperms with non-linear motility move forward but tend to travel in a curved or crooked motion. This grade is referred to as "motility b".
c. Grade 2: These have non-progressive motility because they do not move forward despite the facts that they move their tails.
d. Grade 1: These are immotile sperm and fail to move at all.

2.2.3 Sperm Morphology

Morphology refers to the percentage of sperm in a semen sample that appears to have formed normally. An abnormal sample sperm may have doubled, kinked or coiled tails. The greater the number of sperm with abnormal shape, the less chance of successful fertilization. A sample is normal if at least 30% of the observed sperm have normal morphology (Ellington, 2004). Examples of

abnormalities include headless sperm, flattened head, pin head, bent neck and bent tail. Multiple abnormalities is possible at times.

2.2.4 Ejaculate Volume

Ejaculate volumes between 1.0 ml and 6.5 ml of ejaculate are normal using WHO criteria which specifies that any volume greater than 2.0 ml is normal. However a variety of factors can affect the volume of ejaculate and the quality of ejaculate provided for a semen analysis; for example, the time interval between the last ejaculation to time of sample collection is very important for the quantity subsequently collected. ("Semen analysis" Healthwise. Web MD. http://www.webmd.com/infertility-and-reproduction/guide/semen-analysis. Retrieved 2007)

2.2.5 Other Tests

2.2.5.1 Fructose level of Semen

Measurement of fructose level is done to differentiate between blocked seminal vesicles or non - production of sperms in males. Fructose is indicated in the coagulation and liquefaction process of spermatozoa. Fructose level of 3 mg/ml is regarded as normal. Seminal vesicles produce fructose for nourishment of the spermatozoa (Semen analysis morphology. IVF 2005; http://www.ivfi.com/semen-analyis-morphology/retrieved 2007).

2.2.5.2 pH of Semen

The pH range of 7.1 - 8.0 is accepted as normal (WHO, 2007). Acidic ejaculate (lower pH values) many indicate that one or both of the seminal vesicles are blocked, while a basic ejaculate (higher pH values) may indicate an infection. A pH value outside the normal range is harmful to sperm physiology.

2.2.5.3 Liquefaction of Semen

This is the process by which the gel formed by protein from the seminal vesicles is broken up and the semen made more liquid. It normally takes less than 20 minutes for the sample to change from a thick gel into a liquid. An abnormally long liquefaction time (more than 30 minutes at 37°c) may indicate an infection (WHO, 2007).

2.2.5.4 White Blood Cells Evaluation in Semen

A semen sample containing a higher than usual number of white blood cells, may indicate previous infection or possibly inflammation. While some white blood cells are expected to be found, a sample containing more than a million white blood cells per milliliter is considered to be abnormal (Elington, 2004).

2.3 Hormonal Regulation of Spermatogensis

Spermatogensis in mammals requires the actions of a complex assortment of peptide and steroid hormones, each of which plays an important role in the normal functioning of the seminiferous epithelium (Fig 2.5). These hormonal messengers

are critical not only for the regulation of male germ cell development, but also for the proliferation and functions of the somatic cell types required for proper development of the testis. These include the interstitial stereidogenic leydig cells, whose primary function appears to be production of testosterone (Mendis-Handagarna, 1997; McLachlan *et al.,* 2002a). Follicle-stimulating hormone (FSH) and leutinizing hormone (LH) are glycoprotein hormones secreted by the anterior pituitary that act directly on the testis to stimulate somatic cell function in support of spermatogensis.

Genetic and pharmacological studies in rodents indicate that the primary role of FSH in spermatogenesis is stimulation of sertoli cell proliferation during prepubertal development (Heckert and Griswold, 2002). Regulation of testosterone (T) synthesis seems to be the only indispensable function of LH within the adult testis. Testosterone and its metabolites, dihydrotestosterone (DHT) and estradiol (E2), are collectively referred to as the sex hormones. This is because of their primary role in the regulation of gonadal and germ cell development in both males and females as well as in the sexual differentiation of males.

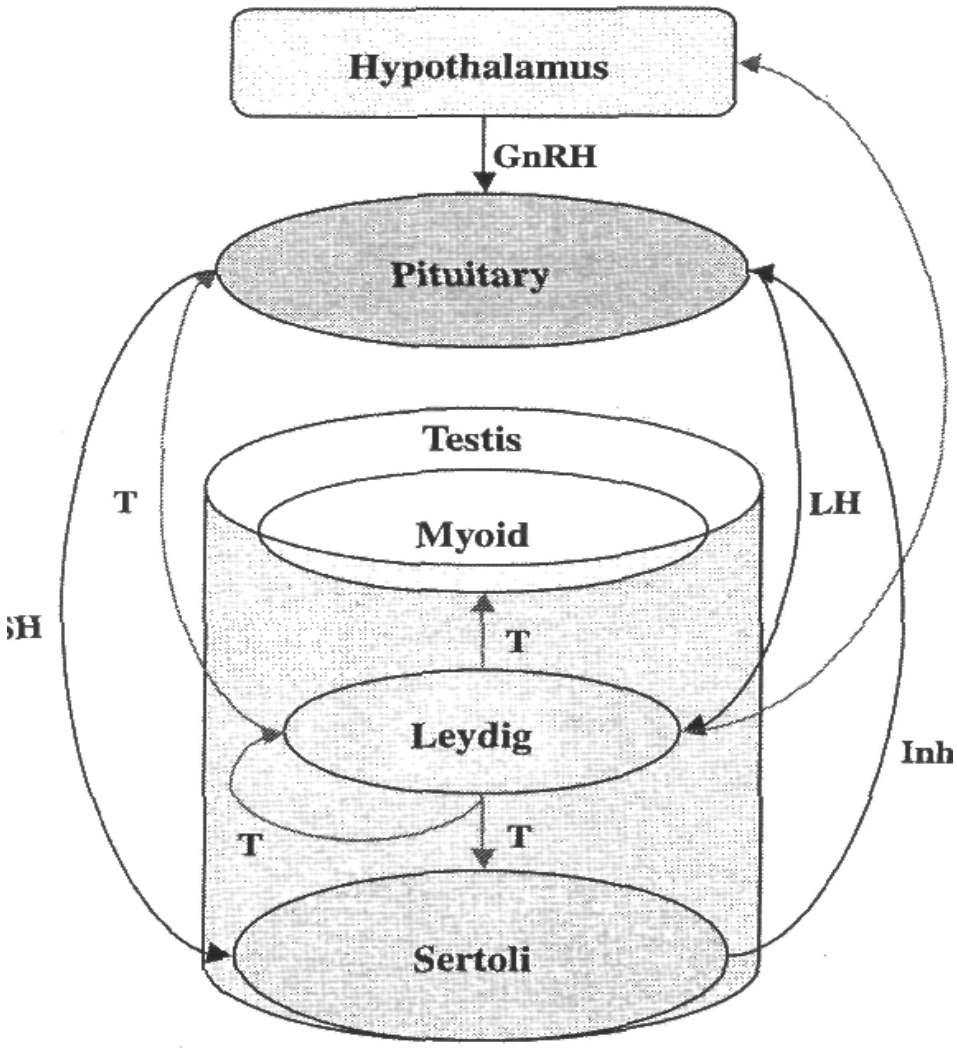


Figure 2.5: Hormonal regulation of the spermatogenesis

Source: McLachlan *et al. (* 2002).

In the male, testosterone assumes the lead role in both morphological development and reproductive function, although E2 and its receptor estrogen receptor (ERα) but not ERβ clearly play some role in the maintenance of male fertility. However, these effects appear to be indirect and secondary (Krege *et al.,* 1998). According to Hess *et al.,* (2000), the primary function of ERα in the male reproductive tract is the regulation of luminal fluid reabsorption in the rat testis and efferent ducts linking the testis and epididymis. Although FSH and testosterone have independent roles in spermatogenesis, they also act in a cooperative manner to promote qualitative spermatogensis by modulation of post-receptor events within sertoli cells. Both FSH and testosterone act in a stage-dependent manner and act at different cellular sites during spermatogenic process (McLachlan *et al.,* 2002a and 2002b). The dual functions of adult testis; namely the production of spermatozoa (fertility) and the secretion of testosterone (virility), are both dependent on stimulation by the pituitary gonadotropins, FSH and LH, which are secreted in response to hypothalamic gonadotropin-releasing hormone (GnRH).

2.3.1 The Acrosome

Cell differentiation in the germinal epithelium of the testis leads to the formation of cells, spermatozoa which uniquiely express genetic information for

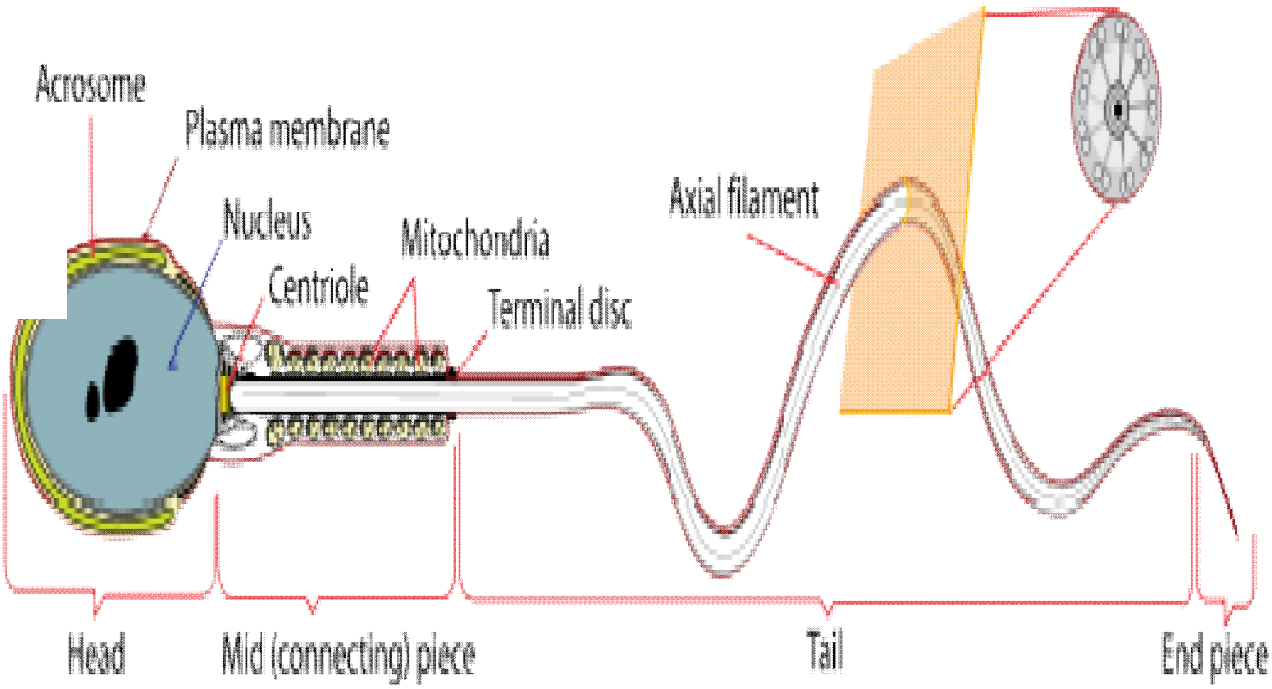


Fig 2.6: Diagram of Human Spermatozoon Showing the Acrosome

Source: Rogers and Bentwood (1982)

such specialized cell structures as the motile flagellum and the acrosome (McLachlan *et al.,* 2002b)

The acrosome is an organelle that develops over the anterior half of the head in the spermatozoa (sperm cell) of many animals. A caplike structure at the anterior end of a spermatozoon that produces enzymes aiding in egg penetration. It is considered to be a lysosome-like structure derived from the Golgi apparatus (Fig. 2.6).

Acrosome formation is completed during testicular maturation. In *Eutherian mammals*, the acrosome contains digestive enzymes some of which are: hyaluronidase, α-glucosidase, acrosin and arylsufatase (Morton, 1976; McRorie & Williams, 1974). These enzymes breakdown the outer membrane of the ovum, called the *Zona pellucida,* allowing the haploid nucleus in the sperm cell to join with the haploid nucleus in the ovum (McRorie & Williams, 1974).

Fertilization is more of chain events than a single, isolated phenomenon. Indeed interruption of any step in the chain will almost certainly cause fertilization failure. Freshly ejaculated sperm are unable to or poorly able to fertilize, rather they must first undergo a series of changes known collectively as capacitation. Capacitation is associated with the removal of adherent seminal plasma protein, reorganization of plasma membrane lipid and protein. It may also involve an influx of extracellular calcium, increase in cycle AMP and decrease in intracellular pH.

The molecular details of capacitation vary among species. Sperm that have undergone capacitation are said to become hyperactivated and display hyperactivated motility. Capacitation appears to destabilize the sperm's membrane to prepare it for the acrosome reaction (Miyazaki, 1990; Yanagimaci, 1976; Rogers *et al.,* 1979).

2.3.2 The Acrosome Reaction

Binding of sperm to the zona pellucida is the easy part of fertilization. The sperm then faces the daunting task of penetrating the zona pellucida to get to the oocyte. Evolutions challenge to this is the acrosome – a huge modified lysosome that is packed with zona-digesting enzymes and located around the anterior part of the sperm's head, just where it is needed.

Acrosome reaction provides the sperm with an enzymatic drill to get through to the zona pellucida. The same zona pellucida protein that serves as a sperm receptor also stimulates a series of events that lead to many areas of fusion between the plasma membrane and outer acrosomal membrane. Membrane fusion (exocytosis) and vesiculation expose the acromosal contents, leading to leakage of acromosal enzymes from the sperm's head (Carver-ward *et al.,* 1997).

As the acrosome reaction progresses and the sperm passes through the zona pellucida, more and more of the plasma membrane and acrosomal contents are lost. By the time the sperm transverses the zona pellucida, the entire anterior surface of

its head, down to the inner acrosomal membrane is denuded. Sperm which lose their acrosomes before encountering the oocyte are unable to bind to the zona pellucida and thereby unable to fertilize. Hence assessment of acrosomal integrity of ejaculated sperm is commonly used in semen analysis. Also the constant propulsive force from the sperm's flagellating tail, in combination with acrosomal enzymes, allow the sperm to create a tract through the zona pellucida. These two factors – motility and zona digesting enzymes allow the sperm to tranverse the zona pellucida, resulting in fertilization (Rogers & Bentwood, 1982).

2.4 Contraceptive Methods in Humans

Contraception is a process whereby pregnancies are deliberately prevented. There are several methods of contraception available in many countries of the world including Nigeria. These methods can be classified into barrier methods, hormonal methods, intrauterine devices in female, male sterilization, emergency contraception and natural methods (Olayemi, 2010) as discussed below:

2.4.1 Physical Barrier Methods

Barrier methods of contraception stop sperm from getting into the womb (uterus). The options here include: male condom, female condom and diaphragm (for females). Barrier methods can be very effective if used correctly and they also decrease the risk of sexually transmitted infections (STIs) but they have high failure rates (Olayemi, 2007).

2.4.2 Hormonal Contraceptives

These are essentially made up of oral contraceptives in form of either combined pill, containing oestrogen and progesterone to prevent ovulation or implantation. The other type of pill is a mini pill containing only progesterone to prevent sperm from entering the uterus by making the cervical mucus thicker. Hormonal contraceptives also include vaginal rings which contain similar hormones to combined-pill and works in the same way. There are also implants and injections which contain hormones. They are more effective than other hormonal contraceptives.

2.4.3 Intrauterine Devices (IUD)

Intrauterine Devices are generally known as coil and are made up of small plastic device with added copper or hormones. An IUD is inserted into the uterus by a doctor or a trained nurse/midwife. Both types are 99 percent effective and they work by changing the lining and environment of the uterus, thus killing off the sperm effectively.

2.4.4 Sterilization

Sterilization is a permanent surgical procedure that requires referral to a specialist. For females, the procedure blocks the fallopian tubes, preventing an egg from passing down the tube and being fertilized. The two common surgical methods are as follows: tubal ligation, involves tubes tying, performed under

general anaesthetic and essure – insertion of small coils, performed under local anaesthetic. The male sterilization, called vasectomy, involves blocking sperm by cutting the tubes they pass through from testes to the penis. Both females and male sterilization are highly effective methods of contraception but they do not protect against STIs (Gupta and Sharma, 2006).

2.4.5 Emergency Contraception

Sometimes, it is necessary to prevent pregnancy after sex, rather than before. For example when a pill is forgotten or a condom breaks, or in the case of rape. Emergency contraception , also known as the "morning after pill" is a hormonal method of contraception that prevents or delays ovulation in that cycle. It may also stop a fertilized egg from sticking to the wall of the uterus. This method prevents 85 percent of pregnancies that would have otherwise occurred.

There are different types of emergency contraception available. The most common form is a single pill containing the hormone progesterone. Emergency contraception is best used within 72 hours of unprotected sex. The sooner within the 72 hours it is taken, the more effective it is (Olayemi, 2010).

2.4.6 Natural Methods

Natural family planning is based on an understanding of the menstrual cycle. Methods include monitoring cervical mucus changes, body temperature changes and rhythm or cycle calculations to help determine when a woman is most likely to

be fertile during each month. The effectiveness of natural family planning varies in relation to whether one or a combination of methods is used. Confidence, correct use and effectiveness improve with time of use. However, natural family planning methods do not protect against STIs.

Choosing the method of contraception will depend on a range of factors such as your general health, lifestyle and relationships, risk of contracting a sexually transmissible infection (STI) and how important it is that one does not become pregnant (Olayemi, 2010).

2.5 Herbal Contraception in Males

The available methods of contraception have been well developed for women, though male injectable contraceptives are on trial runs. But for centuries herbal portions and passaries have been concorted with the goal of preventing and or disrupting pregnancy. Scientific researchers have done some research on botanical anti-fertility agents, some findings have been very interesting and some very promising (Gupta and Sherma, 2006).

There has been a steady accumulation of information regarding the screening of plants having antifertility potential (Bhakuni *et al.,* 1969; Fansworth *et al.,* (I & II) 1975; Gupta and Sharma, 2006). Recently, different parts of plants like seeds, roots, leaves, flowers, stem or stem barks have been evaluated for active principles. However, there are many limitations with regards to the safety and

efficacy of these preparations. Knowledge about active principles of herbal preparations is not well defined and information on toxicity and adverse effects of these formulations are lacking. Information regarding pharmacokinetics and bioavailability in most cases also not available. The relative proportion of phytochemical present in medicinal plants can vary in different ecotypes. Standardization of methods, quality control, data on safety and efficacy are needed for proper understanding and use of the herbal medicines (Gupta and Sharma, 2006). Over a period of 25 years (1980 – 2005), a literature survey revealed that there are about 105 plants which possess antifertility activity in males (Appendix I). Examples of plants that have shown antifertility activities are: *Gossypium herbaceus Linn* (Prasad and Diczfaluzy, 1982), *Tripterygium wilfordi Hook. F.* (Qian, 1986,1987; Qian *et al.,* 1995) and *Azadirachta indica A. Juss*, (Purohit *et al.,* 1990, Joshi *et al.,* 1996).

2.6 Carica papaya Linn (Family - Caricaceae)

Administration of chloroform extract of papaya seeds showed suppression of cauda epididymal sperm motility and counts in rats, suggesting that contraceptive effects are mainly post-testicular in nature, without influencing toxicological profile and libido of animals (Lohiya and Goyal, 1992). According to Lohiya *et al.,* (2002), the extract induced a long term reversible azoospermia in langur monkey. Udoh and Kehinde (1999) reported that an oral dose of crude ripe pawpaw seeds

caused degeneration of the germinal epithelium and germ cells reduction in the number of Leydig cells and vacuoles in the tubules. Pathak *et al.,* (2000) observed sterility in rats due to total suppression of sperm motility. Verma and Chinoy (2001) concluded that the papaya seed extract alters cauda epididymal microenvironment. It was also evaluated that it reversibly reduced the contractile responses of cauda epididymal tubules which possibly retards the sperm transport in cauda epididymides (Verma and Chinoy, 2002). Manivannan *et al.,* (2004) also observed ultrastructural changes in the testis and epididymis of rats following treatment with the benzene chromatographic fraction of the chloroform extract of the seeds. The chloroform extract of seeds has also been shown to decrease the sperm concentration in male adult rabbits (Lohiya *et al.,* 2004). Lohiya et al (1994 a and b) equally reported that the aqueous extract of papaya seeds induced reversible sterility without adverse effects on libido and toxicological profile, and hence could serve as an effective male contraceptive in rodents (Chinoy *et al.,* 1994). However, aqueous extract of the seeds did not exhibit contraceptive activity at any dose regiments tested on male rabbits (Lohiya *et al.,* 2000). In all the cases cited, there was a general observation of unaltered haematological profiles, hence the extract from *Carica papaya* seeds promises to be a potential male contraceptive without adverse side effects. This is supported by its traditional use in different parts of Assam (Tiwari *et al.,* 1982).

2.7 Medicinal and Pharmacological Properties of Carica papaya Plant

Uses of different parts of papaya for medicinal purposes and various pharmacological actions are well reported in literature (Kirtikar and Basu, 2005; Arya, 2005). Crude extracts of different parts of papaya have been used in traditional medicine for the treatment of various diseases (Pathak, *et al.,* 2000). Apart from these, there are several reports on the therapeutic properties and pharmacological actions of papaya based on modern scientific investigations (Gupta and Sharma, 2006).

2.7.1 Antimicrobial Effects

The seed of papaya has antimicrobial activity against *Trichomonas vaginalis* trophozoites. According to Osato *et al.,* (1993) and Calzada *et al.,* (2007), papaya seed can be used in urinogenital disorder like trichomoniasis, while the seed and pulp of papaya was shown to be bacteriostatic against several enteropathogens like *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris* and *Pseudomonas aeruginosa* by the agar plate method.

The aqueous extract of the fruit exhibited antimicrobial activity and promoted significant healing in diabetic rats. The seeds of papaya have bacteriostatic activity on gram positive and gram negative organisms, and hence could be useful in treating chronic skin ulcers. Herbal formulations containing papaya

leaves and root or leaves as one of the constituent has antibacterial activity against *Salmonella typhi, S. paratyphi* and *S. typhimurium* (Dawkins *et al.,* 2003; Nkuo-Akenji *et al.,* 2001).

2.7.2 Anthelmintic Effects

Okeniyi *et al.,* (2007), reported that the air dried papaya seeds given as elixir with honey has shown significant effect on the human intestinal parasites, without significant side effects. It was observed that the consumption of such preparations offers a cheap, natural, harmless, readily available monotherapy and preventive strategy against intestinal parasitosis, especially in tropical communities. This report confirmed the report of Kermanshai *et al.,* (2001) and Krishnakumari and Majimder (1960) who isolated Benzylisothiocyanate as the sole anthelmintic agent present in papaya seeds. In addition, the latex of papaya has anthelmintic efficacy against *Heligmosomoides polygyrus* in experimentally infected mice. This suggests its potential role as an anthelmintic against potent intestinal nematodes of mammalian hosts (Satrija *et al.,* 1995)

2.7.3 Anti-amoebic Effects

The cold macerated aqueous extract of matured papaya seeds has shown anti-amoebic activity against *Entamoeba histolytica* (Tona *et al.,* 1998).

2.7.4 Antimalarial Effects

Bhat and Surolia (2001) observed that the petroleum ether extract of the rind

of raw papaya fruit exhibits significant antimalarial activity.

2.7.5 Antifungal Effects

The latex of papaya and fluconazole has synergistic action on the inhibition of *Candida albicans* growth. This synergic effect results in partial cell wall degradation. Latex alone is statistically effective on *C. albicans* when added to a culture during the exponential growth (Giordani *et al.,* 1996). The degradation of cell wall is due to a lack of polysaccharides constituents in the outermost layer of the fungal cell wall. Latex proteins appear to be responsible for the antifungal action.

2.7.6 Effect on Smooth Muscles

Ethanol extract of papaya seeds showed concentration dependent inhibition of jejunal contractions that was significantly irreversible (Adebiyi and Adaikan, 2005). Hence, it can be deduced that the seed extract of papaya is capable of inhibiting the contractions of isolated rabbit jejunum. Eno *et al.,* (2000) reported that a crude ethanol extract of unripe fruit of papaya produces a significant depression of mean arterial pressure but the extract has about 28% more depression action than hydrallazine in the hypertensive rats. It was therefore concluded that fruit juice of papaya probably contains antihypertensive agent(s), which exhibits mainly alpha adrenoreceptor activity. Also, Adebiyi *et al.,* (2005) observed that ethanol extract of seeds of papaya caused a concentration dependent tocolysis of uterine strips isolated from gravid and non-gravid rats. High concentration extract

is capable of causing irreversible uterine autolysis probably due to the damaging effect of benzylisothiocyanate on the myometrium.

2.7.7 Diuretic Effects

Aqueous root extract of papaya, given orally at a dose of 10 mg/kg to rats produced significant increase in urine output and shows similar profiles of urinary electrolyte excretion to that of hydrochlorothiazide (Bungorn *et al.,* 2001).

2.7.8 Hepatoprotective Effects

According to Adebiyi *et al.,* (2004) both ethanol and aqueous extracts of the fruits possess remarkable hepatoprotective activity against chloroform (CCl_4), induced hepatotoxicity. However, the mechanism as well as the active principles responsible for hepatoprotective activity of papaya is not presently yet known.

2.7.9 Topical Uses

In Spanish Town Hospital, Kingston Public Hospital and University Hospital of the West Indies, Jamaica, papaya fruits are used as topical ulcer dressings and this has produced desloughing, granulation and healing (Imao, *et al.,* 1998). It also reduces the odour in chronic skin ulcers (Hewitt *et al.,* 2002). It is currently used in Gambia at the Royal Victoria Hospital, Banjul in the Pediatric unit as the major component of burns dressings, where it is well tolerated by the children, (Starley *et al.,* 1999). The pulp of the papaya fruit is mashed and applied daily to full thickness on the infected burns. It appears to be effective in

desloughing necrotic tissue, preventing burn wound infection and providing a granulating wound suitable for the application of a split thickness skin graft. Possible mechanisms of action include the activity of proteolytic enzymes chymopapin, papain, as well as an antimicrobial activity. It is cost effective, widely available and it is considered to be more effective than other topical agents in the treatment of chronic ulcers.

2.7.10 Female Antifertility Activity

Sharma and Mahanta (2000) have reported that the composite root extract containing papaya root extract as one of the constituent, induces morphological changes in the endometrial surface epithelium in albino rat uterus. The aqueous extract of the seeds of papaya has shown abortifacient properties on female Sprague Dawley rats (Oderinde *et al.,* 2002), and the petroleum ether, alcoholic and aqueous extracts of the seed of *Carica papaya* inhibit ovulation in rabbits (Imao, *et al.,* 1998). The papaya seed extracts did not exhibit antizygotic, antiimplantation, early abortifacient or antifertility activity in female albino rats (Bodhankar, *et al.,* 1974). Therefore normal consumption of ripe papaya during pregnancy may not pose any significant danger. However, the unripe or semi-ripe papaya (which contains high concentration of the latex that produces marked uterine contractions) could be unsafe in pregnancy (Adebiyi *et al.,* 2002). Also Abdulazeez (2004) reported that unfermented extract of *Carica papaya* seeds, especially at a higher

dose of 150 mg/kg has contraceptive effects on female Wistar rats, causing an increase in post-implantation loss.

CHAPTER THREE

Materials and Methods

3.1 Materials

All chemicals and reagents used in this work were purchased from Riedel-de Hoen, Sigma-Adrich and Labtech Chemicals. The reagent kits used were: testosterone enzyme immunoassay kit, antiacrosin antibodies, (AR Ab ELISA kit), enzychrom Tm Sialic acid assay kit, hyaluronidase assay kit and acid phosphatase activity assay kit.

3.1.1 Plant Sample

Carica papaya fruit were obtained in January 2010 from Basawa village, Zaria (latitude 11^{o} 4' 0'' N and longitude 7^{o} 42' 0'' E), Kaduna State (http://www.travelmath.com/city/Zaria,+Nigeria). The sample was identified, confirmed with a voucher No 755 at the Herbarium, Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria. The fresh seeds were removed, washed with clean water and dried under shade. The dried seeds were then ground at Department of Food Science, Institute of Agricultural Research (IAR), ABU, Zaria and stored in air tight container until used.

3.1.2 Experimental Animals

Over two hundred male albino rats were raised from the stock of animals obtained from National Veterinary Research Institute (NVRI) Jos, Plateau State of Nigeria. The animals were kept in clean plastic cages and kept at the animal house, Department of Veterinary Physiology and Pharmacology, ABU, Zaria, with a 12:12h light-dark schedule. The animals were fed on growers' marsh and water was given *ad libtum*. The animals were allowed to acclimatize under standard laboratory conditions before the commencement of the treatment. Only proven fertile animals (that is those that must have at least initiated one pregnancy) were used.

3.2 Methods

3.2.1 Aqeous Extraction of Pawpaw (Carica papaya) seeds

One hundred gramme (100 g) of the powdered dried seed of *Carica papaya* seeds was suspended in 400 mls of distilled water for 24 hours. The extract was then sieved first with cheese cloth and then with Watmans filter paper No 1. The filterate was concentrated to dryness in a water bath preset at 50^0C.The procedure was repeated ten times. The dried residue was kept in the refrigerator at 4^0C until required.

3.2.2 Phytochemical Screening of the extract of Carica papaya seeds

Phytochemical screening of both the crude aqueous extract and the partially purified extract of powdered *Carica papaya* seed was carried out by employing standard procedures (Trease and Evans, 1989).

Test for Tannins:

Exactly 0.5 g of the sample was boiled in 20 ml of distilled water in a test tube and filtered. A few drops of. 1 % (0.1 g in 100 ml) ferric chloride was added. A blue black coloration which indicated the presence of tannins was found.

Test for saponnins:

Two (2) grams of sample was boiled in 20 ml of distilled water in a water bath and filtered with Watmans No 1 filter paper and 10 ml of the filterate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An emulsion, indicating presence of saponnins, was formed.

Test for flavonoids:

Five (5) ml of dilute ammonia solution was added to a portion of the aqueous fllterate of the sample, followed by addition of concentrated, sulphuric acid. A yellow coloration indicating the presence of flavonoids was obtained. On standing the coloration disappeared. 1% aluminium solution was added to the filterate and yellow coloration developed. This indicated presence of flavonoids, then, a

portion of the filterate was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered with Watmans No 1 filter paper and 1 ml of the filterate was shaken with 1ml of dilute ammonia solution. A yellow coloration indicating the presence of flavoviods was formed.

Test for cardiac glycosides (Keller-killani test):

Five (5) ml of sample was treated with 2 ml glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated, sulphuric acid. A brown ring at the interface indicated a deoxysugar, characteristics of cardenolides. A violet ring appearred below the brown ring while with the acetic acid layer, a greenish ring formed gradually.

Test for alkaloids:

Exactly 0.5 g of sample was dissolved in 5 ml of 1% aqueous hydrochloric acid and placed in a steam bath; it was filtered. To 1ml of the filterate, a few drops of Mayer Drangendorf reagent were added. Development of turbidity or precipitate indicated presence of alkaloids.

Test for anthraquinone:

Exactly 0.1 g of sample was dissolved in 10 ml hot water for 5 minutes and filtered hot. The filterate was re-extracted with 10ml carbon tetrachloride. The carbon tetrachloride layer was removed and washed with 5 ml water, then 5 ml dilute ammoniacal solution was added. There was a formation of pink to red colour in the

ammonia layer which indicated the presence of free anthraquinones.

3.2.3 Lethal Dose (LD_{50}) Determination

This is the determination of the lethal dose known as LD_{50}. According to Lorke (1983) LD_{50} is given by the square root of the highest dose that did not kill, multiplied by the lowest dose that killed. The method of Lorke (1983) was employed. The first stage involved the oral administration of three different doses of 10, 100 and 1,000 mg/kg of the crude extract, to three different groups of adult male wister albino rats. In a fourth group, three adult male wister albino rats were administered with equivalent/volume of distilled water to serve as control. All the animals were orally administered the extract using a curved needle to which a catheter had been fixed. The animals were monitored closely every 30 minutes for the first 3 hours after administration of the crude extract and hourly for the next 6 hours for any adverse effects. Then the animals were left for 72 hours for further observation.

When no death occurred, the second stage of the method (Lorke,1983) was employed for this stage, only one animal was required in each group. Groups 1–3 animals were orally given 1,600, 2,900 and 5,000mg/kg dose levels of the crude extract while group 4 animal, was administered distilled water. All the animals were left for observation as in stage one. The entire protocol was repeated for the chromatographic chloroform fraction of the crude extract.

3.2.4 Partial Purification

Thirty gramme (30 g) of the crude extract of *Carica papaya* seeds was reconstituted in 50 ml of distilled water and chromatographed over deactivated silica gel (60 – 200 mesh) in a glass column (4.0 x 50cm). Different fractions were eluted sequentially with solvents of increasing polarity (Manivannan *et al.,* 2004). It was eluted with benzene followed by chloroform and ethyl acetate. Using 250 mls of each solvent, the eluting continued until it was completely done. The eluting was carried out in the fume cupboard. The elution rate was 50 mls in 20minutes. The eluted fractions were concentrated to dryness in a water bath preset at 50^0C and kept in the refrigerator at 4^0C until required.

3.2.5 Experimental Design and Treatment Groups: Crude Aqueous Extract

The experimental design consisted of two phases, (the Treatment and recovery phases)

Treatment Phase: The treatment was administered for 21 days to three groups of randomly chosen adult male albino rats with 18 animals in each group as follows:

Group I (n = 18): Extract treated (higher dose) received 200 mg/kg body weight

Group II (n = 18): Extract treated (lower dose) received 100 mg/kg body weight

Group III (n = 18): Vehicle treated control received only normal saline

Normal control was not included because the vehicle (normal saline) is assumed not to have activity.

Three animals were randomly chosen in each group and were sacrificed every 7 days for three weeks and all the parameters proposed for this study were assayed.

Recovery Phase: Following 21 days of treatment the animals were withdrawn from the treatment, and 3 animals were randomly chosen from each group and were sacrificed every 7 days for three weeks and all the parameters were measured as in treatment phase.

3.2.6 Experimental Design and Treatment Groups: Partially Purified Fractions of the Aqueous Extract

There were three fractions obtained viz;

Benzene fraction (BF)

Chloroform fraction (CF)

Ethylacetate fraction (EF)

Preliminary Studiesof the Partially Purified Fractions

Three animals were randomly chosen and treated with 200 mg/kg of each fraction for 7 days. Only the chloroform fraction (CF) showed activity, both benzene and ethylacetate fractions did not show any activity. Therefore only the chloroform fraction was used for further studies.

Treatment Phase: Four groups of randomly chosen adult male albino rats consisting of 9 animals each were treated with the chloroform fraction as follows:

Group I: Received 100 mg/kg body weight

Group II: Received 200 mg/kg body weight

Group III: Received 300 mg/kg body weight

Group IV: Control, received only normal saline (vehicle).

Normal control was not included because the vehicle (normal saline) is assumed not to have activity.

All the animals were treated for 14 days and three animals were randomly chosen and sacrificed and all the proposed parameters were determined, every 7 days for two weeks.

Recovery Phase: 7 days after the treatment was stopped, the remaining three animals from each group were sacrificed and all the proposed parameters determined.

3.3 Measurement of Determined Parameters

The following parameters were measured in all the animals during the treatment and recovery phases.

3.3.1 Body Weight

The body weight of all the animals before and after treatment were recorded using digital balance.

3.3.2 Semen Evaluation

3.3.2.1 Sperm Sample Preparation

Each male albino rat was anesthetized with chloroform and opened up to remove the epididymis (Fig. 3.1 and 3.2). The cauda epididymides were cut into small pieces in 5 ml of normal saline. Normal saline, a physiological medium was used to avoid distortion of the sperm. Then 1 ml of the sperm suspension was transferred to a test tube. Two drops each of 1% eosin Y and nigrosine were added to the test tube and mixed by gentle agitation. Sperm were incubated at room temperature for approximately 45 – 60 minutes to allow for staining and then resuspended with a Pasteur pipette.

Slides were cleaned with detergent, washed in water followed by alcohol and dried before use. The stained sperm suspension was mixed gently to avoid potential damage to sperm and a smear was prepared and air dried (Plate 3.1). The slides were prepared in triplicates and are fixed in formalin and a cover slip was applied for storage. The sperm concentration, percentage live sperm and percentage normal sperm were determined using the prepared slides above (WHO, 1992)

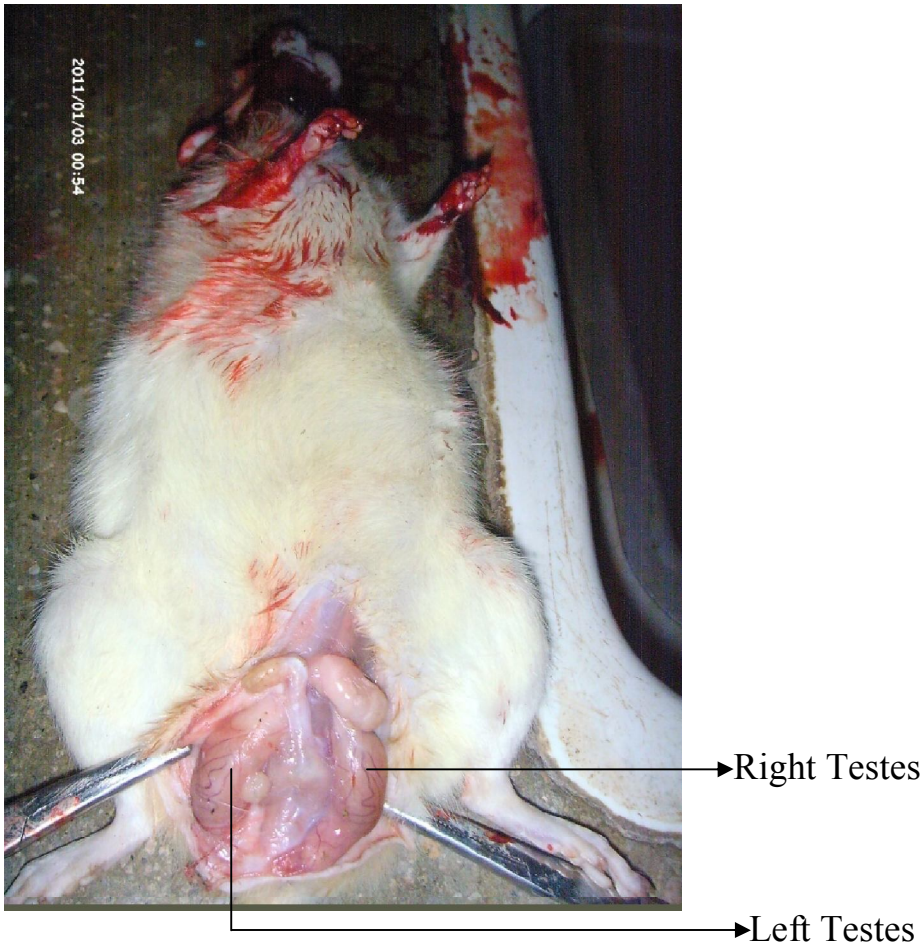


Figure 3.1: Picture of a dissected male albino rat showing the testes

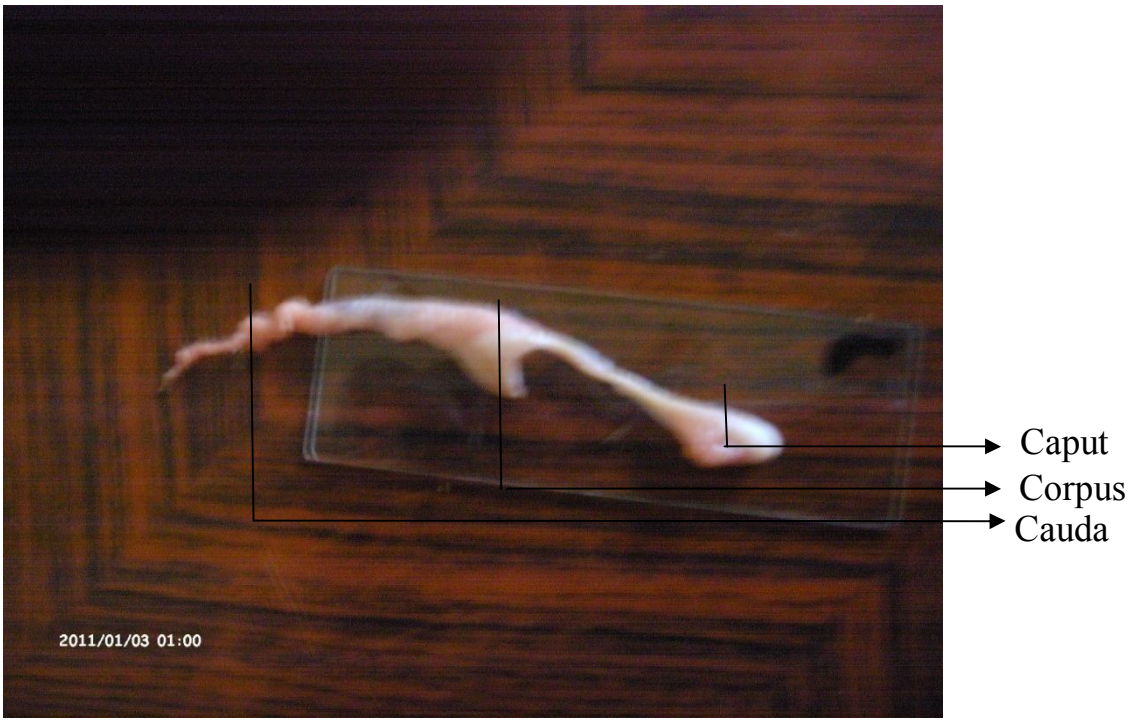


Figure 3.2: Picture of the Epididymis of a male rat

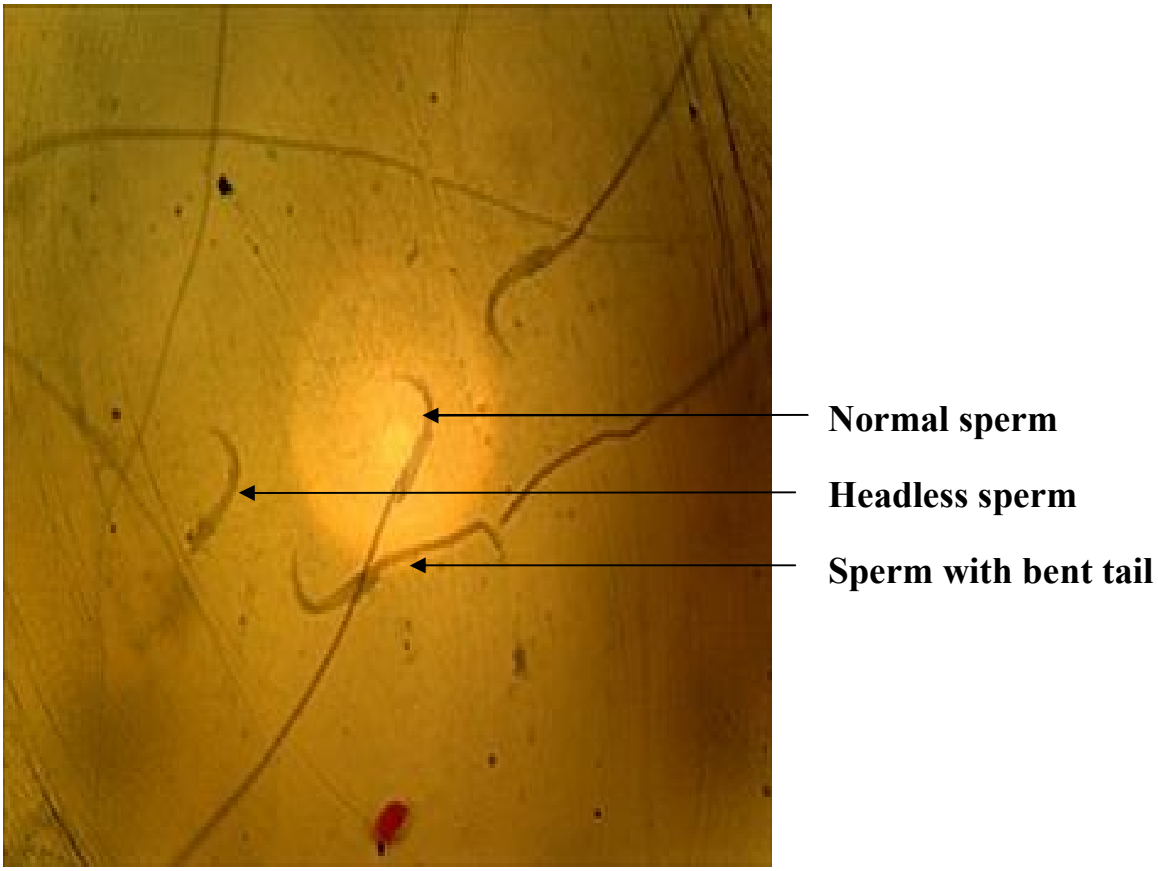


Plate 3.1:Micrograph of spermatozoa X1000

3.3.3 Haematological Studies

Blood was collected by cardiac puncture into EDTA sample bottles from anaesthesised male albino rats and the the following parameters were measured: packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC).

PCV Determination

By the activity of capillarity, blood was drawn into the capillary tubes up to 2/3 of its volume. The tubes were sealed at one end with bunsen burner flame and centrifuged at 10,000g for 5 minutes in microhaematocrit centrifuge. The PCV value was then read using a Hawskley haematocrit reader (Lewis et al, 2001, Provan, 2004)

RBC Determination

Blood sample was drawn up to exactly 0.5 ml mark in the red count pipette and diluted to 101 ml mark with red count diluting fluid, thus making a 1:201 dilution of the blood. The pipette was shaken manually to ensure proper mixing of the blood. The diluted blood was transferred into a Neubauer counting chamber of a haemocytometer using a pipette and the red blood cells counted under microscope at a magnification of x400 (Lewis et al, 2001, Provan, 2004). The value of RBC was then counted from the

$$\text{RBC} = \frac{\text{N x Df x } 10^{-6}}{\text{A x D}}$$

Where:

N = Number of cells counted.

Df = The dilution factor (i.e. 201)

10^{-6} = Convertion to cell per litre

A = Area of the chamber counted (0.2 mm^2)

D = Depth of the chamber i.e. 0.l mm

(Lewis *et al.,* 2001)

Total RBC is expressed as cells x 10^9/litre

WBC Determination

Blood sample was drawn up to exactly 0.5 ml mark in the white count pipette and diluted to 10 ml mark with white count dilution of the blood. The pipette was shaken manually to ensure proper mixing of the blood. The diluted blood was transferred into a Neubauer counting chamber using a pipette and the white blood cells counter under microscope at x400 magnification (Lewis et al, 2001, Provan, 2004). Then the value of WBC was calculated using:

$$WBC = \frac{N \times Df \times 10^{-6}}{A \times D}$$

Where:

N = Number of cells counted.

Df = The dilution factor (i.e. 20)

10^{-6} = Convertion to cell per litre

A = Area of the chamber counted (0.2 mm^2)

D = Depth of the chamber i.e. 0.l mm

(Lewis *et al.,* 2001)

Total WBC expressed as cells x 10^6/litre

3.3.4 Serum Biochemistry

Exactly 2 ml of the blood samples were collected from the anasthesised male albino rats and transferred into sample bottles and properly labeled. The blood samples were allowed to coagulate and the sera collected in another set of properly labeled sample bottles. The collected sera were used to assay for creatinine, glutamate oxaloacetate transaminase (SGOT) also know as aspartate aminotransferase (AST), testosterone and acid phosphatase as follows;

3.3.4.1 Serum Creatinine

Principle:

Creatinine is a nitrogenous waste product formed from the metabolism of Creatine in skeletal muscle. Creatinine is excreted by the kidney, and a normal creatinine range is 60-130 µmol/L Creatinine reacts with picric acid in alkaline medium to produce a yellow red colour which is measured with a spectrophotometer (Cheesbrough,1999). A pair of test tubes was prepared for the reagent blanks, control standard and test samples (sera from treated rat groups). Two milliliter (ml) of freshly prepared creatinine reagents was added to each set of tubes.

Then 0.2 ml of distilled water was added to the reagent blank tubes, 0.2 ml of standard sera to control tubes, 0.2 ml of standard sera to to standard tubes and 0.2 ml of test samples to the appropriate tubes.The control of each tube was mixed together, and left at room temperature for 90 minutes. The absorbances of one tube from each set were read using a spectrophotometer at 490nm. The spectrophotometer was set at zero with the reagent blank. To the second tubes of each set–up, 0.05 ml 0f 60% acetic acid reagent was added and left for 6 minutes. The absorbances were read again for each tube. To obtain the readings for the standard, control and test solutions, the second reading was subtracted from the readings for the first set of tubes.Then using the formulaa below, the concentration of creatinine in the control and test samples were calculated

For test sample $= \frac{AT}{AS} \times 200$

Where AT =absorbance of test solution

AS=absorbance of 200 µmol/l standard solution

For control, AT= absorbance of 200 µmol/l contol solution

(Cheesbrough, 1999)

3.3.4.2 Serum Glutamate Oxaloacetate Transaminase (SGOT)

Principle:

The enzyme glutamate oxaloacetate transaminase (GOT) also known as aspartate aminotransferase (AST) is concerned with amino acid metabolism. GOT is present in liver and has an approximate normal range of up to 42 ìu/l (Cheesbrough, 1999). Kinetic determination of GOT is based upon the following reaction:

L-Aspartate + α-ketoglutamate → Oxaloacetate +L-Glutamate

$$\text{Oxaloacetate} + \text{NADPH} + H^+ \xrightarrow[\textbf{MDH}]{} \text{L-Malate} + NAD^+$$

AST-Aspartate aminotransferase

MDH-Malate dehyrogenase.

The medium is alkaline and gives a red- brown colour and the absorbance can be measured with spectrophotometer at 505nm.

Procedure:

Using Reitman- Frankel (1957) method, twelve test tubes were prepared and the first three were marked as reagent blank, standard, and control respectively. The rest test tubes were marked as test samples.Then 0.5 ml of substrate reagent was pipetted into the test tubes marked for control and test samples only and left for 5 minutes at 37^0C. After, 0.1 ml control serum was added to the control tube and 0.1 ml test samples were added to their appropriate tubes.The contents were mixed together and incubated at 37^0C for 60 minutes then 0.1 ml of distilled water and 0.5

ml substrate reagent were added into reagent blank tube and 0.1 ml distilled water, 0.3 ml substrate reagent and 0.2 ml calibration solution were all added to standard tube.At exactly 60 minutes, 0.5 ml colour reagent was added to all the tubes.The contents of each tube was mixed and left at 25^0C for 20 minutes.Then 5 ml of 0.4 mol/L sodium hydroxide reagent was added to each tube and mixed well.They were all left for 5 minutes.The absorbances of the standard, control and test samples were read in a colorimeter at 505nm using the reagent blank to zero the instrument.The colours obtained were stable for up to 1 hour.The AST (GOT) activity in ìu/l in the control and test samples were read from the calibration graph.

3.3.4.3 Serum Testosterone Assay

Principle:

Testosterone is a steroid hormone and the most important androgen secreted into blood. In males, testosterone is secreted primarily by the Leydig cells of the testes and it is responsible for the development of secondary male sex characteristics and its measurement is helpful in evaluating the hypogonadal states. Expected normal values for adult males ranges from 3.0-10.0 ng/ml (Tietz, 1995)

The testosterone immunoassay is based on the principle of competitive binding between testosterone in the test specimen and testosterone- HRP (Horse Radish Peroxidase) conjugate for a constant amount of rabbit anti–testosterone.

During incubation, a fixed amount of HRP- labeled Testosterone competes with

the endogenous testosterone in the standard, control and test samples for a fixed number of binding sites of the specific testosterone antibody. Thus,the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of testosterone in the specimen decreases. Unbound testosterone peroxidase conjugate is then removed and the wells washed. A chemical that is converted by the enzyme into a colour is applied and the absorbance is measured spectrophotometrically at 450nm.The concentration of the testosterone can be read from a standard curve (Chen et al., 1991; Lequin, 2005)

Procedure:

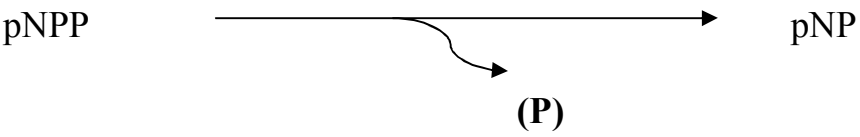
Using diagnostic automation, reagent kit, ten microlitre (10µl) of standards, control and test samples were placed into the 96 microtitre plate according to the animal groupings. Exactly 100 µl of Testosterone-HRP conjugate Reagent was added into each well; followed by addition of 50 µl of rabbit anti-testosterone reagent into each well.The contents of each well was thoroughly mixed together for 30 seconds. And then incubated at 37^0C for 90 minutes.The microwells were rinsed 5 times with distilled water.One hundred microlitre (100 µl) of TMB (3.3^i,5, 5^i tetramethyl-benzidine), a colour developing chemical reagent) was added into each well and the contents of each well were gently mixed together for 10 seconds, and incubated at 25^0C for 20 minutes. The reaction was stopped by adding 100 µl

of stop solution to each well. And then gently mixed together for 30 seconds, until the blue colour changed completely to yellow. The absorbance was read at 450 nm with a microtitre well reader within 15 minutes. The concentration of testosterone in the test samples were obtained from the standard curve (Tietz, 1995; Lequin 2005; Johnson, 2010)

3.3.4.4 Assay of acid phosphatase

Principle:

Acid phosphatases (AP) dephosphorylate phosphate group from phosphate esters under acid conditions. Different acid phosphatase isoenzymes are found in different organs including the prostrate gland. Elevated prostatic acid phosphate levels may indicate the presence of prostrate cancer. The kit uses p-nitrophenyl phosphate (pNPP) as a phosphatase substrate which turns yellow (λ=405nm) when dephosphorilated by AP:



Procedure:

Acid phosphatase in the epidymal supernatant obtained was estimated using acid phosphatase activity assay kit (Biovision) as follows: One hundred microlitres (100 µl) of the test samples were placed in the 96 well microlitre plate according to animal groupings. Fifty microlitre (50 µ1) of pNPP was added to each of the well

and incubated for 60 minutes at 25°C. The absorbances were read at 405nm and the AP activity of the test samples were calculated from:

AP (μ/ml) = A/V/T

Where: A is amount of pNP generated by samples (in μ/mol).

V is the volume of Sample added in the assay well (in ml).

T is reaction time (in minutes)

Unit Definition: One unit AP is the amount of enzyme causing the hydrolysis of one micromole of pPNPP to pNP per minute at pH 5.0 and 25°C (Cheesbrough, 1999).

3.3.5 Seminal Biochemistry

The homogenized cauda epididymides spermatozoa were centrifuged at 13000 g for 3 minutes to remove all suspended spermatozoa. The resultant supernatant was used to assay for:

Sialic Acid

Principle:

Sialic acid is a general name for nine carbon acidic sugars with N- or O-substituted derivatives. The most common member of those sugars is N-acetylneuramic acid (NANA). Sialic acid is widely distributed throughout mammalian tissues and fluids including the seminiferous tubules of the epididymis. The Sialic acid level in the body fluid is an important marker for diagnosing

cancer. Bioassay systems' sialic acid assay uses a simple working Reagent that combines NANA aldolase, pyruvate oxidase and hydrogen peroxide determination in one step.The colour intensity of the reaction product at 570nm is directly proportional to sialic acid concentration in the sample.

Procedure:

Employing the method of Sugahara (1980) and Simpson (1993) the sialic acid concentrations in the epididymal homogenate samples were determined using bioassay sialic acid assay reagent kit: Ten microlitre (10 µl) of standards and 10 µl of test samples were pipitted into separate wells of a clear flat-bottom 96-well microlitre plate. To each well was 90 µl of working reagent (which has been made from 93µl assay buffer, 1µl dye reagent and 1µl enzyme) added and incubated for 60 minutes at room temperature. The absorbance was read at at 570 nm. The absorbance of blank (water) was also determined. The sialic acid concentration in the samples were calculated as standards or sample

$$\text{Sialic acid} = \frac{\lambda_{\text{sample}} - \lambda_{\text{H2O}} \text{ x n}}{\text{Slope}} \text{ nm}$$

Where λ_{sample} is absorbance for sample

λ H_20 is absorbance for water (blank)

slope is the slope of the standard curve in mm

n is the dilution factor of the sample (n=1 for free sialic acid)

Assay of Acrosin

Principle:

Acrosin is a major enzyme found in the acrosome of the spermatozoa.The microtitre plate provided in this kit has been pro-coated with AR antigen. Standards or samples are then added to the appropriate microtitre plate wells with a biotin- conjugated to horse radish peroxidase (HRP) added to each well and incubated. Then a TMB (3, 3^{i}, 5, 5^{i} tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain AR –Ab, biotin-conjugated antigen and enzyme-conjugated Avidin will exhibit a change in colour.The enzyme-substrate reaction is terminated by the addition of a Sulphuric acid solution and colour change is measured spectrophotometrically at a wavelength of 450nm.The concentration of AR-Ab in the samples is then determined by comparing the absorbances of the samples to the standard curve.

Procedure:

Using human anti-acrosin antibodies, AR-Ab ELISA kit, 100 µl of standard, blank and samples were pipetted into separate wells in a 96-well microtitres plate and incubated for 2 hours at 37 ^{0}C. The liquids were removed from the wells and 100µl of detection reagent. A working solution was added to each well and incubated for 1 hour at 37^{0}C. The contents of each well were aspirated and the wells washed with wash buffer (350 µl) using a squirt bottle, multi-channel manifold dispenser. The

whole process was repeated three times, and the plate was inverted blotting it against a clean paper towel to ensure complete removal of liquid. Then 100 µl of detection reagent B working solution was added to each well, and incubated for 1 hour at 37^0C. The wells were aspirated and washed four times and inverted to ensure complete removal of liquid.This is followed by the addition of 90 µl of substrate solution to each well, and incubated for 30 minutes at 37^0C. Fifty microlitres (50 µl) of stop solution was added to each well to stop the reaction and absorbances of all the wells were taken at once using a microplate reader set at 450 nm. The concentration of acrosin inn the test samples were read from the standard curve (Tietz, 1995; Johnson, 2010)

Assay of hyaluronidase

Principle:

Hyaluronidase is an enzyme found in the acrosome of the spermatozoa. It belongs to a group of enzymes that degrade hyaluronic acid (HA). The acid is also known as hyalluronan and it is composed of alternating residue of β-D-(1-3) glucoronic acid and β-D-(1-4)-N-Acetylglucosamine. HA is widely distributed in the body and forms one of the most versatile macromolecoules of the extracellular matrix and plays both structural and biological function. The mammalian hyaluronidase cleaves hyaluronan by hydrolysis of the 1-4 bond between N- acetyl glucosamine and D- glucuronic acid. The product produces a colour which can be measured

Spectrophotometrically.

Procedure:

Enzyme Reaction: Using the methods of Bansal (1982) Watson (1993) and Kazuhiro (2001) and the hyaluronidase assay reagent kit by Razie. Enzyme reaction was initiated by rehydrating the microwells with 100 µl phosphate buffer solution (PBS) and then incubating them at room temperature for 30 minutes. The PBS was removed from the wells and the plate inverted on a clean paper towel to remove the liquid. Fifty microlitre (50 µl) of the reaction buffer was added to all the wells except the positive control wells (two wells).

Additional 50 µl of the reaction buffer was added to four wells as the negative control. Then 50 µl of test samples were added to the wells in duplicates after which 100 µl of the positive control (Bacterial Enzyme) was added to two wells. The 96-well microtitre plate was then incubated for 1 hour at 37^0C on a plate shaker for efficient mixing of the contents of the wells. Microwell strips were washed four times with 200 µl PBS/Tween 20 (0.1% v/v) per well and then twice with 200 µl PBS with thorough aspiration of the microwell contents between washes. The plate was inverted on an absorbent paper towel to remove liquid.

Detection: Immediately after the wash, 100 µl of diluted strep-HRP was added to each well at room temperature and incubated for 60 minutes on a plate shaker. The microwell plate was washed with 200 µl PBS with thorough aspiration

of microwell contents between washes, 100 µl of peroxidase substrate was added per well room temperature. The 96-well microtitre plate was gently shaken to obtain uniform colour. The substrate reaction was stopped by adding 100 µl of 0.12 M HCl into each well, including the blank wells. Then the absorbances of all wells were determined at 450 nm within 5 minutes post stopping using a microplate reader. The activity, R of the test samples (in ng HA removed per minute) was calculated using the following

$$R = \frac{\text{Absorbance of test well}}{\text{Maximum absorbance possible from the plate reader from negative control wells}} \text{ X } 500$$

3.3.6 Histopathology Studies

The testes, caudal epididymides, heart, liver and kidney were removed from all the animals and processed for histological evaluation. The tissues were fixed in Bouin's Fluid for 24 hours, dehydrated in ethanol, cleared in xylem and embedded in paraffin for sectioning at 5 mm. These sections were stained with Harris hematoxylin and eosin for observation under the microscope (Lohiya *et al.,* 2005).

3.3.7 Statistical Analysis

The standard error of mean ± (SEM) of the data was determined. Duncan statistical analysis was employed for statistical comparison and $P < 0.05$ level was considered significant.

CHAPTER FOUR

Results

4.1 Extraction and Percentage Yield of Carica papaya seeds

The crude extract of *Carica papaya* seeds was brownish in colour with an offensive odour which dissolves readily in distilled water. The cumulative weight of the extract was 65.03g which is equivalent to a percentage yield of 6.5. The percentage yield of the partially purified fractions of the crude extract were much lower (Table 4.1).

4.2 Phytochemical Content of the Extract

The crude and chloroform fraction of the aqueous extract of *Carica papaya* seeds were screened for the presence of some classes of phytochemical (alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins and tannis). The quantitative analysis is contained in Table 4.2. The phytochemical content showed that flavonoids and alkaloids are prominently present in both the crude and partially purified extract of *carica papaya* seeds. Other phytochemicals are present in small quantities, 0.24 – 0.68 for crude and 0.04 – 0.08 for partially purified extract from Table 4.2, it is obvious that the purification caused loss in the phytochemical content of *carica papaya* seeds.

Table 4.1: Percentage (%) Yield Contained in Carica papaya Seeds

Extracts	Weight (g)	% Yield
Crude	65.03	6.50
Benzene Fraction of Crude Extract	0.39	0.08
Chloroform Fraction of Crude Extract	1.71	0.37
Ethylacetate Fraction of Crude Extract	0.26	0.06

Table 4.2: Phytochemical Content of Crude and Chloroform Fraction of the Aqueous Extract of *Carica papaya* Seeds

Parameters	Crude Extract (mg/100 g)	Chloroform Fraction of Crude extract (mg/100 g)
Flavonoids	0.42±0.04	0.20±0.001
Saponins	0.24±0.01	0.04±0.003
Tannins	0.14±0.08	0.06±0.001
Anthraquinones	0.24±0.11	0.08±0.002
Cardiac Glycosides	0.68±0.04	0.06±0.001
Alkaloids	0.10±0.01	0.09±0.002

Mean value of triplicate readings ±SEM

4.3 LD_{50} determination of Carica papaya Seed Extract

All the animals orally administered the aqueous crude extract of *Carica papaya* seeds up to 5,000 mg/kg body weight showed no signs of distress and were physically active, even 72 hours post treatment.

Similarly, the male wister albino rats orally administered chromatographic chloroform fraction of the aqueous extract showed no signs of toxicity and were physically active even at 5,000 mg/kg body weight. Hence, for both the crude and partially purified extract, the LD_{50} is greater than 5,000 mg/kg body weight (Table 4.3).

Table 4.3: The Determination of LD_{50} of the Extract of *Carica papaya* Seeds

Group	Dose (mg/kg)	Mortality Ratio	% Survival
1	10	0/3	100
2	100	0/3	100
3	1000	0/3	100
4	Control	0.3	100
5	1600	0/1	100
6	2900	0/1	100
7	5000	0/1	100

Median Lethal Dose (LD_{50}) $\geq$ 5000 mg/kg

4.4 Effects of Crude Aqueous Extract of Carica papaya seeds on Albino Rats

The male wister albino rats orally treated with different dosages (100, 200 mg/kg body weight) showed no significant difference ($P > 0.05$) in their body weights (Table 4.4). The weights of the animals across the groups (rows) were not significantly different before and after the pretreatments constant before and after the treatment.

The sperm concentration (Table 4.5), percentage life sperm (Table 4.6) and percentage normal sperm (Table 4.7) for the treated animals showed reductions in their values. The sperm concentration and percentage live Sperm reduced progressively down the column, that is as the treatment days increased. The dose of 200 mg/kg body weight showed the highest reduction by 21 days of treatment. The reductions observed in percentage normal sperm were not as pronounced for all dosages as those observed for sperm concentration and percentage life sperm.

The packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) were not affected for the two dosages (100, 200 mg/kg body weight) administered to the male wister albino rats. There was no significant difference at $P > 0.05$ between the treated and control animals (Tables 4.8- 4.10).

Serum Glutamate oxaloacetate transaminase (SGOT) or Aspartate amino transferase (AST), (Table 4.11), Serum Creatinine (Table 4.12) and serum testosterone (Table 4.13) and serum acid phosphatase (Table 4.14) were not

significantly ($P > 0.05$) affected for all groups throughout the 21 days of treatment.

The acrosomal enzymes, acrosin and hyaluronidase values were altered at the third week (21 days) of treatment. There was significant ($P < 0.05$) difference between the treated and control animals for all the dosages (Tables 4.15 and 4.16). The Sialic acid values (Table 4.17) were altered significantly at $P < 0.05$ for 200 mg/kg body weight dosage only at 14 and 21 days of treatment.

Table 4.4: Initial and Final Weights of Albino Male Wister Albino Rats treated Orally with Crude Aqueous Extract of *Carica papaya* Seeds

Weight (g)	Group 1 Control(g)	Group 2 (g)	Group 3 (g)
Treatment			
7 days	165.57 ±8.46	156.78 ±7.68	156.54 ±6.04
14 days	163.08 ±10.06	149.57 ±3.38	147.54±0.68
21 days	154.41±1.15	154.67 ±1.20	153.19±1.49
Recovery phase			
7 days	152±1.33	155.67±1.01	153.84±1.83
14 days	149±1.09	149.34±1.34	145.85±18.89
21 days	146.83±12.43	149.10 ±4.95	146.38±3.80
Treatment			
7 days	168.79±1.79	176.99±1.25	161.45±0.08
14 days	171.66±3.26	167.86±2.41	175.06±3.99
21 days	176.10±11.86	165.13±1.86	167.68±6.53
Recovery phase			
7days	156.12±3.50	168.32±4.77	158.83±0.62
14 days	1164.61±4.89	172.86±5.50	169.76±2.22
21 days	183.19±7.45	179.41±8.59	168.79±7.33

Table 4.5: Sperm Concentration of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya* Seeds

Sperm conc. (million/ml)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg
Treatment			
7 days	13.33±0.60	13.33±0.83	12.83±1.09
14 days	13.10±0.95	11.43±0.23	12.90±0.31
21 days	14.50±1.76a	10.33±2.40a	6.95±5.25b
Recovery phase			
7 days	13.69±4.67	10.50±4.75	10.83±4.11
14 days	11.77±0.15	10.50±3.23	10.83±1.18
21 days	12.33±0.17	11.50±4.09	12.50±1.26

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$
* Where there are no superscripts, it shows no significant difference across the groups (rows)

Table 4.6: Percentage Live Sperm of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya* Seeds

Percentage Life Sperm (%)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg
Treatment			
7 days	53.67±2.96	59.00±16.81	59.33±1.76
14 days	50.67±2.29b	19.00±0.58a	23.33±4.41a
21 days	53.67±0.33b	18.33±3.33a	12.50±2.50a
Recovery Phase			
7 days	63.67±3.84	68.67±6.06	62.00±1.73
14 days	54.33±2.73	52.33±8.97	58.00±10.15
21 days	55.67±1.76	68.33±7.69	60.67±2.96

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$
* Where there are no superscripts, it shows no significant difference across the groups (rows)

Table 4.7 Percentage Normal Sperm of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya* Seeds

Percentage normal sperm (%)	Group 1 Control	Group 2 100 mg/kg	Group 3 200mg/kg
Treatment			
7 days	55.00±2.89	62.67±3.28	64.33±2.96
14 days	59.00±3.06b	44.00±4.04a	45.33±3.81a
21 days	58.00±2.08b	49.67±2.33a	49.00±0.00a
Recovery phase			
7 days	63.33±5.90	57.37±2.50	59.00±5.13
14 days	60.00±0.58	59.33±3.38	60.00±5.86
21 days	69.33±0.88	63.67±6.44	69.69±6.12

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$
* Where there are no superscripts, it shows no significant difference across the groups (rows)

Table 4.8: PCV of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya* Seeds

PCV(%)	Group 1 Control	Group 2 100 mg/kg	Group 3 200mg/kg
Treatment			
7 days	43±0.01	43±0.01	45±0.01
14 days	35±0.05	42±0.01	43±0.01
21 days	43±0.01	40±0.03	39±0.01
Recovery phase			
7 days	41±0.02	41±0.01	44±0.20
14 days	40±0.03	40±0.02	44±0.22
21 days	40±3.18	37±0.02	39±0.02

Table 4.9: RBC of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya* Seeds

RBC count($X10^9/mm^3$)	Group 1 Control	Group 2 100 mg/kg	Group 3 200mg/kg
Treatment			
7 days	7.66±0.32	7.71±0.37	8.15±0.33
14 days	7.61±0.83	7.43±0.29	7.92±0.29
21 days	7.97±0.40	7.56±0.82	5.78±0.44
Recovery phase			
7 days	7.39±0.28	7.29±0.27	8.15±0.52
14 days	6.95±0.41	7.25±0.39	7.87±0.30
21 days	7.07±0.41	7.07±0.19	6.88±0.47

Table 4.10: WBC of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya* Seeds

WBC count ($X10^6/mm^3$)	Group 1 Control	Group 2 100 mg/kg	Group 3 200mg/kg
Treatment			
7 days	4.47±0.52	4.10±0.35	4.13±0.18
14 days	5.27±1.09	4.03±0.19	4.50±0.40
21 days	4.34±0.15	4.20±0.35	4.30±0.40
Recovery phase			
7 days	4.57±0.66	4.40±0.23	3.53±0.18
14 days	4.40±0.12	5.40±0.61	4.10±0.15
21 days	4.03±0.32	5.25±0.05	5.47±0.66

Table 4.11 Serum Glutamate Oxaloacetate Transaminase (SGOT) of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya Seeds*

SGOT(iu/L)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg
Treatment			
7 days	16.00±2.08	19.33±2.40	15.67±2.67
14 days	18.00±1.15	21.33±1.33	20.67±2.67
21 days	26.33±1.45	24.67±3.53	21.00±1.00
Recovery phase			
7 days	18.67±2.06	16.00±2.31	18.00±2.31
14 days	14.67±0.88	14.67±1.76	23.67±1.45
21 days	16.33±3.18	14.50±2.50	15.67±2.23

Table 4.12: Serum Creatinine of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya Seeds*

Creatinine (nmol/L)	Group 1 Control	Group 2 100 mg/kg	Group 3 200mg/kg
Treatment			
7 days	61.33±3.0	61.67 ±6.44	55.33±4.84
14 days	62.67±1.20	65.67 ±2.03	65.33±3.18
21 days	68.33±2.03	68.67 ±1.45	67.00±1.00
Recovery phase			
7 days	64.00±2.08	69.67 ±5.04	67.33±5.55
14 days	68.33±1.45	68.33 ±2.19	65.33±4.81
21 days	65.33±2.67	63.50 ±2.50	70.33±1.20

Table 4.13: Serum Testosterone of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica papaya Seeds*

Testosterone (ng/ml)	Group 1 Control	Group 2 100 mg/kg	Group 3 200mg/kg
Treatment			
7 days	4.07±0.32	3.67 ±0.58	4.75±0.64
14 days	4.83±0.46	4.13 ±0.19	43.90±0.21
21 days	4.60±0.17	4.67 ±0.20	4.20±0.10
Recovery phase			
7 days	45.00±0.68	4.90±0.61	4.50 0.26±
14 days	3.57±0.19	3.63 ± 0.33	4.00±0.79
21 days	3.50±0.25	5.05±0.95	3.73±0.48

Table 4.14: Serum Acid Phosphatase of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica Papaya* Seeds

Acid Phosphatase (U/ml)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg
Treatment			
7 days	13.43±0.58	12.13±1.78	14.00±2.19
14 days	12.87±0.99	13.97±0.19	11.67±0.54
21 days	14.00 ±0.18	13.97±0.19	14.40±1.60
Recovery Phase			
7 days	13.80±1.15	14.10±0.84	14.20±1.16
14 days	13.13±1.15	13.23±1.35	13.47±0.98
21 days	14.43±1.15	13.55±0.95	13.07±1.97

Table 4.15: Acrosin Values of Male Albino Rats Treated Orally with Crude Aqueous Extract of *Carica Papaya* Seeds

Acrosin (iu/ml)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg
Treatment			
7 days	77.00±7.55	82.00±1.30	82.67±1.73
14 days	86.67±4.06	70.67±5.70	72.33±3.38
21 days	84.00±3.06b	61.67±4.98a	66.50±2.50a
Recovery Phase			
7 days	77.00±2.87	89.00±1.6	81.33±1.20
14 days	78.33±2.19	86.33±2.13	80.67±1.5
21 days	81.33±3.18	81.00±1.10	84.00±3.71

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$

* Where there are no superscripts, it shows no significant difference across the groups (rows)

Table 4.16: Hyaluronidase Values of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica Papaya* Seeds

Hyaluronidase (iu/ml)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg
Treatment			
7 days	113.3±7.42	116.33±8.37	101.00±6.25
14 days	117.33±4.67	116.33±4.73	101.00±6.25
21 days	115.33±5.46b	94.00±4.11a	98.00±4.00a
Recovery Phase			
7 days	140.00±7.42	143.67±2.45	131.33±7.6
14 days	133.67±4.98	131.33±1.73	131.33±1.49
21 days	137.00±6.66	133.50±6.50	133.33±6.36

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$
* Where there are no superscripts, it shows no significant difference across the groups (rows)

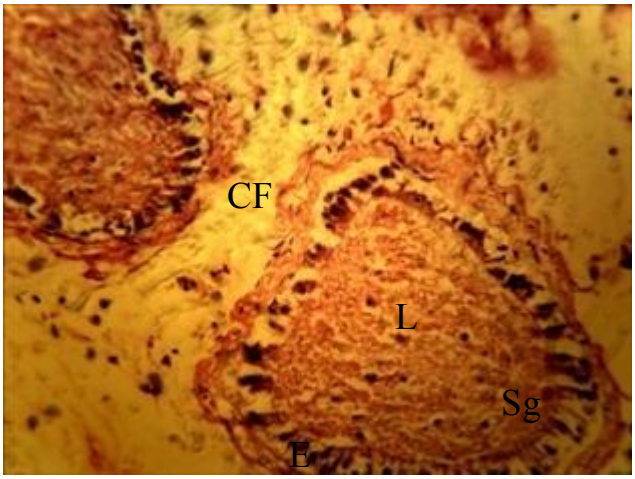
Table 4.17: Sialic Acid Values of Male Wister Albino Rats Treated Orally with Crude Aqueous Extract of *Carica Papaya* Seeds

Sialic acid (µmol/ml)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg
Treatment			
7 days	36.33±4.48	32.00±2.08	34.67±1.76
14 days	37.67±3.18b	31.00±1.15b	23.00±2.08a
21 days	36.00±2.32b	35.33±1.33b	25.00±3.00a
Recovery Phase			
7 days	32.33±3.48	35.00±4.04	34.00±2.08
14 days	36.00±4.15	32.00 ±2.03	29.00±1.53
21 days	32.33±3.48	36.50± 3.50	28.33±2.20

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$
* Where there are no superscripts, it shows no significant difference across the groups (rows)

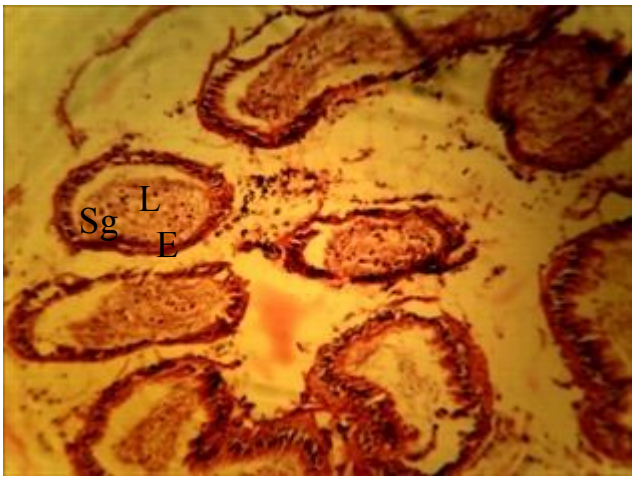
4.5 Histopathology Studies of Rats Treated with Crude Extract

The caudal epididymides and the testes of all the treated animals showed alterations of the spermatogenic cells. There was a gradual reduction of the spermatozoa until the tubules were empty, after 21 days of treatment with the 200 mg/kg body weight dosage. The kidney and the liver showed slight necrosis but the heart was not significantly affected. However, restoration of the cells resumed immediately after withdrawal of the treatment (Plate 4.1 – 4.9).



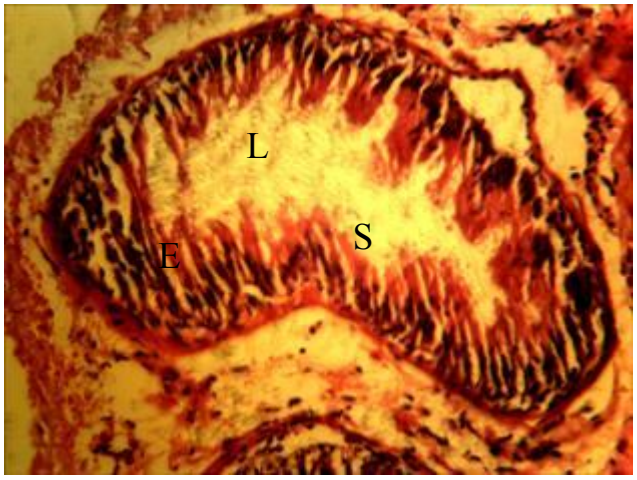
L = lumen packed with spermatozoa
Sg = Secretory granule
E = Epithelium (intact)
Cf = Collagen Fibre

(a) Epididymis Control (*Micrograph of Rat Epididymis at* X250)



L = lumen packed with spermatozoa in loose configuration
Sg = Secretory granule
E = Epithelium (intact)

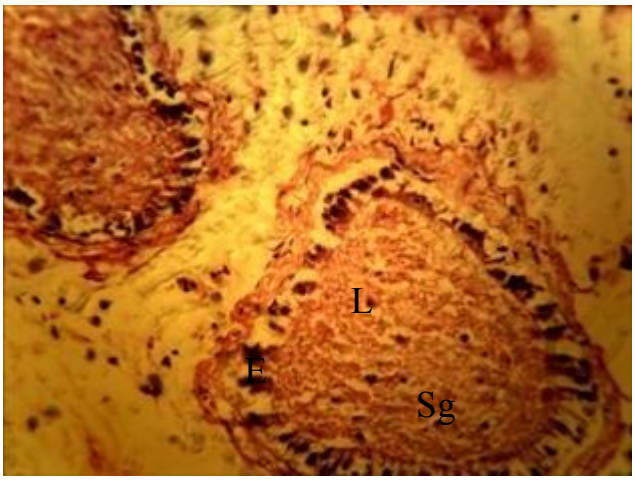
(b) Epididymis Week 3:100 mg/kg (*Micrograph of Rat Epididymis at* X100)



L = lumen (empty)
Sg = Secretory granule
E = Epithelium (intact)

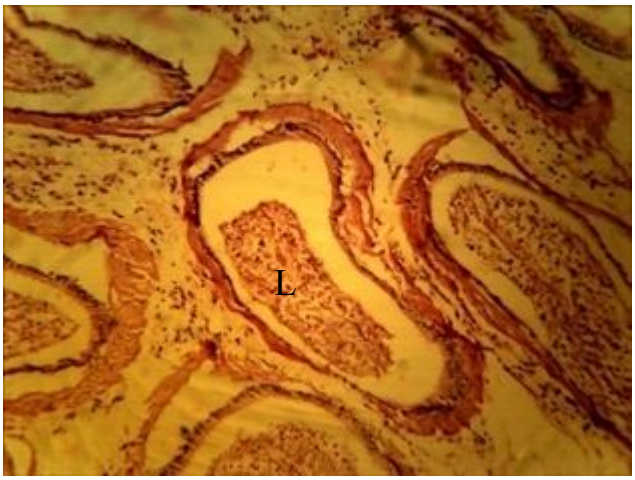
(c) Epididymis Week 3:200 mg/kg (*Micrograph of Rat Epididymis at* X250)

Plate 4.1: Micrographs of Control and Crude Treated Rat Epididymis



L = lumen packed with spermatozoa
Sg = Secretory granule
E = Epithelium (intact)

(a) Epididymis Control (*Micrograph of Rat Epididymis at* X250)



L = lumen in gradual restoration

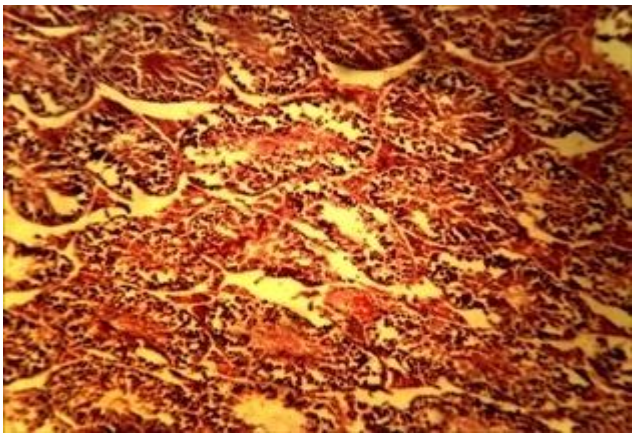
(b) Epididymis Week 5:100 mg/kg (*Micrograph of Rat Epididymis at* X100



L = lumen in gradual restoration

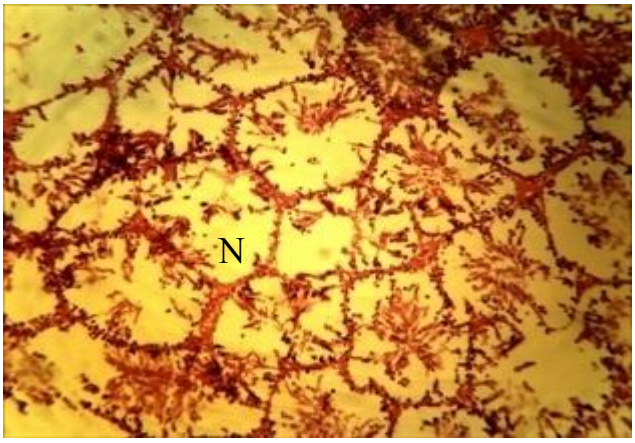
(c) Epididymis Week 5:200 mg/kg (*Micrograph of Rat Epididymis at* X100)

Plate 4.2: Micrographs of Control and Recovering Crude Treated Rat Epididymis



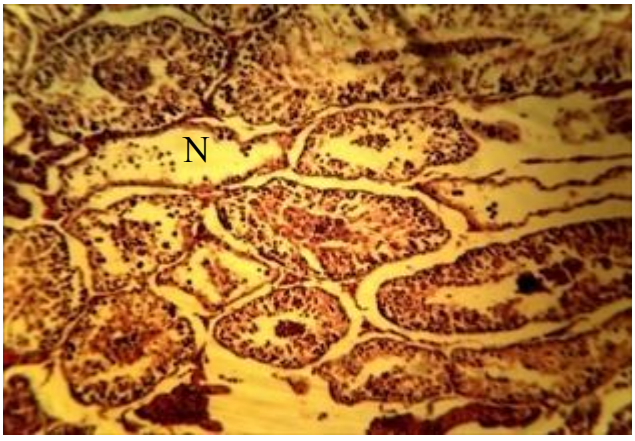
Normal arrangement of the spermatogenic cells in the tubules

(a) Testis Control



N = There is necrosis of spermatogenic cells with fibrous hyperplasia

(b) Testis Week 3:100 mg/kg

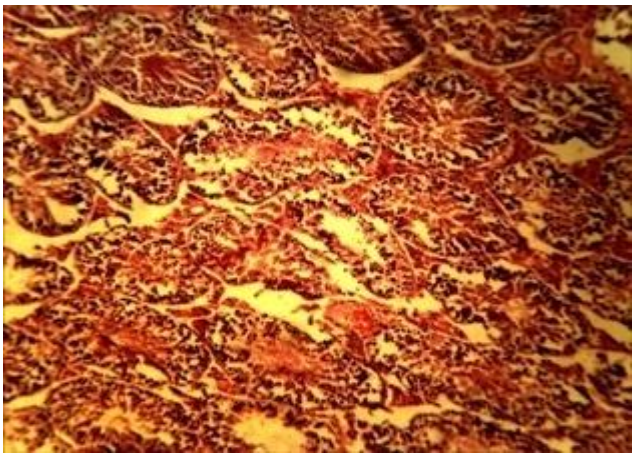


N = There is destruction of the tubular architecture

(c) Testis Week 3: 200 mg/kg

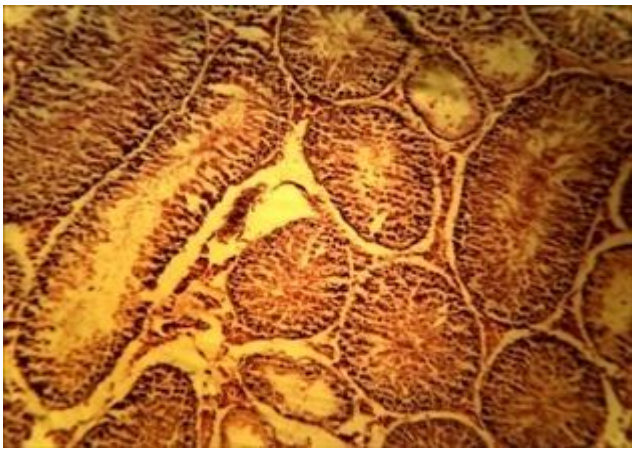
Micrograph of Rat Testis at X1000

Plate 4.3: Micrographs of Control and Crude Treated Rat Testis



Normal arrangement of the spermatogenic cells in the tubules

(a) Testis Control



Restoration of the spermatogenic cells in the tubules

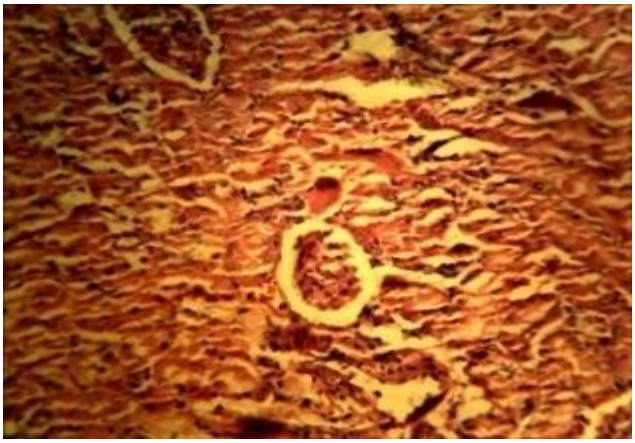
(b) Testis Week 4: 100 mg/kg



Restoration of the spermatogenic cells in the tubules

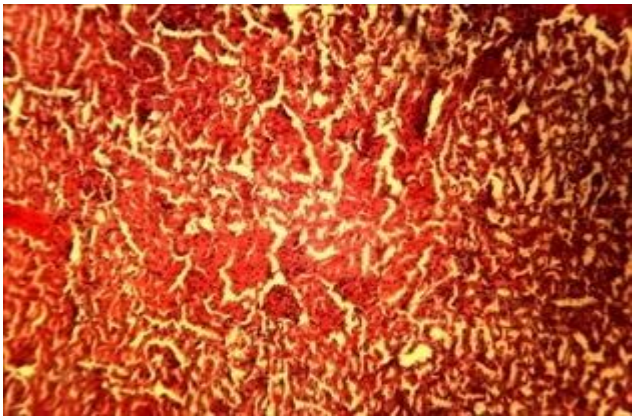
(c) Testis Week 4: 200 mg/kg
Micrograph of rat testis at X100

Plate 4.4: Micrographs of Control and Recovering Crude Treated Rat Testis



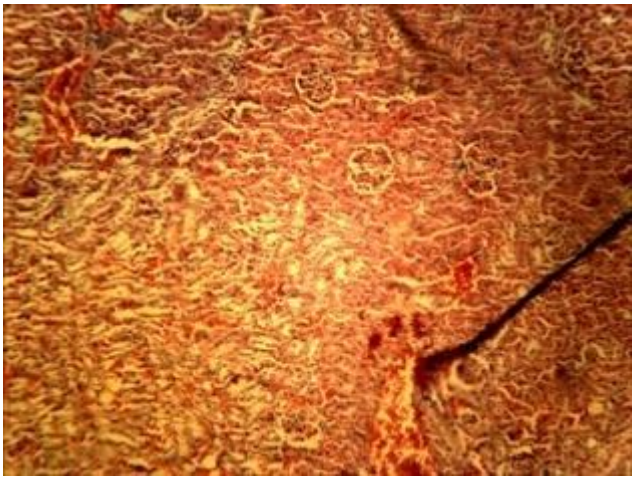
Normal tubular arrangement

(a) Kidney Control



Extensive vascular congestion but with preservation of tubules

(b) Kidney Week 2: 100 mg/kg

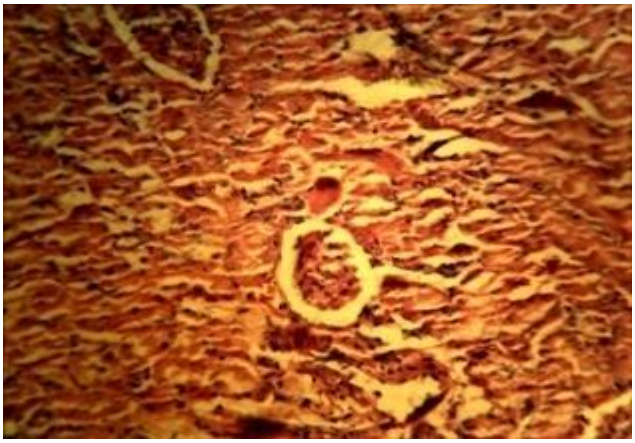


Tubular necrosis with vascular congestion

(c) Kidney Week 2: 200 mg/kg

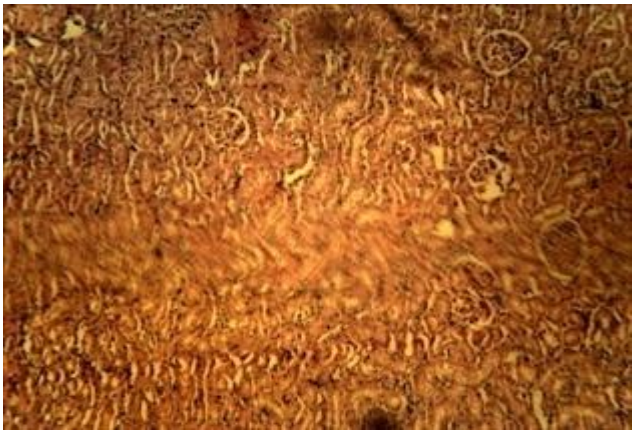
Micrograph of rat Kidney at X100

Plate 4.5: Micrographs of Control and Crude Treated Rat Kidney



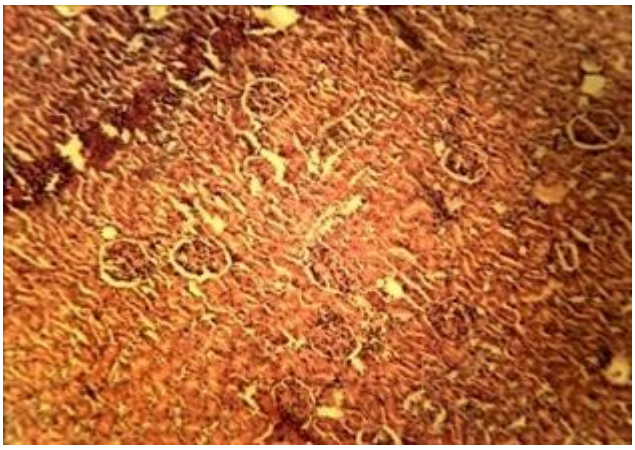
Normal tubular arrangement

(a) Kidney Control



No necrosis with gradual restoration of the tubules

(b) Kidney Week 4: 100 mg/kg

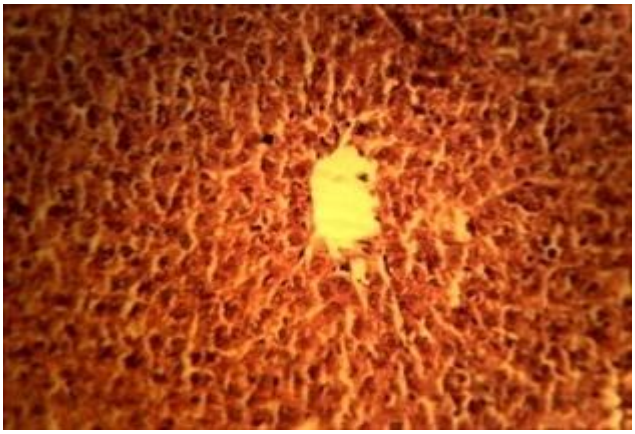


No necrosis with gradual restoration of the tubules

(c)Kidney Week 4 : 200 mg/kg

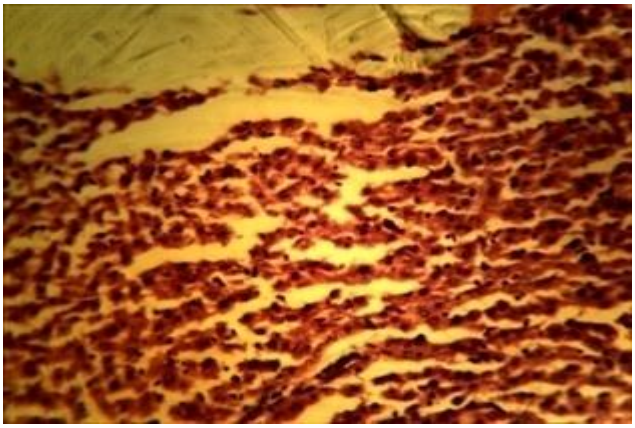
Micrograph of Rat Kidney at X100

Plate 4.6: Micrographs of Control and Recovering Crude Treated Rat Kidney



Normal cells

(a) Liver control



There is cellular vacuolar congestion

(b) Liver Week 3: 100 mg/kg

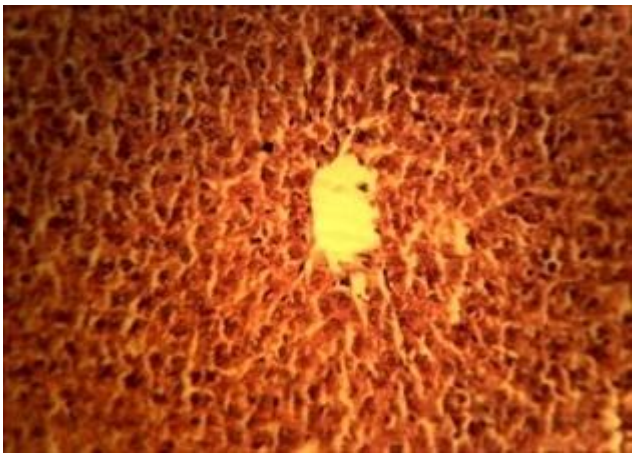


Normal cells with vacuolar congestion

(c) Liver Week 3: 200 mg/kg

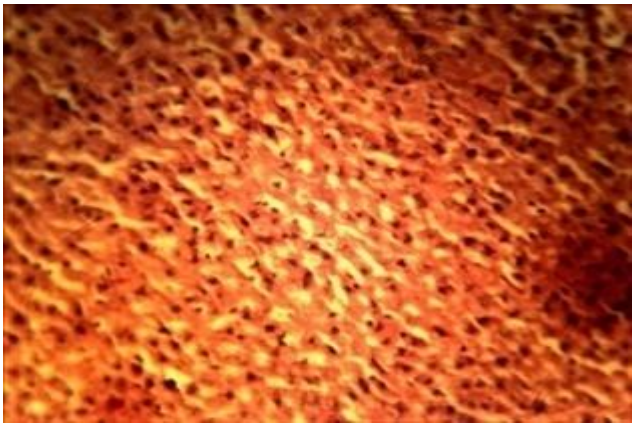
Micrograph of rat Liver at X250

Plate 4.7: Micrographs of Control and Crude Treated Rat Liver



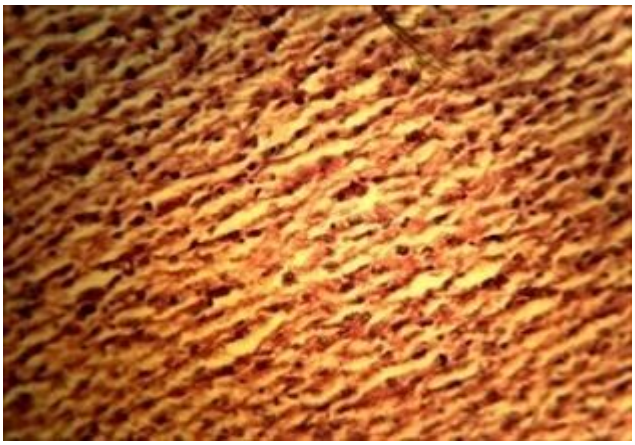
Normal cells

(a) Liver control



Slight cellular necrosis

(b) Liver Week 4: 100 mg/kg

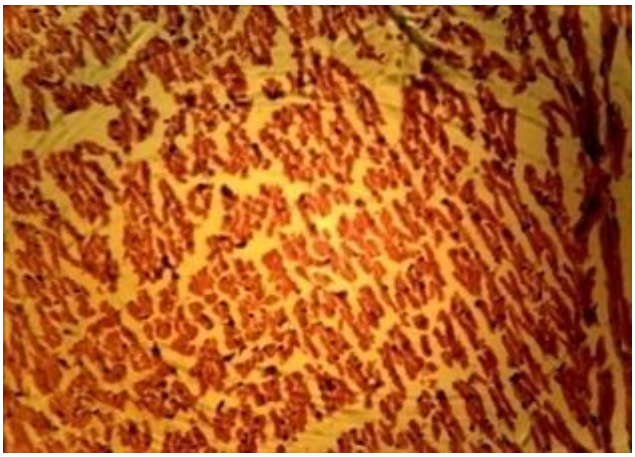


Slight necrosis

(c) Liver Week 4 : 200 mg/kg

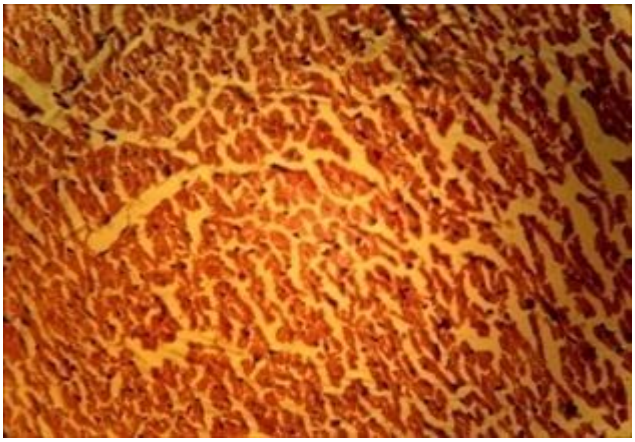
Micrograph of rat Liver at X250

Plate 4.8: Micrographs of Control and Recovering Crude Treated Rat Liver



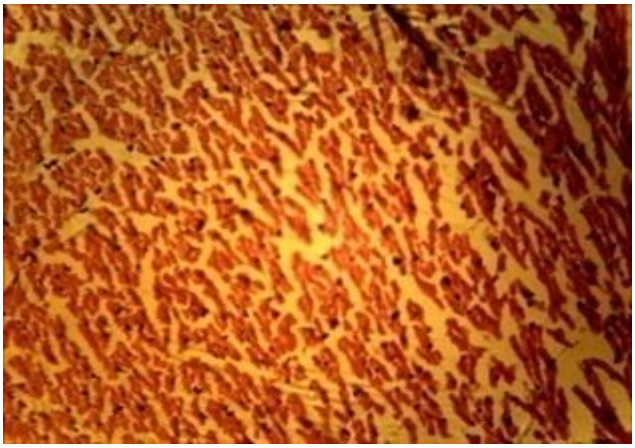
The presentation of the heart was not altered for the control and treated animals

(a) Heart control



The presentation of the heart was not altered for the control and treated animals

(b) Heart Week 3: 200 mg/kg



The presentation of the heart was not altered for the control and treated animals

(c) Heart Week 5: 200 mg/kg

Micrograph of rat Heart at X100

Plate 4.9: Micrographs o control and crude treated rat heart

4.6 Antifertility Properties of Chloroform Fraction of Carica papaya Seed Extract

The weights of all the male wister albino rats treated with chloroform fraction of carica papaya seeds extracts at 100, 200 and 300 mg/kg body weight were not significantly ($P>0.05$) affected (Table 4.18).

The effect of the chloroform fraction on the semen of the treated rats showed a significant reduction at $p <0.05$ in the sperm concentration, percentage live sperm, percentage normal sperm at 200 mg/kg dosage. Whereas at 100 mg/kg and 300 mg/kg, there was no significant difference ($p>0.05$) in the parameters above when compared with the control group (Table 4.19-4.21). This may be that at a lower dose, the threshold concentration to bring about an effective reduction was not attained. However, at the highest dose, other components of the partially purified extract might have constituted a hindrance to its activity.The effect of the chloroform fraction was not dose dependent.

Haematological parameters of PCV, RBC and WBC were not significantly affected at $P>0.05$ (Table 4.22- 4.24).

The serum clinical chemistry of creatinine, SGOT, testosterone and acid phosphatase were not affected adversely ($P>0.05$). From Tables 4.25- 4.28, there was no significant difference between the treated animals and the control group.

The sialic acid and hyaluronidase values were not affected adversely by the oral administration of partially purified extract. Table 4.29 And 4.31 Showed no significant difference at $P>0.05$. But the acrosin values were reduced for 200 and 300 mg/kg body weight at the first week (7 days) of treatment (Table 4.30).

Table 4.18: Initial and Final Weights of Male Wister Albino Rat Treated with Chloroform Fraction of Aqueous Extract

Weight (g)	Group 1 Control	Group2	Group3	Group 4
Treatment 7 days	163.08 ±2.05	163.01±2.07	160.38±1.48	164.97±2.36
14days	164.06±2.48	166.99±0.54	161.25±3.25	161.43±2.99
Recovery Phase 7 days	169.06±3.25	164.30±0.54	168.69±1.88	162.29±1.93

Table 4.19 Sperm Concentration of Male Wister Albino Rats Treated Orally with Chromatographic CF Aqueous Extract of *Carica papaya* Seeds

Sperm conc .(million/ml)	Group1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment 7days	3.33±0.60b	4.67±2.92[a]	1.17±0.17a	5.00±2.25[b]
14days	3.10±0.95	2.00±0.29	3.50±0.50	3.00±0.29
Recovery phase 7 days	4.50±1.76b	3.37±0.23[a]	2.83±0.54[a]	2.20±0.20a

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$
* Where there are no superscripts, it shows no significant difference across the groups (rows)

Table 4.20: Percentage Live Sperm of Male Albino Rats Treated Orally with Chromatographic CF Aqueous Extract of *Carica papaya* Seeds

Percentage Life Sperm (%)	Group1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment 7days	53.67±2.96[b]	56.67±6.22b	17.33±1.73a	54.67±1.45[b]
14days	59.00±3.06[b]	48.67±2.03b	23.00±23.00a	53.00±1.00[b]
Recovery Phase 7 days	63.67±3.84	56.33±7.26	71.00±1.00	58.00±6.00

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$
* Where there are no superscripts, it shows no significant difference across the groups (rows)

Table 4.21: Percentage Normal Sperm of Male Wister Albino Rats Treated Orally with Chromatographic CF Aqueous Extract of *Carica papaya* Seeds

Percentage normal sperm (%)	Group1 Control	Group 2 100mg/kg	Group 3 200mg/kg	Group 4 300mg/kg
Treatment				
7 days	55.00±2.98[b]	59.67±3.53b	17.33±0.33a	59.00±2.08b
14days	62.00±8.02[b]	57.33±2.33b	21.67±11.67a	55.33±1.76b
Recovery Phase				
7 days	58.00±2.08	54.33±2.73	62.00±8.02	54.50±2.50

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$
* Where there are no superscripts, it shows no significant difference across the groups (rows)

Table 4.22: PCV of Male Wister Albino Rats Treated Orally with Chromatographic CF of the Aqueous Extract of *Carica papaya*

PCV (%)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg	Group 4 300mg/kg
Treatment 7days	43±0.01	39±0.01	40±0.02	41±0.02
14days	41±0.02	35±0.01	40±0.02	42±0.01
Recovery Phase 7days	43±0.01	42±0.02	42±0.02	43±0.01

Table 4.23: RBC of Male Wister Albino Rats Treated Orally with Chromatographic CF of the Aqueous Extract of *Carica papaya*

RBC($x10^9/mm^3$)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg	Group 4 300mg/kg
Treatment 7days	7.66±0.32	4.36±0.21	7.30±0.46	7.54±0.54
14 days	7.61±0.81	6.23±0.29	7.44±0.49	6.89±0.40
Recovery Phase 7days	7.97±0.40	7.33±0.23	7.37±0.15	7.69±0.31

Table 4.24: WBC of Male Wister Albino Rats Treated Orally with Chromatographic CF of the Aqueous Extract of *Carica papaya*

WBC count (x 10^6/mm^3)	Group 1 Control	Group 2 100mg/kg	Group 3 200mg/kg	Group 4 300mg/kg
Treatment 7 days	5.27±1.09	5.47±0.94	5.05±0.25	6.30±0.95
14 days	4.47±0.52	5.13±0.55	4.90±0.79	5.10±0.30
Recovery Phase 7 days	4.57±0.66	5.00±0.05	4.43±0.09	4.87±0.20

Table 4.25: Serum Creatinine of Male Wister Albino Rats Orally Treated with Chromatographic Chloroform Fraction of the aqueous extract of *Carica papaya* Seeds

Creatinine (µmol/l)	Group 1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment				
7 days	61.33±3.00	65.67±9.84	57.67±2.02	66.33±1.45
14days	68.33±2.30	71.63±3.18	69.33±1.33	69.50±1.50
Recovery Phase				
7 days	64.08±2.08	64.33±1.20	65.00±3.21	65.00±4.50

Table 4.26: Serum Glutamate OxaloacetateTransaminase (SGOT) of Male Wister Albino Rats Orally Treated with Chromatographic Chloroform Fraction of the Aqueous Extract of *Carica papaya* Seeds

SGOT (iu/L)	Group 1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment 7 days	18.00±1.15	20.67±1.76	23.33±1.76	24.67±1.76
14 days	26.33±1.45	24.67±1.20	26.33±1.20	24.00±2.20
Recovery Phase 7 days	26.33±1.45	27.33±3.52	27.33±1.76	27.50±0.50

Table 4.27: Serum Acid Phosphatase of Male Wister Albino Rats Orally Treated with Chromatographic Chloroform Fraction of the Aqueous Extract of *Carica papaya*

Acid phosphatise (U/ml)	Group 1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment				
7 days	11.43±0.58	11.90±1.45	13.27±1.82	12.83±1.29
14 days	12.87±0.99	15.73±1.73	12.57±1.07	11.50±0.70
Recovery Phase				
7 days	14.00±0.18	11.67±1.73	12.17±0.85	13.40±2.60

Table 4.28: Serum Testosterone of Male Wister Albino Rats Orally Treated with Chromatographic Chloroform Fraction of the Aqueous Extract of *Carica papaya* Seeds

Testosterone (ng/ml)	Group1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment				
7 days	4.07±0.32	4.73±1.06	4.27±0.37	4.73±0.15
14 days	4.83±0.46	5.07±0.52	4.23±0.18	4.00±0.20
Recovery Phase				
7 days	4.6±0.17	4.10±0.17	4.90±0.70	3.65±0.75

Table 4.29: Seminal Sialic Acid of Male Wister Albino Rats Orally Treated with Chromatographic Chloroform Fraction of the Aqueous Extract of *Carica papaya*

Sialic acid (Iu/ml)	Group1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment				
7 days	36.33±4.48	30.33±2.40	35.33±4.51	37.67±2.40
14 days	36.00±2.52	32.33±1.86	31.33±3.38	36.50±3.50
Recovery Phase				
7 days	32.33±3.48	28.33±2.60	30.33±3.48	32.00±3.00

Table 4.30: Seminal Acrosin of Male Wister Albino Rats Orally Treated with Chromatographic Chloroform Fraction of the Aqueous Extract of *Carica papaya*

Acrosin (iu/ml)	Group1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment				
7 days	77.00±7.55b	80.00±7.37b	55.33±6.59a	51.67±4.26a
14 days	86.67±4.06	83.00±2.08	87.33±7.86	85.13±2.76
Recovery Phase				
7 days	84.00±3.06	99.67±6.98	98.00±9.71	98.00±3.18

* Values with different superscript across the groups (rows) are significantly different from each other at $P<0.05$

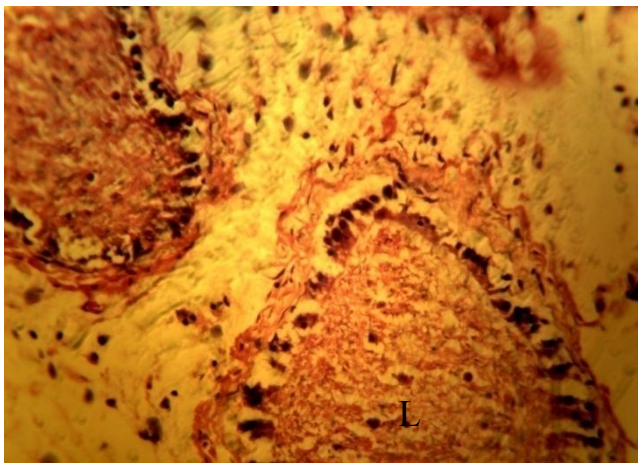
* Where there are no superscripts, it shows no significant difference across the groups (rows)

Table 4.31: Seminal Hyaluronidase of Rats Orally Treated with Chromatographic Chloroform Fraction of the Aqueous Extract of *Carica papaya*

Hyaluronidase (iu/ml)	Group1 Control	Group 2 100 mg/kg	Group 3 200 mg/kg	Group 4 300mg/kg
Treatment				
7 days	113.30±7.42	102.67±4.06	98.67±3.38	101.67±3.38
14 days	86.67±4.06	83.00±2.08	87.33±7.86	85.13±2.76
Recovery Phase				
7 days	95.33±1.46	93.33±1.76	95.33±1.76	95.00±1.00

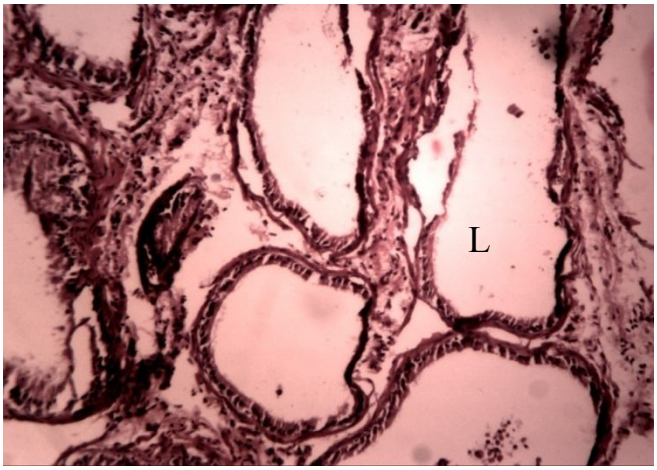
4.7 Histopathology of Rats Treated with Chloroform Fraction

The cauda epididymides and testes of animals treated with chloroform fraction of the aqueous extract of *Carica papaya* seeds showed pronounced alterations of the spermatogenic cells. The kidney and the liver showed slight necrosis with 200 mg/kg body weight dosage. But the heart was not significantly affected. In all cases restorations of the cells and organs were established during the recovery stage (Plate 4.10 – 4.17).



L= Lumen normal presentation of the lumen packed with spermatozoa

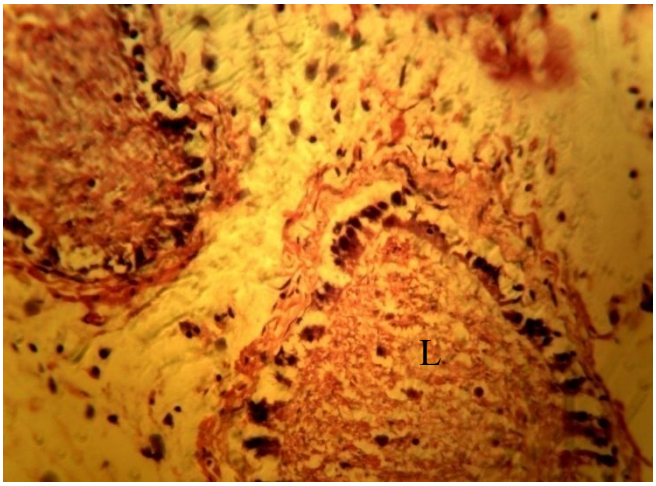
(a) Control Rat Epididymis (micrograph X250)



L= Lumen
The lumen is completely empty of spermatozoa

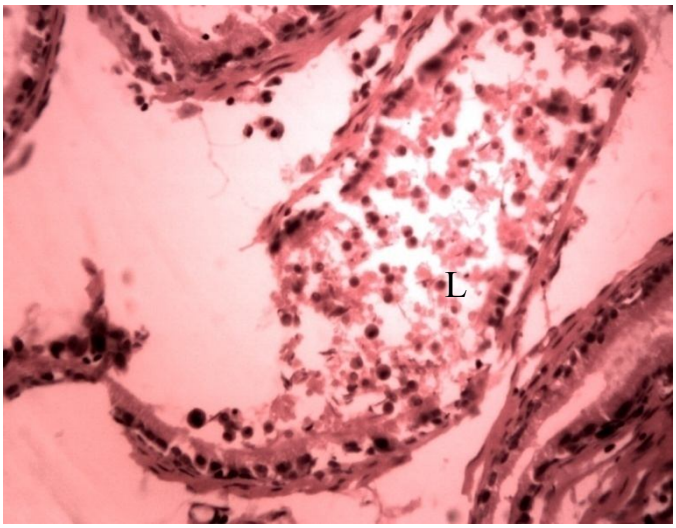
(b) Rat Epididymis wk 1: 200mg/kg (micrograph X250)

Plate 4.10: Micrographs of Control and Chloroform Fraction Treated Rat Epididymis



L= Lumen normal presentation of the lumen packed with spermatozoa

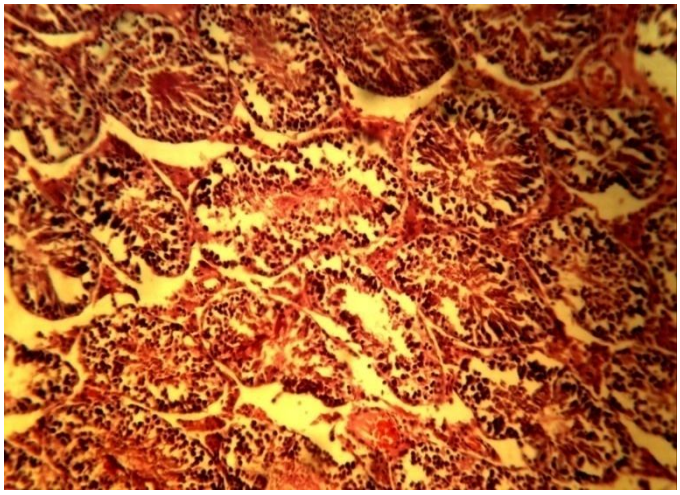
(a) Control Rat Epididymis (micrograph X250)



There is restoration of the s spermatozoa in the lumen

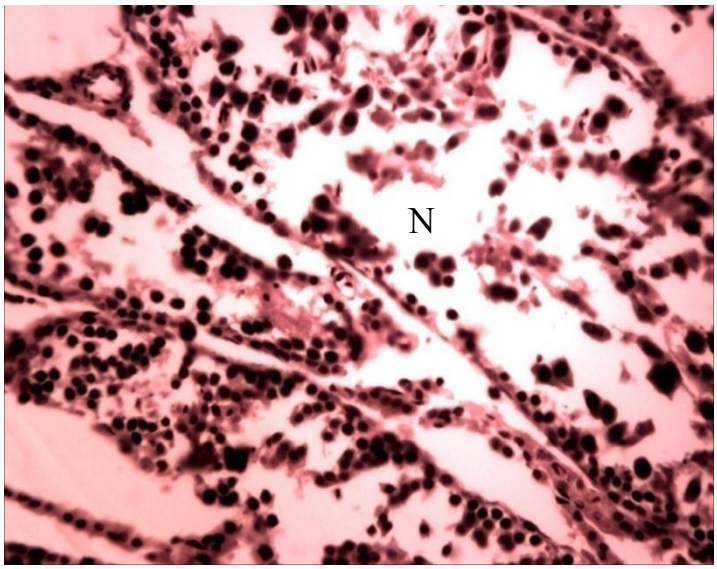
(b) Rat Epididymis wk 3: 200mg/kg (micrograph X250)

Plate 4.11: Micrographs of Control and Recovering Chloroform Fraction Treated Rat Epididymis



Normal spermatogenic cells

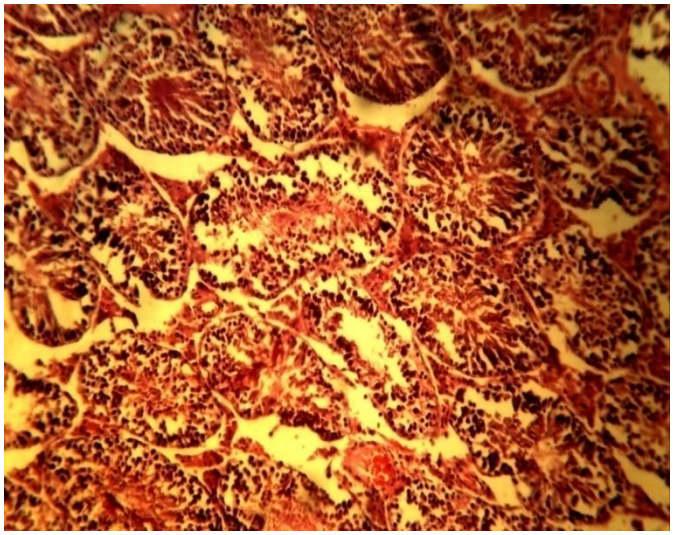
(a) Control Testis (micrograph X100)



N = Necrosis of spermatogenic cells

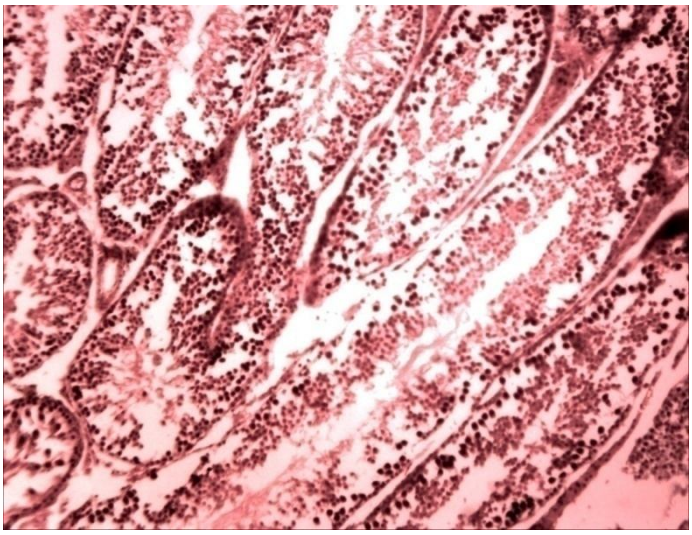
(b) Testis wk 2: 200mg/kg (micrograph X 100)

Plate 4.12: Micrographs of Control and Chloroform Fraction Treated Rat Testis



N = Necrosis of spermatogenic cells

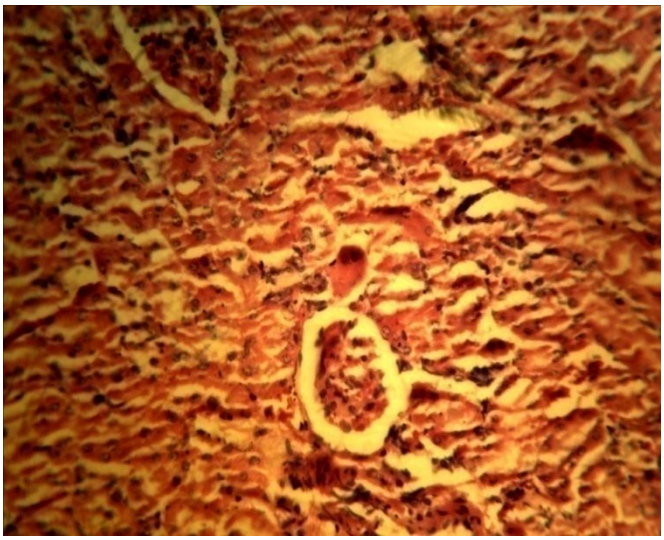
(a) Control Testis (micrograph X100)



Gradual restoration of spermatogenic cells

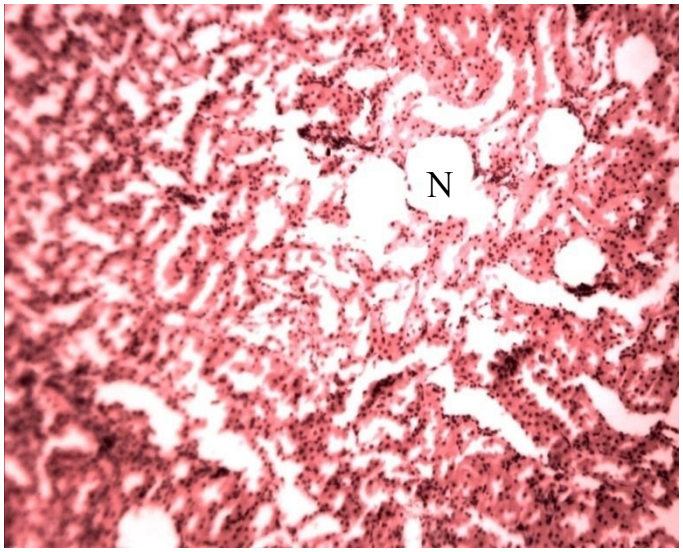
(b) Testis wk 3: 200mg/kg (micrograph X 100)

Plate 4.13: Micrographs of Control and Recovering Chloroform Fraction Treated Rat Testis



Normal tubular arrangement

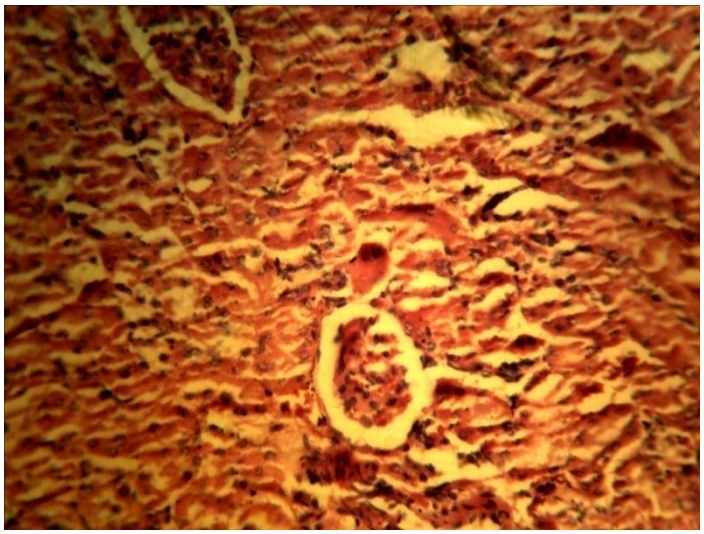
(a) Kidney Rat Control (micrograph X100)



Extensive tubular necrosis

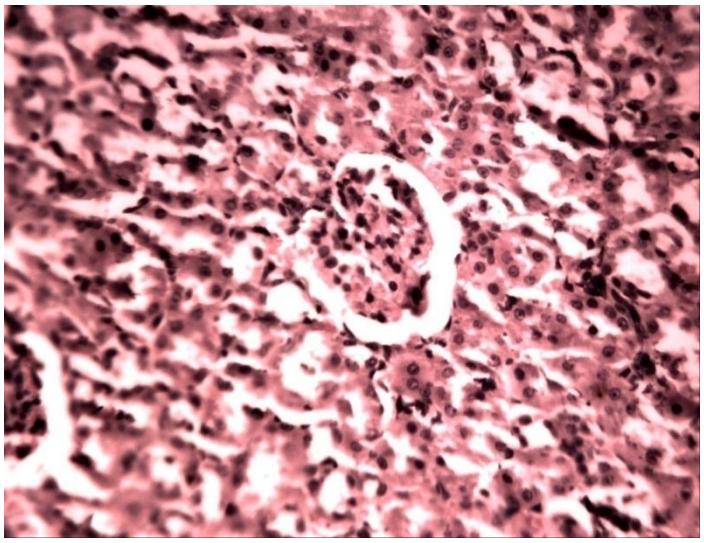
(b) Rat Kidney wk 2: 200mg/kg (micrograph X100)

Plate 4.14: Micrographs of Control and Chloroform Fraction Treated Rat Kidney



Normal tubular arrangement

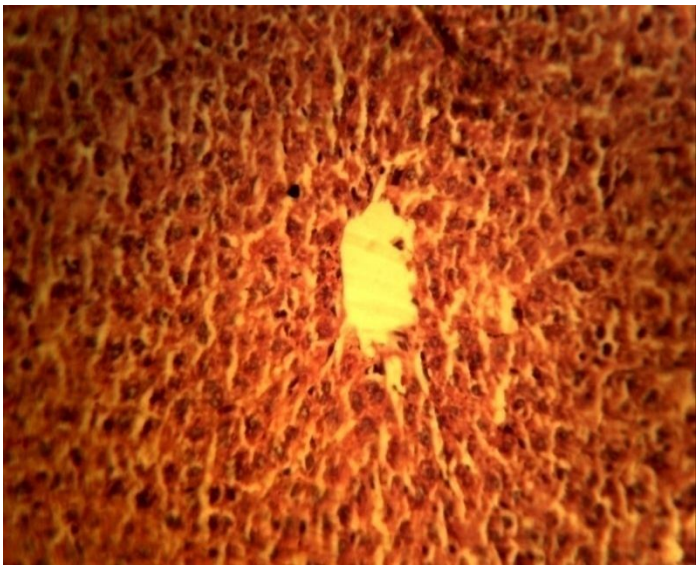
(a) Kidney Control (micrograph X100)



Restoration of the tubules

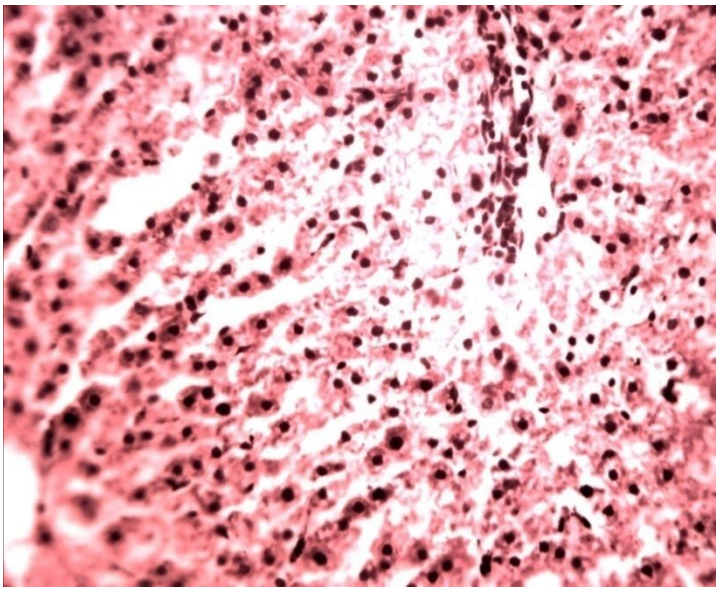
(b) Kidney wk 3: 300mg/kg (micrograph X100)

Plate 4.15: Micrographs of Control and Recovering of Chloroform Fraction Treated Rat Kidney



Normal cells presentation

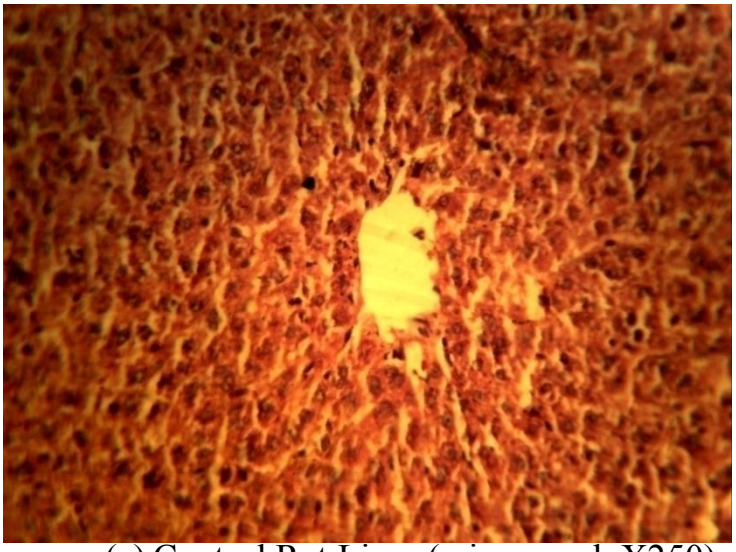
(a) Control Rat Liver (micrograph X250)



Cellular necrosis

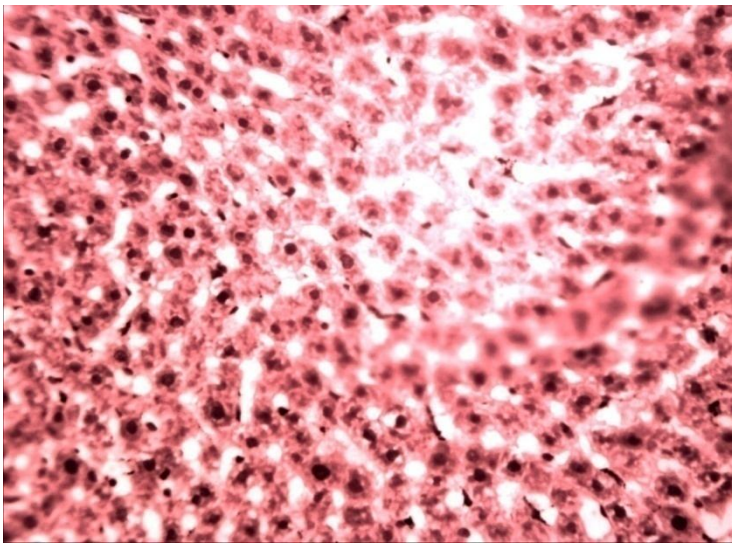
(b) Rat Liver wk 2: 200mg/kg (micrograph X 100)

Plate 4.16: Micrographs Control and Chloroform Fraction Treated Rat Liver



Normal cells presentation

(a) Control Rat Liver (micrograph X250)



Restoration of the cells

(b) Rat Liver wk 3: 200mg/kg (micrograph X100)

Plate 4.17: Micrographs Control and Recovering Chloroform Fraction Treated Rat Liver

CHAPTER FIVE

Discussion

In the earlier studies, it was reported that the seeds of *Carica papaya* induced varied responses according to the kind of extract, dose, duration and mode of administration. Out of the various extracts tested, it was observed that the aqueous and chloroform extracts and the chromatographic fraction of the chloroform extract were found to be more effective (Lohiya and Goyal, 1992; Lohiya *et al.,* 1995, 1994a, b; Chinoy *et al.,* 1995). In all these studies, it was indicated that the effects were post-testicular, mediated through the alterations in the epididymal milieu, thereby altering the functional ability of the spermatozoa without affecting libido and toxicological profile (Lohiya *et al.,* 1994a; Chinoy *et al.,* 1995).

In present study, the aqueous extract of *Carica papaya* seeds induced reduction in sperm concentration, percentage live sperm and percentage normal spserm in male albino rats. The study further confirmed the fact that the antifertility effects were dose and duration dependent. The animals treated with 200 mg/kg body weight of the crude extract showed greater reduction on the sperm concentration and percentage live sperm. This effect was more pronounced at the third week when compared with the first and second weeks. These findings are in agreement with the results obtained by previous investigations. Das (1980)

reported that the dry seed powder of *Carica papaya* suspended in distilled water, fed orally at a dose of 20 mg/ animal per day for 8 weeks produced partial sterility in male albino rats. Aqueous extract of the seeds of *Carica papaya* at 0.5 mg and 5.0 mg/kg body weight per day for 7 days produced complete loss of fertility in male albino mice. Lohiya *et al.,* (1994a&b) reported 100% sterility in male albino rats treated orally with aqueous seed extract at the dose regimen of 10 mg and 50 mg/rat for 90 days. In rabbits, Vyas and Jacob (1984) reported 100% sterility when treated with the aqueous suspension of the seeds of *Carica papaya* orally at 100, 200 mg/animal for 10 and 20 days.

There was no significant different between the values of the PCV, total RBC, and WBC count, obtained from the control and the treated animals throughout the period of the investigation. These suggest that the extract did not have hematotoxic effect. However, the histopathological studies showed some cellular infiltration in the testis, epididymis, liver and kidney.

Copper and Yeung (1999) observed in an earlier investigation that the antifertility effect of *Carica papaya* seed could be directly linked with the epididymal enzymes. Mack *et al,* (1983) also reported that certain enzymes, namely acrosin and hyaluronidase have been linked to spermatozoa function through the acrosome. Activities of other acrosomal enzyme, like α-glucosidase and arylsulfatase have been reported not to give additional information of the

fertility status (Krause and Bohrina, 1999). Monica *et al.,* (2005) observed that among all the sperm acrosomal enzymes studied, acrosin has been identified in all the species evaluated and has been associated with human sperm fertility potential.

The present study showed a significant decrease on the acrosin and hyaluronidase values of crude extract of *Carica papaya* treated groups, when compared with the control group. This may have effect on the spermatozoa ability to penetrate the oozyte. Another important parameter for spermatozoa maturation, the sialic acid, showed a significant decrease in the values obtained in crude extract treated groups. Immediately the treatment was withdrawn, there was a gradual increase in all the parameters studied by day 7. These suggest that the effect of the extract is reversible. This finding correlate with the earlier reports that the antifertility effect of *Carica papaya* seed is reversible.

In this study, it was also observed that the serum creatinine, SGOT, testosterone, acid phosphatase and body weights values of the treated animals (both during and after treatment) showed no significant difference compared to the control animals. Despite the slight cellular infiltration in the histopathology studies, these observations still showed that the extract may not be toxic and does not have effect on the hormone and this correlates with the work done by Pathak *et al.,* (2000).

The chloroform fraction also showed similar activities compared to the crude extract at 200 mg/kg body weight. These activites were only observed in sperm concentration, percentage live sperm, percentage normal sperm and acrosin values, while hyaluronidase and sialic acid values were not affeced. The decrease in the activities of the partially purified chloroform fraction may be as a result of the differences observed in their phytochemical constituents. The antifertility effect of the crude and the chloroform fraction may be associated with the presence of the alkaloid and flavonoid and this is in line with an earlier report by Chaudhury, (1985; 1991) and Nirmal *et al.,* (2000).

The crude aqueous extract of *Carica papaya* seeds showed antifertility effect in male albino rats by reducing the sperm concentration, lowering the percentage life sperm and percentage normal sperm. The investigation showed that the epididymal sialic acid and acrosomal enzymes (acrosin and hyaluronidase) were implicated in the antifertility activity.

An important finding of the present study is that the treatment of male albino rats with the crude and chromatographic chloroform fraction of the aqueous extract of *Carica papaya* seeds showed some antifertility effect within two weeks of treatment and the effect were reversible.

CHAPTER SIX

Summary, Conclusion and Recommendation

6.1 Summary

The present study was conducted to investigate some antifertility potentials of aqueous crude and partially purified extract of *Carioca papaya* seeds and its effects on acrosomal enzymes using male albino rats. At the end of this study, the following results were recorded:

i. The crude and partially purified extract of *Carica papaya* seeds have LD_{50} > 5000 mg/kg body weight.

ii. The phytochemical constituients of crude and chloroform fraction of the aqueous extract showed the presence of flavonoids, saponnis, tannins, anthraquinones, cardiac glycoside and alkaloids in different proportions.

iii. There was no significant ($p>0.05$) difference in the body weights of the male albino rats treated with both crude and chloroform fraction of the aqueous extract of *Carica papaya* seeds.

iv. There were siginifcant ($p<0.05$) decreases in the values of the sperm concentration, percentage live sperm and percentage normal sperm.

v. The hematological parameters of PCV, total RBCs, and WBCs showed no significant ($p>0.05$) difference.

vi. The clinical chemistry parameters of serum creatinine, SGOT, acid phosphatase and testosterone also showed no significant ($p>0.05$) difference.

vii. There was siginificant ($p<0.05$) decrease in the sialic acid, acrosin and hyalurionidase values for the treated animals.

viii. The histological analysis showed a slight cellular infiltration in the testis, liver and kidney of the treated animals.

ix. The effect of the extract was dose and duration dependent.

6.2 Conclusion

The objectives of this study were achieved as stated below:

i. There was significant difference in the phytochemical constituents between the crude and the chloroform fraction.

ii. Both crude and partially purified (chloroform fraction) extract showed some antifertility properities in male albino rats.

iii. Both the crude and the partially purified aqueous extract of *Carica papaya* seeds showed no toxicity in some of the parameters studied.

6.3 Recommendation

i. Further work is required to isolate and characterize the active ingredient in the *Carica papaya* seeds extract.

ii. There is need to elucidate the mechanism of action of the *Carica papaya* seeds extract.

iii. There is need to investigate the effect of the *Carica papaya* seeds extract on other acrosomal enzymes.

iv. Other laboratory animals may be used for the study.

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APPENDICES

APPENDIX 1

Summary of medicinal plants exhibiting antifertility activity in males (1980-2005)

	Name of the plant	Common/English name	Part used	Type of plant extract/active principles	Animal models	Activities
1	***Abrusprecatorius Linn***	Chirmi	Seed	50% Ethanol extract	Rat	Reduces sperm motility and density
				Steroidal fraction	"	Antispermatogenic effect and reduced activity of testicular enzyme
				50% Ethanol extract	"	Post-testicular antifertility Effect
				Alcoholic extract	"	Reduced sperm motility
				Methanol extract	Langur Monkey	Sperm motilityactivity Antispermatogenic and Antiandrogeniceffect
2	***Acacia concinna DC***	Shikakai	Stem bark	Acacic acid	Rat	Spermicidal and semen coagulating activities
3	***Acacia auriculae-form is A. Cunn***			Triterpene, saponins	-	Sperm immobilizing effect
4	***Acacia caesia Wight&Arn..***	Aila	Fruit	Saponins	-	Immobilization of Spermatozoa
5	***Achillea millefoliun Linu.***	Gandna Yarro	Flowers	Ethanol and hydro-alcoholic extract	Mice	Antisperniatogenic effect
6	***Achyranthes aspera Linn.***	Kadaladi	Root	50% Ethanol extract	Rat	Spermicidal action
7	***Albizia lebbek (Linn) benth***	Siris	Pod Bark	Methanol extract saponins	Rat	Antiferility activity
8	***Albizia procera (Roxb) Benth***	Safed siris	Seed and root	Proceric acid and leanic acid	Rat	Spermicidal and semen coagulatig activities.

9	***AlIium sativum Linn***	Lahsun	Pod	Dry Powder	Rat	Antispematogenic activity
10	***Aloe barbadensis Mill***	Gheekunwar	Leaves	50% Ethanol extract	Dog	Antiandrogenic activity
11	***Alstonia scholkaris R.Br***	Saptaparni	Stem bark	Methanol extract □-Amyrine	Rat Rat	Antifertility Effect Suppression of fertility with marked decline in germ cell population
12	***Anagallis arvensis Linn.***	Dhartidhak	Whole plan	-	"	Spermicidal and semen coagulating activityes
13	***Ananas comosus Merr.***	Annanas	Unripe fruit	Alcoholic extract	Rat	Antispematogenic activity
14	***Andrographis paniculata Wall. Ex Nees***	Kirayat	Leaves	Dry powder Andrographilode	Rat	Antispermatogenic and antiandrogenic Effect on spermatozoa
15	***Annona squamosa Linn.***	Sitaphal	Seed	Alcoholic	-	Antispermatogenic activity
16	***Aristolochia indica Linn.***	Hukka bel	Root	Aristolochic acid	Presbytes langur	Antispermatogenic and antiandrogenic effects
17	***Austroplenckia populnea (reiss.) Lundell.***	-	-	Hydro-methanol extract	Rat	`affects the sexual behavior and epididymal sperm concentration
18	***Azadirachta indica A. Juss***	Neem	Seed Leaves	Seed oil Neem oil Seed oil Praneem cream Ethanol extract	Rat Mouse Rat Monkey Human Monkey Rat	Antispermatogenic and antiandrogenic effect Antispermatogenic effects Post-testicular antifertility effects Antispermatogenic effect Spermicidal activity Induced azoospermia Antispermatogenic and antiandrogenic effect Spermicidal activities " Antispermatogenic activity

			Seed “ Leaves “ Bark and flower Bark and seed Leaves	Dry powder Neem oil Dry poweder Dry powder Aqueous suspension 50% ethanol extract 50% ethanol extract and seed oil Aqueous extract		Antispermatogenic activity Antispermatogenic activities and histological changes in tests and epididymides Degeneration of fertilizing ability of sperm Antiandrogenic activity Antispermatogenic and antiandrogenic activity Leydig cell dysfunction Spermicisal activity
19	***Balanites roxburghli Linn***	Hingan	Fruit Fruit putp	Pulp extract Ethanol extract	Dog	Antispermatogenic activity Testicularnecrosls and Atrophy
20	***Bambusa arundinacea Wild.***	Beans	Shoots (tender	Ethanol extract Stem extract	Rat	Impairedthestructural and functional activity of epididymis Reduced sperm motility
21	***Barleria pnonitis Linn.***	Vajradanti	Root	Methanol extract		Antifertility effect
22	***Berberis chifria Buch.-Ham. ex LindI.***		Root	Palmitine hydroxide	Dog	Antispermatogenic action
23	***Bursera sp.***	Mexican copal	Stem leaf	Saponins, leaf extract	Human Bovine	Sperm aggregation
24	***Butea monosperma (Lam) Kuntze***	Palash	Seeds Whole Plant	Butin Water soluble ethanol extract	Rat, Dog and Monkey rat	Effects on testicular function Antispermatogenic effect
25	***Calotropis procera (Ait) R. Br***	Aak	Root Flower	Calotropin Aqueous ethanol extract	Gerbil and Rabbit Mice	Antispermatogenic effect and Leydig cell atrophy Functional alteration in the genital organs and inhibition of fertility
26	***Cannabis sativa Linn.***	Ganja	Leaves	Butin	Presbytis Monkey	Testicular lesions and atrophy of Leydig cells
27	***Carica papaya Linn.***	Papita	Fruit Seed Seed Powder Seed	Dry powder Aqueous extract Aquoeuos extract Chloroform extract Aquoeuos extract Benzene chromatographic sub fraction of chloroform extract	Rat Rat Rabbit Rat Rat Rabbit Mouse Rabbit	Antispermatogenic activity. Induced total sterility Inhibit fertility Reversible post testicular antifertility effects Antifertility actrivity and reversible sterility Contraceptive effect Antispermatogenic effects Induced azoospermia and reversible regressive effect

				Aqueous extract Chloroform extraction Benzene extract	Human Rabbit Langur monkey Rat Rabbit	Induced azoospermia and oligospermia Spermicidal activity No side effects Induced azoospermia Antiferlity effect Induced sterility due to total suppression of sperm motility Alter cauda epididymal microenvironment Ultrastructural changes in the testis and epididymis
28	***Catharanthus roseus G. Don syn. Vinca rosea Linn.***	Sadabahar	Leaves “ “ Whole plant “ “ “	Extract “ Aqueos extract Vincristin “ “ “	Rat Mice Mice Rat	Antispermatogenic activity Antiandrogenic activity Antispermatogenic activity Regression of entire reproductive system Decrease in secretory activity of accessory sex gland Epididymal dysfunction Presence of multinucleated Giant cells, spermatogenic arrest.
29	***Celestrus paniculatus Willd.***	-	Seed	Seed extract	Rat	Antispermatogenic activity
30	***Cichorium intysbus Linn.***	Kasni	Whole plant	Aqueous extract	-	Antispermatogenic activity
31	***Cinnamomum camphora Nees & Eberm.***	-	Seed	-	Sparrow	Arrest and inhibition of spermatogenesis
32	***Citrullus colocynthis Schrad.***	Tumba	Fruit “	50% Ethanol extract “	Rat “	Impairment of sperm Induced reversible antifertility effects
33	***Colebrookia oppositifolla Sm.***		Leaves	Ethanol extract	Rat	Antifertility activity
34	***Convolvulus microphyllus Sieb. a Spreng.***	Shankh pushpin	Whole plant	Ethanol extract	Rat	Antispermatogenic effect
35	***Crotalaria juncea Linn.***		Seeds	Ethanol extract	Mice	Arrest of spermato genesis and antiandrogenic activity
36	***Cuminum cyminum Linn.***	Jeera	Seed	Ethanol extract	Rat	Antisperniatogenic effect
37	***Curcuma lin ga Linn.***	Haldi	Root	50% Ethanol extract	Rat Rat	Interference with spermatogenesis at later stages and antiandrogenh effect Arrest of sperniato genesis and antiandrogenic effect

38	***Cyckamen persicum Mill.***		Whole Plant	Saponins	-	Spermicidal activity
39	***Cynomorum coccineum Linn.***	-	-	Aqueous extract	Rat	Effect on epididymal sperm pattern
40	***Daucus carota Linn.***		Seed	-	Rat	
41	***Desmodium gangeticum DC.***	Chapot	Whole plant	Gangenticum	Rat	Antifertilityt effect
42	***Diploclisia echinatus Linn.***		Stem	Ecdysterone	-	Spermicidal effect
43	***Diploclisia echinatus Linn.***		Stem	Ecdysterone	-	Spermicidal activity
44	***Ecballium elaterium A. Rich***			Contrasperm	Rabbit	Decreases sperm motility
45	***Echeveria gibbiflara DC.***		Whole plant	Aqueous crude extract	Guinea pig	Reduced sperm motility
46	***Echinops echinatus Roxb***	Oontkatalo	Root	50% Ethanol extract	Rat	Sperm antimotility Reduces sperm density In cauda epididymis
47	***Embelia ribes Burm. F.***	Vidang	Berry	Embelin	Rat	Reduced testosterone level Antifertility activity Antispermatogenic and antiandrogenic activity Spermicidal activity Antifertility activity
48	***Epilobiurn angustifolium Linn.***	Rosbay	-	Hexane/Aqueous extract	Rat	Reduction in weight of Accessory Sex organs
49	***Eupatorium brevipes DC.***	-	-	Brevipenin	-	Spermicidal activity
50	***Euphorbia neriifolia Linn.***	Thuar	Root	50% Ethanol extract	Rat	Antispermatogenic effects
51	***Foeniculum vulgare Mill.***	Saunf	Whole plant	Alcoholic extract	Rat	Antiandrogenic activity
52	***Gloriosa superb Linn.***	Shaker pusphi	Tuber	Ethanol extract	Gerbil	Shrinkage of Seminiferous tubules and Leydig cells
53	***Gossypium herbaceum Linn.***	Cotton	Seed	Gossypol	Mice “ Rat Human Mice	Reduced sperm density and weight of reproductive organs Spennicidal activity Reduced motility of spermatozoa, histological changes in epididymis Antispermatogenic effect, muscular paralysis Reduction in sperm Production

					Rat Hamster Human Rat “ Langur monkey Rat Human Human Hamster Mouse	Antispermatogenic activity Arrest and inhibition of spermatogenesis Reduce sperm motility Alteration in spermatozoa Structure Effect on pituitary reproductive axis and antispermatogenic effect Induce oligospermia, decrease sperm motility Adverse effect on epididymal function Effect on secretory activity of accessory sex organs Antifertility effect Reduce sperm motility Inhibition of sperm fertilizing capacity Antifertility effect
54	***Hedera nepalensis K. Koch***	-	Inflorescence	-	-	Immobilization of spermatozoa
55	***Hibiscus rosa-sinensis Linn.***	Gudhal	Flower	50% Ethanol extract Bcnzene extract Benzene/Ether	rat Noiiscmd Bat Mice	Antispetmatogenic and antiandrogenic activity Antlspeiinatogenic Activity Antispermatogenic/ antiandrogenic activity
56	***Hyptis suaveolens Poit.***	Wilayati tulsi	Whole plant	Benzene/Ether extract	Mice	Antifertility effect
57	***Justicia simplex D.Don***	Kala adoosa	Flower	Justicisa saponin	-	Sperm acrosomal membrane stabilizing action
58	***Lepidium. meyenu Walp***	Maca	Root	Aqueous extract	Rat	Invigorates spermalo-genesis by acting on its initial stages (lX-XIV)

59	***Malvaviscus conza*tii Greenm.***	Gercenum	Flower	Methanol extract Alcoholic extract Ethanol extract 50%	Mice Mouse Rat	Antifertility activity Antispermatogenic and antiandrogenic effect AntispermatogenicacuvityLk
60	***Martynia annua Linn.***	Bichchhu	Root	50% Ethanol exract	Rat	Antispermatogenic activity
61	***Melodinus fusiformis Champ. Ex Benth***	-	-	Solasodine (plant steroidal alkaloid)	-	Spermicidal activity
62	***Methan arvensis Linn.***	Pudhina	Leaves	Aqueous extract Pet. Ether extract Methanol extract	Mouse Mice “	Sex organs secretion decreased, antiandrogenic effect Antifertility activity
63	***Millettia auriculata Baker. Ex Brand***	Ganj	Leaves	Alcoholic extract	Rat	Reduction in implantation sites in female mated with Milettia treated male rats.
64	***Momordica charantia Linn.***	Karela	Seeds	-	Rat	Antispermatogenic, antiandrogenic and antisteroidogenic activity
65	***Monda whiteii Skeels***	-	Root bark	-	Rat	Reversible antispermatogenic and antifertility activity
66	***Mucuna urens Medik***	Horse eye bean	Seeds	-	Guinea pigs	Effect gonads and sex accessory glands.
67	***Myristica fragans Hout.***	Jaiphal	Seed	Ethanol extract	-	Premature ejaculation
68	***Nicotiana tabucum Linn.***	Tobacco	Leaves	Nicotine	Rat	Antiandrogenic effects
69	**Ochana jabotapita Linn.**	***Kanakchampa***	Plant (without root)	Ethanol extract	-	Semen coagulating activity
70	***Ocimum sanctum Linn.***	Tulsi	Leaves Leaves	Powder -	Rat Rat	Arrest of spermatogenesis and atrophy of Leydig cells Reduction of sperm motility and sperm count
71	***Oppphiopogon intermediu (D. Don) Maxin***	-	Rhizone	n-Octacosanal □-sitosterol	-	Spermicidal activity
72	***Opuntia dilleni Haw***	Nagphana	Phylloclade	Methanol extract	Rat	Antispermatogenic effect
73	***Piper betle Linn.***	Pan	Petiole Leaf stalk Alcoholic extract	Ethanol extract Water soluble ethanol extract Stalk extract 	Rat Rat Rat Mice	Reuced sperm motility Biochemical changes in epiddymal fluid Antiadrogenic effect, altered testicular histology Antifertility activity
74	***Piper longum Linn.***	Long peeper	Flower and bud	Piperine	Rat	Antispermatogenic effect
75	***Pittosporum neelgherrense Wigh & Arn***	-	Plant (with out root)	Pittosida-A and Pittosida-B	-	Spermicidal and semen coagulating activity
76	***Plumbago zeylanica***	Chitrak	Root, Stem	Plumbagin	Rat	Antiandrogenic activity

	Linn.		bark			
77	***Plumeria alba Linn.***		Leaves	-	Rabbit	Antispermatogenic Effect
78	***Portulaca caeruleum Linn.***	Lunkha	Seed	Alcoholic extract	Mice	Impairment of spermatogenesis
79	***Primula vulgaris Huds***	-	-	Saponins	-	Immobilization of spermatozoa
80	***Pterocarpus santalinus Linn. F.***	-	Stem bark	-	-	Semen coagulating activity
81	***Pueraria tuberose DC.***	Vidharikand	Root	Methanol extract	Rat	Inhibition of spermatogenesis
82	***Pyrus cuspidate Bertol.***	Naspati	Plant (withy out root)	Extract	-	Spermicidal and semen coagulating activity
83	***Quassia amara Linn.***	-	Stem wood Stem bark	Methanol extract Chloroform extract	Rat Rat	Antifertility activity “
84	***Ricinus communis Linn.***	-	-	50% Ethanol extract	Rat	Alteration in the motility, mode of movement and morphology of sperms.
85	***Rubus ellipticus Sm.***	Katsan	Whole plant	-	-	Antifertility activity
86	***Salvia fruticosa Mill.***	Satari	Leaves	Aqueous/Ethanol extract	Rat	No. of implantation site and viable fetuses reduced in female in pregnant by treated male rats.
87	***Sapindus mukorossi Gaertn.***	Ritha	Fruit Pericarp	Aqueous extract pericarp	Rat	Alters the sperm membrane physiology
88	***Sapindus trifoliatus Linn.***	Ritha	Fruit	Ethanol extract	Gerbils	Adverse effect on spermatyogenesis
89	***Sarcostemmma acidum Voigt***	Somlata	Stem	Methanol extract	Rat	Arrest of spermatogenesis
90	***Semecarpus anacardium Linn. F.***	Bhilawa	Seed powder Fruit	Aqueous suspension Ethanol extract “	Rat - Antiandrogenic and antispermatogenic activity Arrest of spermatogenesis	Antiandrogenic effect, degeneration of germ cells
91	***Salanum surattense Burm. F. syn. S. xanthocarpuym Schrad & Wendl.***	Kantkari	Berries Seed Root	Solasodine Alcoholic extract 50% Ethanol extract	Dog Rat Dog Rat Rat	Impairment of spermatogenesis Reversible antifertility activity Epididymal dysfunction due to its antiandrogenic potency Reduced testosterone levels and effect on testes and maturing sperms Antispermatogenic effect
92	***Stephania hernandifolia Wild.***	Aknadi	Leaf	Aqueous extract	Rat	Diminution of the activities of testicular androgenic key enzymes and plasma testosterone with spermatogenesis.
93	***Stevia revaudiana Bertoni***	-	Whole plant	Aqueous extract	Rat	Decrease in testosterone level

94	***Striga orobanchoides Benth.***	Lalagia	Whole plant	Ethanol extract	Rat	Antispermatogenic effect and reduced weight of sex organs.
95	***Syzygium cuminii Linn. Syn. Eugenia jambolana Lam***	Jamun	Seed	Alcoholic extract	-	Antispermatogenic effect
96	***Terminalia arjuna Wight & Arn.***	Arjun	Bark	50% ethanol extract	Rat	Antispermatogenic effect
97	***Tinospora cordifolia (Willd.) Miers ex Hook. F. & Thoms***	Neem giloy	Stem	70% methanol extract	Rat	Antispermatpogenic effect
98	***Trigonella fopenumgraecum Linn.***	-	Seed	-	Rat	Antyiandrogenic effect
99	***Trippterygium hypoglaucum (level) Hutch***	-	Root xylem	-	Human	Reversible regressive effect
100	***Tripterygium wilfordi Hook f.***	-	Root	Glycoside GTW Glocoside GTW Tripchlorolide T4 monomers and R II GTW “ Tripchlorolide	Human and rat Rat Mice Rat Rat Mouse Rat	Antifertility activity Reduced motility of spermatozoa and sperm count Plasma level of testosterone decreased Degenerative changes in seminiferous tubules Antispermatogenic activity Antifertility activity Immunosuppressive effect Antifertility activity No side effect
101	***Tylophora asthmatica Wight & Arn.***	Damabel	Leaf and Stem	Pure alkaloid	Rat	Antispermatogenic activity
102	***Vigna ungiculuta (Linn.) Walp.***	Cowpea	-	-	Rat	Antifertility effect
103	***Vitex negundo Linn.***	-	Seed	Seed extract	Dog	Induced azoospermia

Source: Gupta, R.S. and Sharma, R. (2005).

APPENDIX II

RAW DATA OF SEMEN ANALYSIS

S/No	Sperm count x10^6cell/ml	Active cell	Sluggish cell	Non motile	Normal cell	Head defect	Tail defect	Viable cells	Non viable cells
1	5.0	30	50	20	70	10	20	60	40
2	1.5	10	20	70	60	15	25	56	44
3	2.0	05	10	85	63	25	12	62	38
4	2.5	04	10	86	58	20	22	56	44
5	2.5	0	10	90	61	29	10	49	51
6	5.0	56	34	10	69	15	16	72	28
7	3.0	05	15	80	50	30	20	58	42
8	2.5	0	0	100	55	30	15	48	52
9	4.5	0	0	100	60	10	30	55	45
10	3.5	0	0	100	51	30	19	45	55
11	2.7	0	0	100	55	25	20	40	60
12	2.5	0	0	100	58	15	27	51	49
13	1.5	0	0	100	57	20	23	52	48
14	1.8	0	0	100	61	19	20	39	61
15	1.0	0	0	100	58	18	24	41	59
16	1.3	0	0	100	59	19	22	43	57
17	3.5	0	0	100	55	25	20	51	49
18	4.5	0	0	100	57	18	25	53	47
19	1.5	0	0	100	60	10	30	59	41
20									
21	12.0	0	25	75	65	15	25	59	41
22	15.0	0	0	100	55	25	20	62	38
23	7.0	0	0	100	65	15	20	62	38
24	9.0	0	0	100	67	15	18	55	45
25	8.0	0	0	100	56	24	20	45	55

26	3.0	0	10	90	56	24	20	47	53
27	2.5	0	25	75	59	23	18	52	48
28	8.0	0	0	100	52	20	28	61	39
29	21.0	10	50	40	69	13	18	65	35
30	9.5	10	60	30	56	24	20	59	41
31	18.0	05	40	55	62	18	20	79	21
32	3.5	0	10	90	53	19	28	58	42
33	4.0	0	05	95	58	22	20	69	31
34	2.5	0	05	95	56	23	21	62	38
35	3.5	0	05	95	59	13	28	58	42
36	35.0	10	60	30	75	12	13	71	39
37	0.5	0	0	100	51	15	34	35	65
38	5.0	0	0	100	58	18	24	41	59
39	27.0	0	0	100	71	10	19	68	32
40	2.5	0	0	100	57	20	23	57	43
41	2.0	0	5	95	55	20	25	35	65
42	12.0	0	15	85	66	20	14	65	35
43	1.5	0	0	100	59	23	18	56	44
44	1.8	0	5	95	60	18	22	58	42
45	2.0	0	0	100	61	20	19	49	51
46	5.0	0	03	97	71	10	19	45	55
47	1.5	0	0	100	50	35	15	52	48
48	1.0	0	0	100	58	20	22	55	45
49	15.0	0	0	100	68	15	17	65	35
50	12.0	0	09	91	72	09	19	67	33
51									
52	1.5	0	0	100	51	20	29	43	57
53	2.5	0	0	100	61	25	14	49	51
54	2.0	0	0	100	59	16	25	43	57
55	2.5	0	0	100	58	18	24	45	55

56	2.0	0	02	98	53	30	17	49	51
57	1.5	0	0	100	61	18	21	52	48
58	10.5	0	25	75	65	15	20	69	31
59	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0
61	3.5	0	0	100	52	27	21	52	48
62	2.5	0	0	100	58	24	18	55	45
63	3.0	0	01	99	56	25	19	52	48
64	2.9	0	0	100	58	26	16	43	57
65	3.6	0	0	100	56	20	24	58	42
66	3.6	0	0	100	49	22	29	68	32
67	2.2	0	0	100	46	33	21	72	28
68	3.9	0	0	100	71	22	07	69	31
69	2.4	0	0	100	69	28	0.3	72	28
70	2.0	0	0	100	57	32	21	64	36
71	2.4	0	0.1	9.9	52	38	10	52	48
72									
73	1.9	0	0	100	53	29	18	31	69
74	4.9	0	0	100	49	29	22	76	23
75	1.0	0	0	100	65	27	08	66	34
76	1.8	0	0	100	66	25	09	70	30
77	1.4	0	0	100	56	33	11	69	31
78	1.6	0	0	100	66	29	05	53	57
79									
80	0.4	0	0	100	71	24	06	71	29
81	0.2	0	0	100	76	20	04	76	24
82	9.5	0	03	97	63	20	17	57	43
83	3.2	0	0	100	59	27	14	60	40
84	4.3	0	0	100	47	29	14	60	40
85	2.1	0	0	100	47	42	11	67	33

APPENDIX III

RAW HAEMATOLOGICAL DATA

S/No	PCV L/L	Hb	RBC xlO^{12}	Mcv xlO^{15}	MCH $io-^{12}$(PS)	MCHC gm/dl	WBC xlO^{9}
1	.43	14.0	7.53	57	18.6	32.6	4.4
2	.47	15.2	8.66	54	17.6	32.3	3.8
3	.45	14.8	8.26	51	17.9	32.9	4.2
4	.45	15.2	8.40	53	18.0	33.8	4.8
5	.43	14.3	7.60	56	18.8	33.3	3.7
6	.42	13.9	7.13	58	19.5	33.0	3.8
7	.43	14.2	7.58	56	19.9	33.0	4.4
8	.42	14.1	7.15	58	19.7	33.6	5.4
9	.44	15.0	8.24	53	18.2	34.0	3.6
10	.44	14.8	8.46	52	17.5	33.6	3.8
11	.41	13.5	7.81	52	17.3	33.9	5.2
12	.43	14.4	7.48	57	19.3	33.5	4.5
13	.43	14.2	7.92	54	17.9	33.5	4.4
14	.42	14.0	7.60	55	18.2	33.3	3.9
15	.40	13.4	6.93	57	19.3	33.5	3.8
16	.41	13.5	7.11	57	19.0	32.9	4.6
17	.25	8.1	4.26	58	19.0	32.4	7.4
18	.39	13.2	5.48	71	24.1	33.8	3.8
19	.38	13.0	5.34	71	24.3	34.2	4.7
20							

21	.39	13.0	6.21	62	20.8	33.3	3.9
22	.37	12.0	6.66	55	18.0	32.4	3.6
23	.45	15.2	9.20	48	16.5	33.8	4.2
24	.37	12.6	6.82	54	18.5	34.1	4.8
25	.41	13.5	7.18	57	18.5	32.9	4.1
26	.44	14.5	8.41	52	17.2	33.0	4.6
27	.43	14.2	8.33	52	17.0	33.0	4.4
28	.47	15.8	8.70	54	18.1	33.6	3.8
29	.41	13.9	7.10	66	19.6	33.9	3.6
30	.45	15.2	8.64	52	17.6	33.8	3.2
31	.42	14.3	7.81	54	18.3	34.0	4.0
32	.40	13.5	7.11	56	19.0	33.8	4.4
33	.41	13.7	6.94	59	19.4	33.4	4.8
34	.43	14.4	7.86	55	18.3	33.5	5.5
35	.38	13.0	6.88	55	18.9	34.2	4.9
36	.42	14.2	7.42	57	19.1	33.8	3.3
37	.46	15.4	8.44	55	18.2	33.5	3.9
38	.44	15.0	7.62	58	19.7	34.1	4.4
39	.42	14.2	7.48	56	19.0	33.8	4.0
40	.37	12.4	6.66	56	18.6	36.5	5.8
41	.41	13.0	7.10	58	18.3	31.7	6.2
42	.43	14.5	7.98	54	18.2	33.7	4.2
43	.42	14.1	7.34	57	19.2	33.6	4.6
44	.44	15.0	7.38	60	20.3	34.1	4.4

45	.33	12.0	6.13	54	19.6	36.4	4.2
46	.35	12.2	6.15	57	19.8	34.9	5.8
47	.43	14.4	7.76	55	18.6	33.5	4.2
48	.38	13.1	6.73	56	19.5	34.5	6.4
49	.35	13.0	6.94	50	18.7	37.1	3.2
50	.39	13.3	7.32	53	18.2	34.1	3.3
51							
52	.40	13.3	7.10	56	18.7	33.3	4.5
53	.41	13.7	6.34	55	21.6	33.4	4.2
54	.39	13.2	7.77	50	17.0	33.8	3.4
55	.40	13.6	6.97	57	19.5	34.0	3.6
56	.37	13.0	7.40	50	17.6	35.1	6.2
57	.39	13.6	7.71	51	17.6	34.9	6.6
58	.42	14.1	7.84	54	18.0	33.6	
59	.41	13.9	7.68	53	18.0	33.9	5.3
60	.37	12.3	6.38	58	19.3	33.2	4.8
61	.39	13.4	6.90	57	19.4	34.4	8.2
62	.40	13.6	7.10	56	19.2	34.0	5.3
63	.45	16.0	8.62	52	18.6	35.6	5.4
64	.33	10.8	5.70	58	18.9	32.7	6.2
65	.35	12.1	6.30	55	19.2	34.6	4.8
66	.37	12.5	6.70	55	18.7	33.8	4.4
67	.40	14.0	7.52	53	18.6	35	5.2
68	.44	15.2	8.25	53	18.4	34.5	6.1

69	.37	12.4	6.55	56	18.9	33.5	3.4
70	-	-	-	-	-	-	-
71	.42	14.3	7.28	58	19.6	34.0	4.8
72	.42	12.6	6.49	56	19.4	30.0	5.4
73	.40	13.5	7.10	56	19.0	33.8	5.5
74	.43	14.6	7.55	57	19.3	34.0	4.5
75	-	-	-	-	-	-	-
76	.41	14.2	7.28	56	19.5	33.8	4.4
77	.40	13.4	7.18	56	18.7	33.5	4.3
78	.45	14.8	7.66	59	19.3	32.9	4.6
79	.43	14.5	7.86	55	18.4	33.7	4.9
80	.45	15.5	8.12	55	19.1	34.4	4.5
81	.40	13.2	7.10	56	18.6	33.0	5.2
82	.40	13.2	6.82	59	19.3	33.0	4.6
83	.38	13.0	6.20	61	21.0	34.2	5.3
84	.45	15.2	8.42	55	18.1	33.8	6.4
85	.39	13.5	7.62	51	17.7	34.6	4.4

APPENDIX IV

RAW DATA OF BIOCHEMICAL ANALYSIS

S/N	Fructose	Acid phos	Sialic Acid	Acrosin	Hyluronidase	SGOT	LDH	Testosterone	Ceatinine	glucos	Weight	
											Initial	Final
1	7.4	10.6	28	106	96	16	180	3.9	65	12.3	147.37	153.59
2	12.2	18.1	24	93	106	19	207	6.0	51	16.3	154.31	149.31
3	16.1	13.3	22	49	92	12	140	4.36	50	11.5	167.93	181.45
4	8.3	16.4	26	58	102	24	141	4.8	49	12.3	171.32	177.17
5	5.7	15.8	28	82	116	18	138	3.3	70	19.8	145.20	174.74
6	7.1	10.8	33	106	131	16	167	2.9	66	12.9	153.83	179.05
7	12.2	12.6	39	92	126	15	165	4.1	69	11.9	175.94	199.74
8	7.0	10.9	24	68	102	13	172	4.6	71	14.4	148.80	142.03
9	8.3	10.8	28	71	106	20	204	3.5	61	13.4	171.96	164.60
10	10.4	12.1	31	70	113	18	210	3.5	65	24.2	148.74	181.18
11	9.0	10.6	38	68	92	18	167	4.0	60	15.8	146.37	167.56
12	11.6	12.3	36	79	98	26	134	4.2	71	14.3	147.51	176.45
13	12.2	13.9	39	84	101	20	180	4.5	62	17.3	156.18	172.38
14	11.3	15.6	29	86	111	24	160	4.0	66	16.7	147.50	164.15
15	16.1	12.9	30	102	137	20	155	3.9	69	16.2	145.04	167.05
16	12.1	10.9	28	94	122	18	206	5.6	65	12.0	179.08	177.21
17	13.3	13.7	24	86	122	16	210	4.0	61	11.9	144.53	170.11
18	16.3	14.0	26	80	108	20	206	4.9	62	10.9	165.62	197.67
19	8.6	16.0	28	69	102	22	106	4.3	49	11.8	146.70	154.63
20												
21	12.4	12.8	22	64	94	20	193	4.1	65	13.0	149.17	174.52
22	16.1	14.2	24	60	90	18	166	4.7	66	12.6	154.04	165.28
23	10.3	13.6	24	54	86	30	204	5.0	69	18.9	147.73	168.28
24	7.1	14.1	28	71	106	26	229	4.3	71	9.2	162.23	161.83
25	7.1	13.8	31	68	92	24	165	3.3	65	10.5	121.60	Dead
26	9.0	12.7	38	78	88	26	251	3.6	68	8.9	158.00	164.24
27	8.3	15.5	139	76	106	29	206	3.9	72	6.7	153.64	187.96
28	9.0	12.3	38	79	106	18	186	4.6	66	12.1	169.69	158.16
29	8.1	14.0	31	83	127	14	128	4.0	47	9.7	152.64	160.07
30	2.6	16.3	33	82	161	22	147	4.9	59	16.9	139.18	158.25
31	3.1	11.5	43	121	192	20	166	5.9	60	7.1	145.81	158.85
32	3.9	8.6	30	70	112	12	189	3.8	72	13.6	159.09	172.05
33	4.7	10.2	32	76	127	16	210	5.0	77	19.7	162.11	174.07
34	5.6	16.8	26	49	96	26	206	4.0	68	5.8	110.50	129.52

35	11.3	12.6	33	69	106	12	224	4.7	61	10.3	121.95	153.79
36	16.3	12.0	38	183	218	18	149	6.3	63	4.8	154.72	176.28
37	7.1	13.9	26	49	101	21	151	2.8	68	11.6	172.42	172.17
38	6.4	11.6	30	62	127	26	160	3.7	72	12.0	155.77	179.52
39	12.2	14.9	31	101	136	24	141	5.5	56	9.7	109.35	127.60
40	16.3	14.0	26	46	96	18	166	3.0	54	12.4	152.88	173.05
41	13.3	15.1	28	44	86	12	182	3.8	60	12.1	170.49	182.13
42	10.2	10.6	42	109	122	14	133	4.1	61	8.1	124.65	163.10
43	16.1	10.9	26	56	103	16	192	3.7	66	14.5	161.13	Dead
44	8.4	14.1	24	49	86	13	146	3.2	71	8.3	157.12	160.72
45	12.1	14.4	28	55	92	15	144	3.8	68	9.4	144.26	170.50
46	16.0	16.8	29	63	106	18	139+	4.7	71	9.9	122.65	154.56
47	13.3	12.3	30	38	86	11	182	3.2	68	8.6	158.85	170.65
48	12.1	10.1	26	31	88	18	180	3.3	72	9.9	187.63	181.15
49	11.3	9.6	38	112	137	17	141	6.0	61	6.8	154.27	167.57
50	4.9	11.5	35	90	118	12	134	4.1	66	13.1	139.20	174.55
51												
52	5.6	15.1	33	41	109	13	171	3.2	68	9.8	169.33	183.07
53	10.2	16.0	38	47	96	18	138	4.0	60	13.8	128.40	170.34
54	8.6	12.2	26	36	86	24	141	3.3	68	7.9	172.82	196.15
55	6.9	12.1	27	67	96	20	146	4.1	4.8	12.8	178.45	193.73
56	8.3	9.3	35	84	102	18	159	6.8	67	14.6	159.34	148.51
57	4.7	14.3	29	92	110	24	187	3.3	82	10.1	151.23	153.67
58	4.4	10.2	26	76	87	20	139	3.0	54	11.1	151.44	193.72
59	6.6	16.5	38	49	96	24	152	1.8	58	16.4	152.25	160.85
60	10.2	13.1	42	41	98	26	169	2.0	61	14.4	147.45	164.15
61	7.1	13.7	33	46	95	24	164	4.0	66	10.8	148.13	216.70
62	5.8	14.5	41	49	104	28	178	3.5	69	8.7	146.44	153.90
63	9.2	10.3	39	60	106	22	159	3.7	64	7.8	140.35	145.57
64	6.8	12.5	30	84	98	23	151	4.7	77	13.4	145.25	269.24
65	7.3	18.4	36	86	94	27	147	4.4	66	16.8	134.47	295.74
66	7.5	16.3	31	79	102	24	139	6.1	71	5.4	131.25	104.75
67	9.2	12.1	38	92	86	28	142	4.3	68	3.8	144.15	169.71
68	13.3	11.0	27	98	94	27	144	3.9	72	3.7	150.33	160.97
69	8.5	14.6	29	72	107	24	139	4.5	68	3.6	129.28	162.91
70	10.2	12.2	33	86	98	26	169	3.8	68	11.5	144.42	155.58
71	17.2	10.8	40	84	92	22	155	4.2	71	5.9	138.44	173.02
72												
73	4.9	9.6	28	109	94	22	177	4.1	62	3.8	135.22	141.56
74	6.8	10.3	33	86	96	26	164	4.4	65	9.0	134.33	147.59

75	6.8	15.1	24	104	90	34	171	3.8	66	6.5	133.35	249.41
76	7.0	10.5	24	104	96	28	160	4.1	60	16.0	137.47	267.07
77	10.3	12.7	36	92	92	24	152	6.3	71	3.1	142.39	160.52
78	6.9	13.3	31	98	98	30	149	4.3	64	22.4	136.22	208.75
79												
80	10.0	16.0	29	100	86	38	168	2.9	70	9.1	123.37	162.42
81	4.7	10.8	35	96	84	37	177	4.4	61	5.6	141.22	167.79
82	10.5	14.0	33	98	109	26	182	3.9	58	9.4	241.35	181.35
83	7.7	8.6	29	72	131	28	161	4.0	70	4.1	236.45	154.25
84	8.3	15.0	39	94	86	28	171	4.6	66	6.8	296.15	171.38
85	12.0	11.1	26	103	90	24	176	7.0	64	20.4	238.36	169.15